Abstract:
The invention relates to lithium treatments for treating microbial infections in human subjects. Uses of compositions containing compounds that liberate lithium ions are described, including adjuvants and devices for administration. The lithium treatment(s) can be used in combination with other treatments, such as mupirocin, for treating microbial infections. The combination treatment(s) may be administered concurrently with, or before and/or after the lithium treatment. The invention also relates to lithium treatments for scar revision and wound healing in human subjects. The invention also relates to dermabrasion treatments for skin conditions and novel dermabrasion tips for carrying out such treatments.

[Continued on next page]

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LITHIUM TREATMENT FOR MICROBIAL INFECTIONS

Cross-Reference to Related Applications


1. INTRODUCTION

[0002] The invention relates to lithium treatments for microbial infections and for colonization by microbes in human subjects. Compositions containing compounds that liberate lithium ions are described, including formulations comprising lithium carbonate for topical administration. The lithium treatment(s) can be used in combination with other treatments for microbial infections and for reduction of colonization by microbes.

[0003] The invention also relates to lithium treatments for scar revision and wound healing in human subjects. The lithium treatments may be administered as a single pulse treatment or intermittently. Compositions containing compounds that liberate lithium ions are described, including formulations comprising lithium carbonate for topical administration. Uses of compositions containing compounds that liberate lithium ions are described, including adjuvants and devices for administration. The lithium treatment(s) can be administered via a drug sprayer device. The lithium treatment(s) can be used in combination with other treatments for scar revision, wound healing, or for hair growth or hair development and follicle neogenesis. Such combination treatments may involve mechanical or physical treatments that modulate scar revision or wound healing, or that cause integumental perturbation (e.g. such as laser, surgical treatments, including skin graft or full thickness wounding, or dermabrasion, dermatome planing, etc.); and/or chemical treatments that modulate wound healing, scar revision, or hair growth or hair follicle development, or that cause integumental perturbation or immune stimulation (e.g., such as adjuvants, antigens, cytokines, growth factors, etc.) for the treatment of wounds or revision of scars. The combination treatment(s) may be administered concurrently with, or during the "holidays"
between, cycles of intermittent lithium treatments; or concurrently with, or before and/or after the pulse lithium treatment.

[0004] The embodiments of the present invention also involve mechanical or physical treatments that cause integumental perturbation (e.g., dermabrasion) which may be used as part of a treatment for modulating hair growth in human subjects. Additionally, the embodiments of the present invention may involve mechanical or physical treatments that cause dermabrasion which may be used as part of a treatment for different skin conditions.

2. BACKGROUND

[0005] The skin usually provides a remarkably good barrier against pathogenic microorganisms, such as pathogenic bacteria and fungi. The skin is colonized with resident bacterial flora. Although these bacteria are mostly non-pathogenic, colonization with potential pathogens also occurs, such as with Staphylococcus aureus, including methicillin-resistant strains. Although many microbes come in contact with or reside on the skin, they are normally unable to establish an infection. Cutaneous microbial infections often arise when there is a break in the continuity of the skin because the skin is torn, cut, or punctured, resulting in a wound. Microbial infections can also arise as a part of systemic infection.

[0006] Bacterial infections can be caused by several types of bacteria, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Streptococcus pyogenes*, *corynebacterium species*, enterococci, *Proteus mirabilis*, group D streptococci, other gram-positive aerobes, and *Bacteroides fragilis*. The most common anti-bacterial agents prescribed to patients with bacterial infections are antibiotics. However, infections caused by antibiotic resistant strains have become a global problem. The increasing prevalence of multi-drug resistant organisms with few or no treatment options such as methicillin resistant Staphylococcus aureus (MRSA), vancomycin resistant enterococci (VRE), multidrug-resistant *Acinetobacter baumannii*, and the extended spectrum beta-lactamase (ESBL) producing Gram-negative bacilli are a serious cause for concern. Thus, there is an urgent need for improved therapies to treat bacterial infections.

[0007] Mycosis is a condition in which fungi pass the resistance barriers of the human or animal body and establish infections. *Superficial mycoses* are limited to the outermost layers of the skin and hair. *Cutaneous mycoses* extend deeper into the epidermis, and also include invasive hair and nail diseases. Anti-fungal agents that are currently used to treat cutaneous
fungal infections include imidazoles, polyenes, thiocarbamates, ciclopirox, terbinafine, amorolfine, undecylenic alkanolamide and griseofulvin. Cutaneous fungal infections can be persistent and can often reoccur. Thus there is a need for alternative treatments to effectively and safely treat fungal infections, including novel topical treatments for conditions where current therapies generally require systemic administration (e.g. onychomycosis and tinea capitis).

[0008] Microbial biofilms are increasingly being implicated in the failure of antimicrobial agents to treat wound infections, which leads to delayed healing. The presence of biofilms in various types of wounds has been shown directly by microscopic evaluation, revealing biofilms to consist of bacteria, fungi and mixed-species biofilms. Several studies have shown that organisms in biofilms act synergistically, modulating the action of antimicrobial agents and other toxins, improving survival of organisms in various situations. To date, no antimicrobial class of compounds are available that effectively treat wound-related biofilms.

2.1 **ACNE**

[0009] Acne is a general term used for acneiform eruptions. It is usually used as a synonym for acne vulgaris. Acne vulgaris (or cystic acne) is a common human skin disease, characterized by areas of skin with seborrhea (scaly red skin), comedones (blackheads and whiteheads), papules (pinheads), pustules (pimples), nodules (large papules) and possibly scarring. Acne affects mostly skin with the densest population of sebaceous follicles; these areas include the face, the upper part of the chest, and the back.

[0010] Acne is a multifactorial disease: genetic factors, stress, androgens, and excess sweating all influence its development and/or severity. Corticosteroids, oral contraceptives, iodides, bromides, lithium, and chemicals such as dioxins are known to induce acne eruptions.

[0011] Acne is a disease of the pilosebaceous units in the skin. A changed keratinisation pattern in the hair follicle leads to blockage of sebum secretion. It is probable that hyperresponsiveness to the stimulation of sebocytes and follicular keratinocytes by androgens leads to the hyperplasia of sebaceous glands and seborrhea that characterise acne. The enlarged follicular lumen attributable to inspissated keratin and lipid debris forms a closed comedone (whitehead). When the follicle has a portal of entry at the skin, the semisolid mass protrudes forming a plug, producing an open comedone (blackhead).

[0012] *Propionobacterium acnes* colonises the follicular duct and proliferates, breaking down the sebum to triglycerides, irritants that probably contribute to the development of
inflammation. When the follicular epithelium is invaded by inflammatory cells it ruptures, releasing sebum, micro-organisms, and keratin into the dermis. Neutrophils, lymphocytes, and foreign body giant cells accumulate and produce the erythematous papules, pustules, and nodular swelling characteristic of inflammatory acne.

[0013] Acne may also refer to acne aestivalis (multiple, uniform, red, papular lesions reported to occur after sun exposure), acne conglobata (a highly inflammatory disease presenting with comedones, nodules, abscesses, and draining sinus tracts), acne cosmetica (acne caused by or made worse by cosmetics), acne fulminans (a severe form of acne, which can occur after unsuccessful treatment for another form of acne, acne conglobata), acne mechanica (an acneiform eruption that has been observed after repetitive physical trauma to the skin such as rubbing), acne medicamentosa (drug-induced acne), acne miliaris necrotica (acne consisting of follicular vesicopustules, sometimes occurring as solitary lesions that are usually very itchy), acne necrotica (acne in which the primary lesion is a pruritic or painful erythematous follicular-based papule that develops central necrosis and crusting and heals with a varioliform scar), acne rosacea (a red rash predominantly on the face), infantile acne/neonatal acne (a rash seen on the cheeks, chin, and forehead of infants) and occupational acne (acne caused by exposure to various industrial compounds).

2.2 ONYCHOMYCOSIS

[0014] Onychomycosis is used as a general term to denote any fungal nail infection. The nail plate is a hard, thin (0.25-0.6 mm for fingernails and up to 1.3 mm for toenails) plate derived of highly disulfide-linked keratin. The structure is made up of approximately 25 layers of dead, keratinized, flattened cells, which are tightly bound to one another via numerous intercellular links, membrane granules and desmosomes. The nail plate is hydrophilic and is permeable to water and aqueous-based solutions, as opposed to oil, alcohol and hydrophobic molecules.

[0015] The term "dermatophytosis" is used to describe infection by members of the genera Microsporum, Trichophyton and Epidermophyton. The species that cause onychomycosis most often are T. rubrum, T. mentagrophytes and E. floccosum. Dermatophytes account for 90% of the cases of onychomycosis of the toenails and at least 50% of fingernail infections. The fungal cells associated with dermatophytes manufacture keratinolytic proteases, which provide a means of entry into living cells.

[0016] Dermatophytoses of the fingernails and toenails, in contrast to those at other body sites, are difficult to eradicate with drug treatment. The hard, protective nail plate sequesters
pathogens between the nail bed and plate. Many fungal nail infections have proven to be very resistant to any type of treatment. Systemic administration of anti-fungal drugs, such as the azoles (ketoconazole, fluconazole) and the allylamines (terbinafine, butenafine), is hindered by limited blood circulation in the nail bed and poor transport to the nail plate, requiring high dosage levels for long periods of time. Such high drug dosages can have adverse side effects, and it has been found that clearance of the infection is often only temporary. Systemic treatment must often be continued indefinitely, thereby also increasing the potential for antimicrobial resistance.

[0017] Topical therapy for onychomycosis might be the treatment of choice, since it does not lead to adverse systemic effects or drug interactions. In order to be effective, antifungal drugs need to penetrate the nail plate to reach the infection sites under the nail. The nail plate is a relatively thick structure that inhibits penetration of the drug being applied. Nails that have been infected with onychomycosis have thicker nail plates, sometimes > 500 µm. The topical application of creams, solutions, lotions and gels is often dissipated in relatively short periods of time. Attempts have been made to incorporate topically active antifungal drugs into film-forming compositions (e.g. nail polishes or lacquers to improve drug persistence). The removal of the nail (nail avulsion) can improve topical drug treatment. Another recent means of treating onychomycosis include laser therapy on the affected nail, followed by topical administration of an antifungal agent.

[0018] It is desirable to provide an effective system and composition for topically treating nail fungal infections. Specifically, a need exists to deliver a potent antimycotic agent through the nail to attain appropriate concentrations so that the targeted fungal organism is eradicated. A delivery system to achieve this need must possess the necessary chemical, mechanical or physical properties to allow penetration of the antimycotic through the three layers of the nail such that therapeutic levels are attained.

[0019] The current standard of care for onychomycosis is Penlac® (Cyclopinox nail lacquer 8% solution). It is the only topical therapy for onychomycosis approved by the FDA. Penlac® works by disruption of DNA, RNA and protein synthesis, thereby killing the fungus responsible for these infections. It requires long term treatment, and although about 1 out 15 patients demonstrate improvement, recurrence is common.

2.3 WOUND HEALING AND SCAR FORMATION

[0020] Wound healing, or wound repair, is an intricate process in which the skin (or some other organ) repairs itself after injury. In normal skin, the epidermis (outermost layer) and
dermis (inner or deeper layer) exist in a steady-state equilibrium, forming a protective barrier against the external environment. Once the protective barrier is broken, the physiologic process of wound healing is immediately set in motion. The classic model of wound healing is divided into three or four sequential, yet overlapping, phases: (1) hemostasis (not considered a phase by some authors); (2) inflammation; (3) proliferation; and (4) maturation and remodeling. For reviews on wound healing, see Lorenz & Longaker, 2003, Chapter 7 in Surgery: Basic Science and Clinical Evidence, pp. 77-88.

The phases of wound healing normally progress in a predictable, timely manner; if they do not, healing may progress inappropriately to either a chronic wound, such as a venous ulcer, or pathological scarring such as a keloid scar and other forms of scarring. When wound edges are directly next to one another, and there is little tissue loss, wounds may heal by primary intention. Such wounds may be referred to as "closed wounds." Scarring is often minimal, but can be variable depending on the size and location of the wound, the tension on tissue and other factors. Healing by secondary intention occurs when the extent of skin separation or skin tissue removed is too great for the edges of the wound to be placed in proximity (e.g., by bandages or sutures). Such wounds may be referred to as "open wounds." Healing by secondary intention follows the same basic steps as wounds that heal by primary intention, but each sequence may take much longer, especially the proliferative phase. In healing by secondary intention, there is much more granulation tissue formation and contraction, which carries a greater risk of scarring. In wound healing by tertiary intention (delayed primary closure), the wound is initially cleaned, debrided, and observed, and typically 4 or 5 days elapse before closure. The wound is purposely left open. Examples include healing of wounds by use of tissue grafts.

A major component of wound healing in humans is scar formation. A scar ("cicatrix"; plural, "cicatrices") is an area of fibrous tissue that forms as part of the healing process to replace normal skin after injury. A hallmark of scars is altered extracellular matrix, notably a reduction of elastin fibers (De Vries et al., 1995). Scars result from damage to the dermis, and with the exception of very minor lesions, every wound results in some degree of scarring. Scars generally form in proportion to the extent of damage.

Human skin appendages, also referred to as "adnexal" structures, include hair and hair follicles, sebaceous glands (which secrete sebum onto hair follicle to oil the hair), eccrine and apocrine sweat glands, and nails. The importance of hair follicles to skin biology is now known not to be restricted to production of hair shafts and sebum. Rather, the hair follicle and other adnexal structures appear to be regenerative organs that play a central role in
normal skin homeostasis and in response to wounding. Several lines of evidence suggest that hair follicles and other skin adnexal structures have the potential to provide skin with stem cells and other elements that are important for skin regeneration. These data suggest that hair follicles participate in the regeneration of normal dermis and epidermis in healing wounds, thereby preventing or possibly reversing scarring or contributing to the formation of scars that are not disfiguring or dysfunctional.

**[0024]** Acute treatment of wounds is generally focused on hemostasis and antimicrobial considerations. The treatment depends on the type, cause, and depth of the wound as well as whether other structures beyond the skin are involved. Appropriate treatment of chronic wounds seeks to address the problems at the root of chronic wounds, including ischemia, hypoxia, bacterial load, and imbalance of proteases. Scar revision and wound management are limited by the limited regenerative capacity of adult human skin. Current therapies (skin grafting, pressure application) have modest functional and cosmetic results and are limited by the availability of donor skin, and by morbidity of donor and graft sites. There is an urgent need for improved therapies to treat wounds and scars.

### 2.4 INCREASING EPIDERMAL THICKNESS

**[0025]** The epidermis is the outer layer of the skin providing the body’s major barrier against the environment. In humans, the epidermis is thinnest on the eyelids at 0.10 mm and thickest on the palms and soles at 1.5mm. Epidermal thickness is reduced by certain factors (age, smoking status). If the epidermis is thinning because of age, smoking, or other environmental factors, it is desirable to increase epidermal thickness. The present invention provides methods for increasing epidermal thickness.

### 2.5 COSMETIC COMPOSITIONS

**[0026]** Many cosmetic compositions have been developed to enhance the appearance of the human body. In particular, there exists a plethora of topical lotions and creams that promise to tighten and plump aging skin, and which at best provide temporary and short-lived results. The alternative for many is expensive plastic surgery procedures that are accompanied with serious risks. There is a need for cosmetic compositions that effectively enhance the appearance of the skin with long lasting results, while being safe, stable and, cost-effective.
2.6 HAIR FOLLICLE MORPHOGENESIS AND REGENERATION

[0027] It has been proposed that hair follicle neogenesis can be associated with wound healing in animals (e.g., rabbits, mice). See, Stenn & Paus, 2001, Physiol. Revs. 81:449-494. In a mouse study, Dr. George Cotsarelis showed that physically disrupting the skin and existing hair follicles, in a defined fashion, can lead to hair follicle neogenesis (Ito et al, 2007, Nature 447:3 16-321). Despite earlier suggestions of the regenerative capacity of the adult mammalian skin to recreate the embryonic follicle, hair follicle neogenesis was never proven because of an incomplete understanding of the fundamental biology of the follicle and the lack of tools needed to demonstrate the occurrence or hair follicle neogenesis (see, Argyris et al, 1959, Dev. Biol. 1: 269-80; Miller, 1973, J. Invest. Dermatol. 58:1-9; and Kligman, 1959, Ann NY Acad Sci 83: 507-511). More recently, a series of murine experiments definitively showed that hair follicle-derived epithelial stem cell progenitors migrate out of the follicle and contribute to the re-epithelialization of injured skin (see, Morris et al, 2004, Nature Biotechnology 22:41 1-417; Ito et al, 2004, Differentiation 8:548-57; and Ito et al, 2005, Nature Medicine 11:1351-1354.)

[0028] Cotsarelis showed, in mice, that following wound closure of large healed wounds created by full thickness excision (FTE) (1 cm² square wounds) new hairs are formed at the center of the wound (Ito et al, 2007, Nature 447:316-321). Cotsarelis' findings (Nature, 447, p.7142, 2007) constitute a breakthrough in biology because they change the understanding of organ regeneration in mammals. It was previously thought that adult mammals did not have the ability to regenerate new hair follicles. Cotsarelis' work shows that, after wounding, the epidermis of mice can regenerate new follicles during a specific time window. Observations dating back to the 40's and 50's already indicated that mice, rabbits, and humans grew some hair follicles after wounding (Kligman, A. M. & Strauss, J. S. J. Invest. Dermatol. 27, 19-23 (1956) ; Billingham, R. E. & Russel, P. S. Nature 111, 791-792 (1956); Breedis, C. Cancer Res. 14, 575-579 (1954); Lacassagne, A. & Latarjet, R. Cancer Res. 6, 183-188 (1946)). However, these observations were discounted because they were not conclusive. By using advanced techniques, Cotsarelis has been able to create a mouse model where the fate of hair follicle stem cells can be traced and the chain of events leading to follicle neogenesis is conclusively established.

[0029] Other studies have identified a therapeutic window after epithelial disruption where the skin reverts to an embryonic state, allowing manipulation of skin and follicle phenotype by addition of compounds. For example, because new hair patterns after
wounding are not predetermined, the regulatory pathways relevant to follicle formation (e.g., Wnt, EGFR) can be influenced dramatically, e.g., to increase the number and size of follicles.

As described above, a major insight is Cotsarelis' identification of a therapeutic window after epithelial disruption wherein the skin reverts to an embryonic state, allowing manipulation of skin and follicle phenotype by addition of compounds.

The process described above occurs in two steps where the skin is first disrupted and then a drug is applied topically. For the first step, motorized devices for performing dermabrasion for skin resurfacing and scar restoration have been around for decades. Over these years, the traditional embodiment of a motorized rotating grinding wheel hasn't changed much. Essentially, when power is applied to an abrasive wheel it grinds off stratum corneum and epidermis until the desired clinical effect is achieved.

The rotating wheel, however, presents significant challenges when used in areas of thinning hair as part of a follicular growth treatment. Specifically, as can be seen in Figures 61 and 62, because the traditional dermabrasion wheel 202 rotates through 360 degrees, the rotating wheel 202 tends to wind up and pull out existing hair 204. Also, the rotational inertia of a rotating wheel becomes transferred to blood and debris thereby causing the blood and debris created by the dermabrasion process to splatter, raising safety concerns and visual unpleasantness. Further, as can be seen in Figure 63, a rotating wheel 202 tends to track and move or "walk" in the direction of rotation, resulting in poor overall control by the technician and also causing the skin 206 to deform 208, which makes the procedure consistency more difficult.

Additionally, the axial orientation of conventional dermabraders provides for poor ergonomics. As can be seen in Figure 64, with conventional dermabrasion hand pieces 210, the clinician's hand continually interferes with the patient and standard human factors engineering teaches that this is a poor way to hold a finesse instrument.

Accordingly, disclosed herein is a device directed to addressing the above-discussed drawbacks with conventional dermabrasion units. The embodiments of the present invention describe a skin disruption system leveraging traditional dermabrasion technology but modifying it for use on the scalp and other hairy areas.
3. **SUMMARY OF THE INVENTION**

[0035] Novel lithium treatments for microbial infections, *i.e.*, bacterial, fungal, or parasitic infections, are described. The lithium treatments described herein can be administered to treat cutaneous microbial infections, *e.g.*, cutaneous bacterial infections or cutaneous fungal infections. In particular, the lithium treatments can be administered to treat infected wounds, *e.g.*, wounds infected by bacteria or wounds infected by fungi, and to treat skin colonized by microbes.

[0036] Described herein are methods for treating microbial infections and microbial colonizations, comprising administering a lithium composition to a human subject in need thereof. Also described herein are methods for treating microbial infections and colonizations, comprising administering a lithium composition in combination with mupirocin. Any pharmaceutically acceptable compound that releases the lithium ion (also referred to herein as lithium cation, Li+, or ionized lithium) can be used for the lithium treatment; such compounds include, but are not limited to lithium gluconate, lithium succinate, lithium salt of mupirocin, and other organic salts/acids; and lithium chloride and other inorganic salts/acids, as described in Section 5.1, *infra*. Preferably, the lithium composition administered for treatment of a microbial infection or colonization comprises lithium carbonate. In an embodiment, mupirocin and lithium carbonate are co-mixed as a simple cream.

[0037] Alternatively, the lithium composition administered for treatment of a microbial infection or colonization comprises lithium gluconate or lithium succinate. In some instances, the lithium composition administered for treatment of a microbial infection does not comprise lithium chloride.

[0038] The microbial infections and colonizations treated with the lithium compositions described herein can be bacterial infections or bacterial colonizations. In some embodiments, the bacterial infection or colonization that is treated with the lithium composition is a cutaneous bacterial infection or colonization. In some embodiments, the cutaneous bacterial infection is an infected wound. The bacterial infections that are treated with the lithium compositions described herein can be caused by any bacteria, including, but not limited to, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Streptococcus pyogenes*, *corynebacterium species*, *enterococci*, *Proteus mirabilis*, group D *streptococci*, *other gram-positive aerobes*, and *Bacteroides*.
fragilis. In some embodiments, the cutaneous bacterial infection is an infected rash, including but not limited to eczema, cellulitis, or erysipelas. The bacterial infections that are treated with the lithium compositions described herein can be caused by any bacteria, including, but not limited to, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Streptococcus pyogenes*, *corynebacterium species*, *enterococci*, *Proteus mirabilis*, group D streptococci, other gram-positive aerobes, and *Bacteroides fragilis*. In some embodiments, the cutaneous bacterial colonization is on clinically non-wounded skin. The bacterial colonizations that are treated with the lithium compositions described herein can be caused by any bacteria, including, but not limited to, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Streptococcus pyogenes*, *corynebacterium species*, *enterococci*, *Proteus mirabilis*, group D streptococci, other gram-positive aerobes, and *Bacteroides fragilis*.

[0039] The microbial infections treated with the lithium compositions described herein can also be fungal infections. In some embodiments, the fungal infection that is treated with the lithium composition is a cutaneous fungal infection. In some embodiments, the cutaneous fungal infection is an infected wound. The fungal infections that are treated with the lithium compositions described herein can be caused by any fungus, including, but not limited to, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Microsporum gypseum*, *Microsporum gypseum*, *Microsporum canis*, *Epidermophyton floccosum*, *Candida albicans*, and *Candida parapsilosis*, *Malassezia furfur*, and *Aspergillus fumigatus*.

[0040] In some embodiments, the lithium compositions described herein are not used to treat infections caused by viruses. In a particular embodiment, the lithium compositions described herein are not used to treat infections caused by DNA viruses. In a specific embodiment, the lithium compositions described herein are not used to treat infections caused by Herpes Simplex Virus (HSV).

[0041] In an embodiment, the lithium compositions described herein are administered to a subject to prevent, reduce, or eradicate colonization of one or more microbes, including but limited to *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, and drug-resistant *Enterococci*, and multi-drug resistant *Acinetobacter*. In another embodiment, a combination treatment comprising a lithium compound and mupirocin is administered to a subject to prevent, reduce, or eradicate
colonization of one or more microbes, including but limited to *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, and drug-resistant *Enterococci*. In an embodiment, the lithium compositions described herein are administered to a subject to prevent, reduce, or eradicate colonization of the nares, axillae, perineum, groin, chronic wounds or decubitus ulcer surface, around gastrostomy and tracheostomy sites, and in the sputum or urine. In another embodiment, a combination treatment comprising a lithium compound and mupirocin is administered to a subject to prevent, reduce, or eradicate colonization of the nares, axillae, perineum, groin, chronic wounds or decubitus ulcer surface, around gastrostomy and tracheostomy sites, and in the sputum or urine.

[0042] The lithium treatment can be administered topically, transdermally, intradermally, cutaneously, subcutaneously, intramuscularly, intravenously, orally, sublingually, or can be bucchal. Topical lithium treatment is a preferred embodiment because high local concentrations can be achieved while minimizing systemic exposure. In one such embodiment, lithium gluconate 8% weight/weight (w/w) gel (e.g., Lithioderm 8% gel) commercially available in France for the treatment of seborrheic dermatitis (Dreno B, 2007, Ann Dermatol Venereol. 134:347-351, incorporated herein by reference) can be used in the treatment methods described herein. In certain preferred embodiments, lithium is formulated into a modified release form that allows controlled release, over time, into the skin. In another preferred embodiment, the lithium is formulated as part of a mesh scaffold that delivers lithium into the skin. More details on these and other lithium formulations and delivery methods for use in the treatment methods described herein are described in Sections 5.1-5.4 infra.

[0043] The lithium compositions described herein can be administered using an intermittent lithium treatment protocol, which involves multiple courses of lithium treatment interrupted by lithium treatment "holidays" (periods during which no lithium treatment is administered). A lithium treatment holiday is a period of time during which the patient stops the lithium treatment with the intent of resuming treatment. Lithium compositions can also be administered using a single pulse protocol, wherein a dose of lithium is administered over a short period of time.

[0044] The lithium treatments can be administered alone or in combination with other agents to enhance treatment of a microbial infection or a microbial colonization. The other agent can be administered before, concurrently with, or after the lithium composition is administered. In some embodiments, a lithium composition is administered in combination
with an antibiotic to treat a bacterial infection or a bacterial colonization. In some embodiments, a lithium composition is administered in combination with an anti-fungal agent to treat a fungal infection.

[0045] Human subjects who are candidates for lithium treatments described herein include any subject in need of treatment or prevention of a microbial infection or reduction of a microbial colonization, particularly with bacteria or fungi. Human subjects who are candidates for such treatments include any subject for whom improved wound healing is desired. Such human subjects include, but are not limited to, subjects with bacterial infections caused by any bacteria including, but not limited to Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Propionibacterium acnes, Staphylococcus epidermidis, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae, Streptococcus pyogenes, corynebacterium species, enterococci, Proteus mirabilis, group D streptococci, other gram-positive aerobes, and Bacteroides fragilis. Human subjects who are also candidates for such treatments include, but are not limited to, subjects with fungal infections caused by any fungi including, but not limited to Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans, Microsporum gypseum, Microsporum gypseum, Microsporum canis, Epidermophyton floccosum, Candida albicans, and Candida parapsilosis, Malassezia furfur, and Aspergillus fumigatus.

[0046] Topical formulations of the lithium treatments described herein can be administered as a skin sanitizer and/or hand sanitizer to prevent infection by bacteria including, but not limited to, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Propionibacterium acnes, Staphylococcus epidermidis, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae, Streptococcus pyogenes, corynebacterium species, enterococci, Proteus mirabilis, group D streptococci, other gram-positive aerobes, and Bacteroides fragilis. Topical formulations of the lithium treatments described herein can be administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi. Topical formulations of the lithium treatments described herein can be administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi and bacteria. In an embodiment, the skin sanitizer formulation and/or hand sanitizer formulation is aqueous based or glycerol based. In another embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.

[0047] In an embodiment, a combination treatment comprising a lithium compound described herein and chlorhexidine is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant Staphylococcus aureus. In an embodiment, a
combination treatment comprising a lithium compound described herein and iodine is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*. In an embodiment, a combination treatment comprising a lithium compound described herein and povidone-iodine is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*. A combination treatment comprising a lithium compound described herein can be formulated as a hand or body wash, a wound dressing, which can be in the form of barriers, membranes, or films, a gel, a lotion, a cream, an ointment, a surgical scrub, a hand sanitizer, a skin sanitizer, or a spray that can be sprayed on to skin or a wound as a protective layer.

[0048] Any pharmaceutically acceptable compound that releases the lithium ion (also referred to herein as lithium cation, Li+, ionized lithium) can be used for a lithium treatment described herein; such compounds include, but are not limited to lithium gluconate, lithium succinate, and other organic salts/acid, lithium carbonate, lithium chloride and other inorganic salts/acid, and lithium hydroxide, as described in Section 5.1, *infra*.

[0049] Provided herein are pharmaceutical compositions formulated for topical administration, comprising a source of lithium ions formulated into aqueous formulations (*e.g.*, hydrogels), ointments, or creams (*e.g.*, emulsions) for topical administration. In one embodiment, provided herein, is a topical lithium ointment formulation comprising a source of lithium ions, petrolatum, mineral oil, and lanolin alcohol. In another embodiment, provided herein is a topical lithium cream, comprising a source of lithium ions in an emulsion of petrolatum and water. In another embodiment, provided herein is a topical lithium aqueous hydrogel, comprising a source of lithium ions, Carbopol 980, methyl paraben, propyl paraben, propylene glycol, glycerine, and water. Sources of lithium ions include, for example, lithium carbonate, lithium citrate, lithium gluconate, lithium chloride, lithium succinate, or lithium hydroxide. In one embodiment, the source of lithium ions is lithium carbonate. In another embodiment, the source of lithium ions is lithium citrate. In another embodiment, the source of lithium ions is lithium hydroxide. In certain embodiments, regardless of the source of lithium ions, the topical formulation comprises 2.74 mg Li+/gram.

[0050] Also provided herein are pharmaceutical compositions formulated for topical administration, wherein lithium hydroxide is the source of the Li+. In one embodiment, the pharmaceutical composition based on lithium hydroxide is formulated as a hydrogel. In one such embodiment, the hydrogel formulation comprises lithium hydroxide monohydrate, citric acid, CMC, methyl paraben, propyl paraben, allantoin, alginate, and water.
Also provided herein are pharmaceutical compositions formulated for topical administration, comprising lithium carbonate and a pharmaceutically acceptable carrier or excipient. In one embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 0.18%-5.66% w/w lithium carbonate, glycerol, carboxymethyl cellulose, allantoin, sodium alginate, methyl paraben, and propyl paraben. In another embodiment, the pharmaceutical composition is formulated as a cream comprising 0.18%-5.66 % w/w lithium carbonate, Citric Acid, Carbopol 980, Tween 20, Cetearyl Alcohol, Silicon 350 CSt, Silicon 12,500 CSt, Span 80, Lanolin Alcohol, and Emulsifier 10. In another embodiment, the pharmaceutical composition is formulated as a cream comprising 0.18%-5.66 % w/w lithium carbonate, Citric Acid, Carbopol 980, Tween 20, Cetearyl Alcohol, D350 Mineral Oil, Span 80, Lanolin Alcohol, and Emulsifier 10. In another embodiment, the pharmaceutical composition is formulated as a cream comprising 0.18%-5.66 % w/w lithium carbonate, Citric Acid, Carbopol 980, Tween 20, Lecithin, Silicon 350 CSt, D350 Mineral Oil, Span 80, Lanolin Alcohol, and Emulsifier 10.

In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising carboxymethylcellulose, allantoin, alginate, glycerol, citric acid, distilled water and lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and 1.46% lithium carbonate.

In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising carboxymethylcellulose, allantoin, alginate, glycerol, citric acid, distilled water, mupirocin and lithium carbonate. In a further embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 0.5% to 3% mupirocin. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin, and 11.5% lithium carbonate. In another embodiment, the
pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 1.46% lithium carbonate.

[0054] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 0.5% mupirocin, and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 0.5% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 5.66% lithium carbonate.

[0055] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin, and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin and 1.46% lithium carbonate.
In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 3% mupirocin, and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 3% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 3% mupirocin and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 3% mupirocin and 1.46% lithium carbonate.

Provided herein are devices that can be used to deliver the therapeutic compound to the skin site, including drug spraying devices. In certain embodiments, the drug spraying device comprises a drug cartridge having two separate chambers that keep drug components isolated until the therapeutic compound is to be dispensed. In one embodiment, provided herein is a device for spraying a therapeutic compound comprising:

(A) a control unit;
(B) a foot piece;
(C) a power module;
(D) a hand piece comprising:
(i) a housing;
(ii) a first plunger having a first connecting portion; and
(iii) a second plunger having a second connecting portion; and
(E) a drug cartridge comprising:
(i) a housing;
(ii) a first chamber containing a first liquid component, wherein the first liquid component is rearwardly confined by a first piston having a first cavity formed in an end thereof;
(iii) a second chamber containing a second liquid component, wherein the second liquid component is rearwardly confined by a second piston having a second cavity formed in an end thereof;
(iv) a static mixer; and
(v) a nozzle;
(F) wherein the first connecting portion engages the first cavity such that movement of the first plunger moves the first piston within the first chamber in a corresponding manner, and
(G) wherein the second connecting portion engages the second cavity such that movement of the second plunger moves the second piston within the second chamber in a corresponding manner.

In another embodiment, a device for spraying a therapeutic compound comprises:
(A) a control unit;
(B) a foot piece,
(C) a power module;
(D) a hand piece including a housing and a plunger having a connecting portion; and
(E) a drug cartridge comprising:
   (i) a drug cartridge housing having a front end and a back end;
   (ii) a first chamber containing a liquid component, wherein the liquid component is confined at a first end by a first piston and at a second end by a first one-way valve;
   (iii) a second chamber containing a solid component, wherein the solid component is confined at a first end by a second piston having a cavity formed in an end thereof and at a second end by the first one-way valve and a second one-way valve;
   (iv) a bottom chamber; and
   (v) a nozzle;
(F) wherein the connecting portion engages the cavity in the second piston such that movement of the plunger moves the second piston within the second chamber in a corresponding manner, and
(G) wherein movement of the piston away from the front end of the drug cartridge housing creates negative pressure within the second chamber, and
(H) wherein the negative pressure created in the second chamber pulls the liquid component through the first one-way valve into second chamber.

[0059] In certain embodiments, a drug cartridge for use in a device for spraying a therapeutic compound comprises:

(A) a housing having a front end and a back end;
(B) a first chamber containing a liquid component, wherein the liquid component is confined at a first end by a first piston and at a second end by a first one-way valve;
(C) a second chamber containing a solid component, wherein the solid component is confined at a first end by a second piston having a cavity formed in an end thereof and at a second end by the first one-way valve and a second one-way valve;
(D) a bottom chamber; and
(E) a nozzle;
(F) wherein movement of the second piston away from the front end of the housing creates negative pressure within the second chamber, and
(G) wherein the negative pressure created in the second chamber pulls the liquid component through the first one-way valve into second chamber.

[0060] In certain embodiments, the drug spraying device disclosed herein enables the sustained release of ionized lithium (Li+), without the use of highly hydrophobic, occlusive matrices. In particular, the drug spraying device enables the delivery of ionized lithium in microspheres (e.g., PLG microspheres) such that the microspheres stay at the wound site for a prolonged period of time and are not cleared rapidly by phagocytosis. A prolonged period of
time can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at least 20 days.

[0061] In certain embodiments, the drug spraying device disclosed herein enables the sustained release of ionized lithium (Li+) and uptake by the skin through a scab. In particular, the drug spraying device enables the delivery of ionized lithium such that the delivery system is incorporated into the scab. This can be accomplished by placing a lithium containing thin, gauze-like, pliable biodegradable scaffold on the fresh wound. The material properties of the scaffold will be adjusted such the gauze is able to absorb the blood and other exudates from the wound. In certain, more specific embodiments, the biodegradable scaffold has high content of void space, to absorb blood, fibrin and fibrinogen. In some embodiments, after placement of the drug-containing biodegradable scaffold into the wound, an in-situ crosslinking hydrogel is applied on top to cover the entire site as a wound dressing.

[0062] In certain embodiments, the drug spraying device disclosed herein enables the concurrent delivery of two or more drugs with different solubility properties and/or physical/chemical incompatibilities (such as different excipient requirements; binding and/or reaction of the two or more drugs with each other).

[0063] In certain embodiments, the drug spraying device disclosed herein enables the cleansing and administration of one or more drugs with one single device. In these embodiments, the contents of each chamber could be sprayed separately. Once chamber can contain the cleansing solution; the liquid in the other chamber contains lithium. Any wound-cleansing solution known to the skilled artisan can be used with these embodiments.

[0064] With regard to the concentrations of lithium (including its concentration in formulations, in tissue, in serum, etc., and as a salt form, as ionized lithium in solution, etc.) described herein, since ionized lithium is a monovalent cation, the concentration of lithium expressed in millimolar units (mM) is equal to its concentration expressed in milliequivalents (mEq) \(i.e.,\) to avoid any doubt, 1 mM Li+ = 1 mEq Li+, as is sometimes used in the art.

[0065] In one embodiment, the lithium treatments, combination treatments and/or pharmaceutical compositions described herein are administered to treat, reduce, or prevent a microbial biofilm. In an embodiment, the microbial biofilm is a wound-related microbial biofilm. In another embodiment, the microbial biofilm is caused by bacteria and/or fungi. In one embodiment, the lithium treatments, combination treatments and/or pharmaceutical compositions described herein are administered to prevent planktonic forms of bacteria and/or fungi from developing to biofilms. In an embodiment, the lithium treatments,
described herein result in 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% reduction of a biofilm.

[0066] Novel lithium treatments, such as a single pulse lithium treatment or intermittent lithium treatments, that promote wound healing, scar prevention, and scar revision are described. The lithium treatments can be administered in situ to acute wounds, chronic wounds, to scars, and/or surrounding skin. The lithium treatments can be administered to the wound site or surrounding skin before, at the time of, and/or subsequent to, either acute wounding or, more typically, the wounding that is induced in scar revision. The lithium treatments may also be administered to skin-derived cells or skin tissue ex vivo.

[0067] Methods for using lithium treatment, such as pulse or intermittent lithium treatments, to enhance deposition of skin adnexal structures into wound sites (e.g., by inducing hair follicle neogenesis in the site of a scar revision), which in turn enhances wound healing, are described. For example, a lithium treatment may be used to enhance hair follicle neogenesis or enhance the re-association of dissociated hair follicle cells into follicles and facilitate their growth and expansion either in situ, or, alternatively, in culture for their implantation into fresh wounds and scar revisions.

[0068] With these methods, traditional approaches to scar revision, such as human skin transplantation, can be efficiently replaced with transplantation of follicular units or other smaller appendage structures from skin. Thus, hair follicles can be introduced to the wound by migration or de novo hair follicle neogenesis, or by transplanting one or more of the following skin elements: full skin (xeno-; autologous human), follicular units, dissociated cells (donor dominance; recipient effects), ex vivo-expanded skin and/or follicular units, or human skin equivalents in vivo (universal donors). Engineered human skin, or human skin equivalents, can also be used for hair follicle neogenesis and scar revision platforms.

[0069] Lithium treatments are used to revise scars and heal wounds in human subjects. For example, provided herein are intermittent lithium treatment protocols involving multiple courses of lithium treatment interrupted by lithium treatment "holidays" (periods during which no lithium treatment is administered). A lithium treatment holiday is a period of time during which the patient stops the lithium treatment with the intent of resuming treatment. Also provided herein are single pulse lithium treatment protocols, in which a dose of lithium is administered over a short period of time.

[0070] The lithium treatment can be administered topically, transdermally, intradermally, cutaneously, subcutaneously, intramuscularly, intravenously, orally, sublingually, or can be buccal. Topical lithium treatment is a preferred embodiment because high local
concentrations can be achieved while minimizing systemic exposure. In one such embodiment, lithium gluconate 8% weight/weight (w/w) gel (e.g., Lithioderm 8% gel) commercially available in France for the treatment of seborrheic dermatitis (Dreno B, 2007, Ann Dermatol Venereol. 134:347-351, incorporated herein by reference) can be used in the treatment methods described herein. In some embodiments, adjuvants that target the Compound to the desired hair follicles or site on the skin may be included in the formulations used. Adjuvants may be incorporated into the formulation to recruit cells from surrounding tissue to the target tissue. In certain preferred embodiments, lithium is formulated into a modified release form that allows controlled release, over time, into the skin. For example, the modified release form may be formulated to release lithium in a sustained fashion, or in a "pulsed" fashion. In order to achieve a pulsed mode of administration, the lithium formulation may be administered multiple times. Another way to achieve pulsed delivery is to formulate the lithium in beads with multiple coatings, with the lithium contained in alternate coating layers. In another preferred embodiment, the lithium is formulated as part of a mesh scaffold that delivers lithium into the skin. More details on these and other lithium formulations and delivery methods for use in the treatment methods described herein are described in Sections 5.1-5.4 infra. For example, in some embodiments, the lithium formulations, including various modified release forms, may be delivered topically as additives to shampoos and other hair products, as a lotion, cream, or ointment, may be delivered using devices such as iontophoresis, micro-needle injection arrays, auto-injector, or drug sprayer devices.

[0071] The lithium treatments can be administered alone to wounded skin (e.g., prior to, during, or subsequent to scar revision, or acute skin wounding, or chronic skin wounding) or in combination with other treatments to enhance wound healing or scar revision, or with other antimicrobial treatments or treatments to enhance the texture or appearance of skin. The lithium treatments can also be administered in combination with other treatments that facilitate hair follicle development and deposition into the wounded skin. Embodiments of the invention include combination therapies, involving the addition of other treatment(s) concurrently with, or during the breaks between, the cycles of intermittent lithium treatments; or the addition of other treatment(s) concurrently with, or before and/or after the pulse lithium treatment. Such combination therapies can include, but are not limited to, the concurrent or sequential use of other chemical agents, or mechanical or physical treatments including but not limited to, laser (e.g., Fraxel), dermatome planing, laser abrasion, low voltage electric current, electrolysis, intense pulsed light, or surgical treatments (e.g., hair
transplant, skin graft, strip harvesting, scalp reduction, or follicular unit extraction (FUE), etc.) that promote scar revision or wound healing.

[0072] Provided herein are lithium treatments in combination with perturbation (e.g., debridging, peeling, or wounding) of the skin and/or other tissues of the integumentary system by methods such as laser treatment, dermabrasion, needling (using, e.g., microneedles), electromagnetic disruption, electroporation, or sonoporation; chemically (e.g., to induce inflammation); or by any other method described herein or known in the art, prior to or concurrent with administration of a lithium formulation described herein. For example, the integumental perturbation procedure can be any "wounding" procedure used for scar revision. The procedure can be controlled to limit perturbation to the epidermis, or extend deeper into the dermis and/or hypodermis. The occurrence of pinpoint bleeding would indicate removal of the epidermis and portions of the upper layer of the dermis. The occurrence of increased bleeding would indicate deeper penetration (and thus perturbation) into the dermis layer.

[0073] Described herein are lithium treatments administered in combination with laser treatment or another approach to scar revision. Lasers, particularly fractional lasers, and skin graft, follicular unit, and skin component transplant technologies have the capacity to induce regenerative changes in skin that mimic wounding and have applications in revision of scars. In particular, and without being bound by any theory of how the invention works, laser techniques may "mimic" the plastic, embryonic-like, state of the epidermis created by other wound signals, but with laser's precision, versatility, and demonstrated efficacy in small scars. Consequently, when laser treatments are combined with lithium treatment, the outcome of revising extensive scars, particularly those that limit function (e.g., eye or mouth closure; joint contractions), may be vastly improved.

[0074] Also described herein are lithium treatments, e.g., intermittent lithium treatments and pulse lithium treatments, administered concurrently or in sequential/alternating combination with other agents or treatments that modulate the wound healing process. The lithium treatments may be administered with treatments that either promote or delay the wound healing process, such as described in Section 5.5 infra.

[0075] The lithium treatments described herein can be administered concurrently or alternating sequentially with one or more treatments that promote hair growth or prevent or delay hair loss. For example, a lithium treatment described herein may be used with a treatment that prevents follicle senescence (for example, anti-oxidants, mTOR inhibitors, sirtuins); promotes hair growth (for example, minoxidil, kopexil (for example, the product Keranique™), finasteride, bimatoprost (Latisse), CaCl₂, or adenosine); or techniques of
integumental perturbation. Treatments that promote hair growth, or, alternatively, treatments that prevent hair growth, may also be used in combination with a lithium treatment, e.g., an intermittent lithium treatment or a pulse lithium treatment, described herein in order to promote the establishment of desired hair patterning in the healed wound or revised scar, thereby improving the appearance of the treated skin. For example, a lithium treatment may be used concurrently or in sequential combination with either a treatment that enhances hair growth (described above) or a cytotoxic drug, a hair growth retardant, such as efalornithine HCl (Vaniqa), 5-fluorouracil (5-FU) (e.g., Efudex 5% cream), or other epilation or depilation methods to prevent or reduce hair growth.

Success of a lithium treatment, such as a pulse or intermittent lithium treatment, described herein can be measured by one or more of the following outcomes:

- improvement of pigmentation of the scarred or wounded area
- improved surface contour of the scarred or wounded area
- improved texture of the scarred or wounded area
- improved thickness of the scarred (if the scar started out as depressed) or wounded area
- improved overall cosmetic outcome
- subjective patient measures of improved outcome
- presence of elastin
- proper collagen orientation
- improvement in viscoelasticity
- increased number of hair germs
- hair follicle neogenesis or regeneration
- increased proportion of hair follicles in anagen or decreased proportion of follicles in telogen
- increased numbers of follicular units with 3 or more hair follicles
- reduction in the size of the wound or appearance of the scar compared to a wound or scar not treated with lithium
- conversion of the dermal epidermal junction from a flat junction between the dermis and epidermis (typical of a scar) to rete pegs (epithelial extensions that project into the underlying connective tissue) with interdigitating dermis, as assessed by in vivo scanning laser microscopy
- normalization of blood vessels as assessed using laser Doppler analysis.
Human subjects who are candidates for the lithium treatments, e.g., intermittent lithium treatments and pulse lithium treatments, described herein include any subject in need of improved wound healing, particularly wound healing without scarring, or scar revision. Human subjects who are candidates for such treatments include any subject for whom improved wound healing or scar revision is desired. Such human subjects include, but are not limited to, subjects with photodamaged skin, acne scars, chicken pox scars, scarring (cicatricial) alopecia, chronic non-healing wounds or scars due to, e.g., diabetes, venous or arterial disease, old age or senescence, infection, medication, chemotherapy, trauma, burns, stress, autoimmune disease, malnutrition, or endocrine dysfunction. Surgical subjects who are candidates for such treatments include, but are not limited to, patients with skin graft, hair transplantation, skin cancer surgery, or Mohs surgery. Subjects who are candidates for such treatments also include subjects with any other form of wounding or scarring or disease or disorder associated with wounding or scarring as discussed infra and/or known in the art. In some embodiments, the subject has a wound or scar on a cosmetically sensitive location, such as the face or neck.

The invention is based in part on the recognition that the timing of the administration of lithium is important for it to function as an effective modulator of wound healing (and thus, scar revision) in human subjects. For example, lithium treatment results, indirectly, in increasing Wnt signaling, but agents that increase Wnt signaling have had conflicting effects on hair follicle development and wound healing. When continuously present, they stimulate follicle morphogenesis but also induce hair follicle tumors (Gat et al., 1998, Cell 95: 605-614), and lead to decreased hair growth (Millar et al., 1999, Dev. Biol. 207:133-149). In the case of lithium, it has been shown to arrest mitosis (Wolniak, 1987, Eur. J. Cell Biol. 44: 286-293; and Wang, 2008, World J. Gastroenterol. 14:3982-3989), cause pathological hair loss when systematically administered (see, e.g., Mercke et al., 2000, Ann. Clin. Psych. 12:35-42), induce a psoriatic (i.e., chronically wounded) state (see, Wolf et al., 2000, J. Eur. Acad. Dermatol. Venereol. 14:97-99; Stojadinovic et al., 2005, Am. J. Pathol. 167:59-69) or, at best, stimulate the generation of only rudiments of hair follicles (which also leads to the formation of epithelial cysts) (Fathke et al., 2006, BMC Cell Biol. 7:4). These apparently discrepant roles of lithium as a stimulator of Wnt signaling and a negative regulator of the cell cycle are resolved in the present invention. Without being bound by theory, by using lithium in formulations for intermittent or pulse treatments described herein, for example, before, concurrently with, or after integumental perturbation or another treatment that modulates wound healing, it functions as an effective treatment for
wounds and scar revisions in humans. It is thus also possible that the timing of exposure to other compounds with Wnt agonist activity may be important for wound healing and scar revision.

[0079] The invention is also based, in part, on the principle that human skin is replenished by bone-marrow derived and tissue-derived stem cells throughout life. In some embodiments, the lithium treatment(s) is used in combination with methods that mobilize tissue stem cells (e.g., using integumental perturbation) and/or methods that mobilize bone marrow-derived stem cells (e.g., growth factors such as G-CSF and/or chemical agents such as plerixafor (Mozobil®)). In some embodiments, the lithium treatments described herein are used together with methods that regulate the differentiation of these stem cells into specialized human hair follicles in order to facilitate the desired hair patterning at the acceptor site, using agents such as finasteride, fluconazole, spironolactone, flutamide, diazoxide, 11-alpha-hydroxyprogesterone, ketoconazole, RU58841, dutasteride, fluridil, or QLT-7704, an antiandrogen oligonucleotide, cyoctol, topical progesterone, topical estrogen, cyproterone acetate, RU58841, combination 5 alpha reductase inhibitors, oral contraceptive pills, and others in Poulos & Mirmirani, 2005, Expert Opin. Investig. Drugs 14:177-184, incorporated herein by reference, or any other antiestrogen, an estrogen, or estrogen-like drug (alone or in combination with agents that increase stem cell plasticity; e.g., such as valproate), etc., known in the art. Such combination treatments can further include the use of agents that modulate hair growth or that modulate wound healing.

[0080] In certain embodiments, uses of the lithium compounds, alone or in combination with another treatment, described herein are disclosed. In one embodiment, disclosed is the use of a lithium compound described herein in the manufacture of a medicament for treatment and/or prevention of a microbial infection and/or prevention of microbial colonization. In one embodiment, disclosed is the use of a lithium compound described herein in the manufacture of a medicament for treatment and/or prevention of acne. In one embodiment, disclosed is the use of a lithium compound described herein in the manufacture of a medicament for wound healing or scar revision. In one embodiment, disclosed is the use of a lithium compound described herein in the manufacture of a medicament for enhancing the appearance or texture of skin, e.g., by epidermal thickening.
4. DESCRIPTION OF THE FIGURES

[0081] Figure 1 depicts the minimum inhibitory concentrations (mM) of lithium gluconate, lithium succinate, and lithium carbonate against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, Methicillin-resistant *Staphylococcus aureus*, *Propionibacterium acnes*, *Candida albicans*, *Malassezia furfur*, and *Aspergillus fumigatus*.

[0082] Figure 2A-D depicts the minimum inhibitory concentrations (mM) of lithium carbonate and lithium gluconate, tested with or without serum, with the following wound-associated micro-organisms: *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus aureus*, *Candida albicans*, *Enterobacter cloacae*.

[0083] Figure 3 depicts a time-dependent kill curve (log reduction of colony forming units (CFU)/mL) of Methicillin-resistant *Staphylococcus aureus* treated with lithium carbonate. The time points depicted are 30 minutes, 2 hours, and 4 hours.

[0084] Figure 4 depicts a time-dependent kill curve (log reduction of colony forming units (CFU)/mL) of *Escherichia coli* treated with lithium carbonate. The time points depicted are 30 minutes, 2 hours, and 4 hours.

[0085] Figure 5 depicts a time-dependent kill curve (log reduction of colony forming units (CFU)/mL) of *Pseudomonas aeruginosa* treated with lithium carbonate. The time points depicted are 30 minutes, 2 hours, and 4 hours.

[0086] Figure 6 depicts a standard calibration curve for Li+ determination in polyvinyl alcohol (PVA) film.

[0087] Figure 7. In vitro release profiles from Petrolatum/Mineral Oil/Lanolin Alcohol formulations containing 8% lithium gluconate. Diamond=Petrol. Form#1 with mineral oil/petrolatum ratio of 8.6; asterisk=Petrol. Form#2, mineral oil/petrolatum ratio of 2.85; square=Petrol. Form#3 with mineral oil/petrolatum ratio of 0.925; triangle=Petrol. Form#4 with mineral oil/petrolatum ratio of 0.283.

[0088] Figure 8. In vitro cumulative percent Li+ from formulations with varying hydrophobic/hydrophilic ratios. In vitro release experiments were performed, n=3, with error bars as standard deviation.

[0089] Figure 9. Cumulative release profiles of Li+ of various salt forms. Each in vitro experiment was performed for n=3, error bars are standard deviation.

[0090] Figure 10. Calibration curve (0-0.2 ppm).

[0091] Figure 11. Range of linearity calibration curve (0-2 ppm).
[0092] **Figure 12.** Calibration curve (0-10 ppm).

[0093] **Figure 13.** Percent cumulative in vitro release rates from Lithioderm® and lithium carbonate hydrogel Formulation A (lithium carbonate at the indicated strength, glycerol (10.4%), carboxymethyl cellulose (2% w/w), allantoin (0.16%), sodium alginate (0.12%), methyl paraben (0.1%), propyl paraben (0.04%), water (Q.S.), and sodium hydroxide (pH adjusted to 6.5-7.5)). Error bars are expressed as standard error. "mg/g" refers to mg Li+/g.

[0094] **Figure 14.** Pharmacokinetics study design of lithium carbonate hydrogel Formulation A compared to Lithioderm®. Samples were taken at 1h, 24h, 25h, 28h, 48h, 49h, 52h, 192h. Two tissue samples were taken per time point.

[0095] **Figure 15.** Pharmacokinetic profile in skin of lithium carbonate hydrogel Formulation A at various doses (skin levels, DA). The 192 h data point was taken 18h after 6 days of twice daily administration.

[0096] **Figure 16.** Comparison of skin and blood concentrations of Li+ from lithium carbonate hydrogel Formulation A at the following concentrations compared: 0.34 mg Li+/g, 2.74 mg Li+/g, 5.48 mg Li+/g.

[0097] **Figure 17.** Cmax (A) and trough (B) values of Li+ in skin, from the following concentrations of lithium carbonate hydrogel Formulation A: 0.34 mg Li+/g, 2.74 mg Li+/g, 5.48 mg Li+/g. Cmax values were measured 1 hour post-dosing after the first dose each day. Trough values were measured approximately 18 hours after the previous day's dose.

[0098] **Figure 18.** Cmax (A) and trough (B) values of Li+ in blood, from the following concentrations of lithium carbonate hydrogel Formulation A: 0.34 mg Li+/g, 2.74 mg Li+/g, 5.48 mg Li+/g. Cmax values were measured 1 hour post-dosing after the first dose each day. Trough values were measured approximately 18 hours after the previous day's dose.

[0099] **Figure 19.** Pharmacokinetic profiles of lithium carbonate hydrogel Formulation A and lithium gluconate in skin (A) and blood (B). A. Pharmacokinetic profile of Li+ concentrations in skin with twice-daily dosing of lithium gluconate (2.74 mg Li+/g) compared with lithium carbonate hydrogel (2.74 mg Li+/g). Li+ concentrations in skin (Y-Axis, x=Lithium gluconate, square=Lithium carbonate) are plotted as a function of time in hours (h) (X-Axis). Data are mean ± range, of 2 animals per time point. B. Pharmacokinetic profile of Li+ concentrations in blood with twice-daily dosing of lithium gluconate (2.74 mg Li+/g) compared with lithium carbonate hydrogel (2.74 mg Li+/g). Li+ concentrations in blood (Y-Axis, asterisk=Lithium gluconate, square=Lithium carbonate).

[00100] **Figure 20.** The absorbance vs. lithium ion concentration in standard solutions.
Figure 21. Matrix effect evaluations of other ions on the measurement of Li+ in a lithium carbonate cream formulation.

Figure 22. Release profile of Li+ from immediate release lithium carbonate cream formulation Lot# TH-00 L-081.

Figure 23. Release profile of Li+ from intermediate release lithium carbonate cream formulation Lot # TH-00 L-084b.

Figure 24. The release profile of sustained release lithium carbonate cream formulation with lot # TH-00 L-084a.

Figure 25 is a cross-linking reaction between PEG-AM and PEG-NHS.

Figure 26 is a graph depicting the gel time as a function of pH for PEG-NHS/PEG-AM hydrogels.

Figure 27 is a graph depicting the gel time as a function of PEG concentration for PEG-NHS/PEG-AM hydrogels.

Figure 28 is a graph depicting the gel time as a function of total PEG concentration for PEG-NHS/PEG-AM hydrogels.

Figure 29 is a graph depicting gel time as a function of PEI concentration in Phosphate buffer (0.1M, 0.2M and 0.5M) for PEG-NHS/PEI hydrogels.

Figure 30 is a graph depicting the log reduction of bacterial counts against untreated (y-axis), as a function of 5.66% Li-Carb, 11.5% Li-Carb, Vehicle and 2% Mupirocin (x-axis). *p value < 0.05, compared to untreated control N=10/group; CD-I mice.

Figure 31 is a scanning electron micrograph of lithium carbonate-containing PLG microspheres lyophilized with PEG3.3K-NHS.

Figure 32 is a graph depicting the in vitro release of ionized lithium from PLG microspheres.

Figure 33 is a graph depicting the gel time as a function of total percent crosslinkable solids (PEG-AM, PEI, PEG-NHS).

Figure 34 is a graph depicting the degree of swelling for PEG-NHS/PEG-AM hydrogels, PEG-NHS/PEI hydrogels, and PEG-NHS/PEG-AM/Chitosan hydrogels.

Figure 35 is a graph depicting the viscosity of PVA solutions as a function of temperature. The left-hand bar for each concentration (x-axis) represents the viscosity at 25 °C and the right-hand bar for each concentration (x-axis) represents the viscosity at 30 °C.

Figure 36 shows the release of lithium gluconate can be controlled by blend ratio of polymers. Shown is a plot of Percent Cumulative Release of Li+ as a Function of Time in
Days, which demonstrates release profiles of four different blends. The micrographs to the left and bottom of the graph show two different polymer blends.

[00117] Figure 37A is a front perspective view of a spraying device, according to an embodiment of the present invention.

[00118] Figure 37B is a rear perspective view of a spraying device, according to an embodiment of the present invention.

[00119] Figure 38 is a front perspective view of a spraying device, according to an embodiment of the present invention.

[00120] Figure 39A is a plan view of showing the components of a hand piece depicted in Figures 33A and 33B.

[00121] Figure 39B is a rear perspective view of a hand piece, according to an embodiment of the present invention.

[00122] Figure 40A is a plan view of a drug cartridge, according to an embodiment of the present invention.

[00123] Figure 40B is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

[00124] Figure 40C is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

[00125] Figure 40D is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

[00126] Figure 41A is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

[00127] Figure 41B is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

[00128] Figure 41C is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

[00129] Figure 41D is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

[00130] Figure 41E is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

[00131] Figure 42 depicts the log reduction of colony forming units (CFU)/mL) of MRSA ATCC 33591 treated with (1) 5.66% w/w Lithium Carbonate gel (10.64 mg Li+/g); (2) 11.5% w/w Lithium Carbonate gel (20 mg Li+/g); (3) 5.66%> w/w Lithium Carbonate
emulsion (10.64 mg Li+/g); (4) 11.5% w/w Lithium Carbonate emulsion (20 mg Li+/g); (5) Vehicle; and (6) 2% Mupirocin.

[00132] Figure 43 depicts the log reduction of colony forming units (CFU/mL) of MRSA ATCC 33591 treated with (1) 2% Mupirocin; (2) Aqueous gel (Vehicle Control); (3) 2% Mupirocin + Placebo (aqueous gel); (4) 2% Mupirocin + 1.46% w/w Lithium Carbonate (2.74 mg Li+/g); (5) 2% Mupirocin + 5.66% w/w Lithium Carbonate (10.64 mg Li+/g); (6) 2% Mupirocin + 11.5% w/w Lithium Carbonate (20 mg Li+/g).

[00133] Figure 44 depicts the log reduction of colony forming units (CFU/mL) of MRSA strain ATCC 33591 in two duplicate experiments and MRSA strain NEMC 89-4 treated with (1) 2% Mupirocin + 11.5% w/w Lithium Carbonate (20 mg Li+/g); (2) 2% Mupirocin; and (3) Aqueous gel (Vehicle Control).

[00134] Figure 45 depicts the percent inhibition of various concentrations (Fold-MIC) of lithium carbonate and ciprofloxacin on S. aureus DNA synthesis.

[00135] Figure 46 depicts the percent inhibition of various concentrations (Fold-MIC) of lithium carbonate and linezolid on S. aureus protein synthesis.

[00136] Figure 47 depicts the percent inhibition of various concentrations (Fold-MIC) of lithium carbonate and cerulenin on S. aureus lipid synthesis.

[00137] Figure 48 depicts the percent inhibition of various concentrations (Fold-MIC) of lithium carbonate and vancomycin on S. aureus cell wall synthesis.

[00138] Figure 49 depicts the percent inhibition of various concentrations (Fold-MIC) of lithium carbonate and rifampicin on S. aureus RNA synthesis.

[00139] Figure 50 depicts the percent inhibition of lithium carbonate on S. aureus DNA, lipid, protein and cell wall synthesis at 1.2-, 4-, and 8-fold the MIC.

[00140] Figure 51 shows the Franz Cell set-up for diffusion studies.

[00141] Figure 52 shows Li+ flux from formulation TH-003-070a, as a function of diffusion time through the nail, as a function of nail thickness, with red bars denoting Li+ flux through a nail of thickness of 0.448 cm and blue bars denoting Li+ flux through a nail of thickness of 0.04775 cm. The nails received no iontophoretic treatment.

[00142] Figure 53 shows Li+ flux from formulation TH-003-070b, as a function of diffusion time through the nail, as a function of nail thickness, with red bars denoting Li+ flux through a nail of thickness of 0.0625 cm and blue bars denoting Li+ flux through a nail of thickness of 0.248 cm. The nails received no iontophoretic treatment.

[00143] Figure 54A compares the flux between formulations TH-003-070a, TH-003-070b and TH-003-070C.
Figure 54B shows percent Li\textsuperscript{+} permeated per time interval, for all three formulations, without iontophoresis.

Figure 55A shows a comparison of diffusive flux of Li\textsuperscript{+} via formulation TH-003-070a with, and without iontophoresis.

Figure 55B shows the percent Li\textsuperscript{+} permeation using via formulation TH-003-070a over each time interval.

Figure 55C shows a comparison of diffusive flux of Li\textsuperscript{+} across the nail plate with iontophoresis, plotted as a function of formulation composition (TH-003-070a, TH-003-070b and TH-003-070c).

Figure 55D shows the Li\textsuperscript{+} concentrations in nail and nail bed using formulation TH-003-070a over each time interval.

Figure 56A is a schematic Schematic representation of human nail mounted in a gasket with the relative position of the formulation and organism.

Figure 56B compares the percentages of ATP recovered (mean ± range) following the dosing regimen summarised in Example 23 for 14 days against T. rubrum.

Figure 57 depicts a prior art dermabrasion rotating wheel ejecting blood and debris.

Figure 58 depicts a prior art dermabrasion rotating wheel in use.

Figure 59 depicts a prior art dermabrasion rotating wheel in use.

Figure 60 depicts a prior art dermabrasion hand piece and wheel in use.

Figure 61 depicts a dermabrasion tip according to an embodiment of the present invention attached to a conventional dermabrasion hand piece.

Figure 62 depicts a conventional dermabrader.

Figure 63 is a schematic drawing of a dermabrasion tip according to an embodiment of the present invention.

Figure 64 is a schematic drawing of a linkage assembly according to an embodiment of the present invention.

Figure 65 depicts a dermabrasion tip according to an embodiment of the present invention.

Figure 66 depicts a dermabrasion tip in use, according to an embodiment of the present invention.

Figure 67 depicts a dermabrasion tip in use, according to an embodiment of the present invention.
[00162] Figure 68 depicts a portion of the transmission assembly according to an embodiment of the present invention.

[00163] Figure 69 is a schematic drawing of a dermabrasion tip according to an embodiment of the present invention.

[00164] Figure 70 is a schematic drawing of a dermabrasion tip according to an embodiment of the present invention;

[00165] Figure 71 depicts a dermabrasion tip according to an embodiment of the present invention.

[00166] Figure 72A is a rear view of a dermabrasion tip according to an embodiment of the present invention.

[00167] Figure 72B is a side view of a dermabrasion tip according to an embodiment of the present invention.

[00168] Figure 73 depicts a linear reciprocating converting dermabrasion tip according to an embodiment of the present invention.

[00169] Figure 74 depicts a combination radial and linear reciprocating converting dermabrasion tip according to an embodiment of the present invention.

[00170] Figure 75. Topical LiCl 8% and 16% decreases the area of healed full thickness excision (FTE) wounds. 1.5 cm² wounds were induced by FTE, and then allowed to heal. Shown are median + first and third quartiles. P-values for comparisons of lithium treatments to placebo are for one-sided tests for superiority, and should be p < 0.0125 for statistical significance with a family-wise error rate of α = 5% adjusted by the Bonferroni method for 4 comparisons to placebo. In the right graph, a Hodges-Lehman estimate of median difference is shown, with simultaneous 90% confidence intervals, corrected for 4 comparisons by the Bonferroni method.

[00171] Figure 76. Increased total number of new hair follicles (NHF) is a factor that accounts for the increased coverage of wounds with LiCl 8% as compared to placebo. Shown is mean ± SEM. p-values for comparison of lithium treatments to placebo for are for one-sided tests for superiority, and should be p < 0.0125 for statistical significance with a family-wise error rate of α = 5% adjusted by the Bonferroni method for 4 comparisons to placebo. Shown in the right graph: Mean ± SEM.

[00172] Figure 77. Representative histological images showing that 8% lithium gluconate treatment results in an increased number and area of neogenic hair follicles (regeneration zone) and a reduction in the scar zone.
Figure 78. Elastin immunohistochemistry indicates that elastin is associated with neogenic hair follicles (NHF) in the center of the wound (regeneration zone) (left), whereas elastin is undetectable in the scar zone (middle). Adjacent (non-wounded) skin is shown on the right. Dashed line: dermal-epidermal junction; NHF, neogenic hair follicle; HF, hair follicle.

5. DESCRIPTION OF THE INVENTION

5.1 LITHIUM COMPOSITIONS

Any compound or composition that can release a lithium ion (also referred to herein as lithium cation, Li+, or ionized lithium) is suitable for use in the compositions and methods. Such compounds include but are not limited to a pharmaceutically acceptable prodrug, salt or solvate (e.g., a hydrate) of lithium (sometimes referred to herein as "lithium compounds"). In another embodiment, the lithium ion is provided by lithium hydroxide, such as, e.g., lithium hydroxide monohydrate. Optionally, the lithium compounds can be formulated with a pharmaceutically acceptable vehicle, carrier, diluent, or excipient, or a mixture thereof. Additionally, lithium-polymer complexes can be utilized to developed various sustained release lithium matrices.

Any form of lithium approved for pharmacological use may be used in the lithium treatments described herein. For example, lithium is best known as a mood stabilizing drug, primarily in the treatment of bipolar disorder, for which lithium carbonate (Li2CO3), sold under several trade names, is the most commonly used. Other commonly used lithium salts include lithium citrate (Li3C6H5O7), lithium sulfate (Li2SO4), lithium aspartate, lithium orotate, lithium succinate, and lithium gluconate. A lithium formulation well-suited for use in the methods disclosed herein is lithium gluconate, for example, a topical ointment of 8% lithium gluconate (Lithioderm™), is approved for the treatment of seborrheic dermatitis, can be used in the methods disclosed herein. See, e.g., Dreno and Moyse, 2002, Eur J Dermatol 12:549-552; Dreno et al., 2007, Ann Dermatol Venereol 134:347-351 (abstract); and Ballanger et al., 2008, Arch Dermatol Res 300:215-223, each of which is incorporated by reference herein in its entirety. A lithium formulation well-suited for use in the methods disclosed herein is lithium carbonate, for example, a topical ointment of 1.46 % lithium carbonate, 2.91 % lithium carbonate, 5.66% lithium carbonate or 11.5% lithium carbonate. Another lithium formulation for use in the methods disclosed herein is lithium succinate, for example, an ointment comprising 8% lithium succinate, which is also used to treat seborrheic
dermatitis. See, e.g., Langtry et al., 1996, Clinical and Experimental Dermatology 22:216-219; and Cuelenaere et al., 1992, Dermatology 184:194-197, each of which is incorporated by reference herein in its entirety. In one embodiment, the lithium formulation is an ointment comprising 8% lithium succinate and 0.05% zinc sulfate (marketed in the U.K. as Efalith). See, e.g., Efalith Multicenter Trial Group, 1992, J Am Acad Dermatol 26:452-457, which is incorporated by reference herein in its entirety. Examples of lithium succinate formulations and other lithium formulations for use in the lithium treatment described in U.S. Patent No. 5,594,031, issued January 14, 1997, which is incorporated herein by reference in its entirety.

Surprisingly, in some embodiments, lithium carbonate is more effective in its antimicrobial activity than lithium gluconate and lithium succinate. In vitro minimum inhibitory concentration (MIC) assays for six microbial species revealed that lithium carbonate was more effective at inhibiting bacteria, by a factor of 2 to 4 over lithium succinate and by a factor of 8 to 32 over lithium gluconate (see Example 1). Minimum inhibitory concentration (MIC) assays for nine microbial species that are associated with wounds revealed that lithium inhibited the growth of all the organisms tested and lithium carbonate was a more potent anti-microbial than the gluconate salt form with respect to P. aeruginosa and S. aureus (see Example 2).

Moreover, in wounded mice infected with methicillin resistant S. aureus (MRSA), treatment with lithium carbonate at 10.73 mg Li+/g (5.66% w/w Li₂C₀₃) reduced the MRSA microbial load by 1.54 CFU/g of skin (see Example 4). Lower concentrations of lithium carbonate at 2.74 mg Li+/g (1.46% w/w Li₂C₀₃) and 5.48 mg Li+/g (2.91% w/w Li₂C₀₃) reduced the MRSA microbial infection by 0.70 and 0.74 CFU/g of skin, respectively (see Example 4). Lithium gluconate at 2.74 mg Li+/g (8% lithium gluconate) was not efficacious against the microbial MRSA infection with a reduction of 0.10 CFU/g of skin (see Example 4).

In one embodiment, a lithium composition formulated for topical administration comprises lithium carbonate. In another embodiment, a lithium composition formulated for topical administration comprises lithium hydroxide. In one embodiment, the lithium ions in a composition formulated for topical administration are provided by lithium carbonate. In another embodiment, the lithium ions in a composition formulated for topical administration are provided by lithium hydroxide, such as, e.g., lithium hydroxide monohydrate.
5.1.1 LITHIUM SALTS

[00179] Any pharmaceutically acceptable lithium salt may be used as a source of lithium ions in the lithium treatment. It will be understood by one of ordinary skill in the art that pharmaceutically acceptable lithium salts are preferred. See, e.g., Berge et al., J. Pharm. Sci. 1977, 66:1-19; Stahl & Wermuth, eds., 2002, Handbook of Pharmaceutical Salts, Properties, and Use, Zurich, Switzerland: Wiley-VCH and VHCA; Remington’s Pharmaceutical Sciences, 1990, 18th eds., Easton, PA: Mack Publishing; Remington: The Science and Practice of Pharmacy, 1995, 19th eds., Easton, PA: Mack Publishing.

[00180] In some embodiments, the compositions used for a lithium treatment comprise mixtures of one or more lithium salts. For example, a mixture of a fast-dissolving lithium salt can be mixed with a slow dissolving lithium salt proportionately to achieve the release profile. In certain embodiments, the lithium salts do not comprise lithium chloride.

[00181] In some embodiments, the lithium salt can be the salt form of anionic amino acids or poly(amino) acids. Examples of these are glutamic acid, aspartic acid, polyglutamic acid, polyaspartic acid.

[00182] By reciting lithium salts of the acids set forth above, applicants do not mean only the lithium salts prepared directly from the specifically recited acids. In contrast, applicants mean to encompass the lithium salts of the acids made by any method known to one of ordinary skill in the art, including but not limited to acid-base chemistry and cation-exchange chemistry.

[00183] In another embodiment, lithium salts of anionic drugs that positively affect hair growth, such as prostaglandins can be administered. In another embodiment, a large anion or multianionic polymer such as polyacrylic acid can be complexed with lithium, then complexed with a cationic compound, such as finasteride, to achieve a slow release formulation of both lithium ion and finasteride. Similarly, a lithium complex with a polyanion can be complexed further with the amines of minoxidil, at pHs greater than 5.

[00184] Lithium compounds for use in the methods provided herein may contain an acidic or basic moiety, which may also be provided as a pharmaceutically acceptable salt. See, Berge et al., J. Pharm. Sci. 1977, 66:1-19; Stahl & Wermuth, eds., 2002, Handbook of Pharmaceutical Salts, Properties, and Use Zurich, Switzerland: Wiley-VCH and VHCA.
5.1.2 ORGANIC LITHIUM SALTS

[00185] In some embodiments, the lithium salts are organic lithium salts. Organic lithium salts for use in these embodiments include lithium 2,2-dichloroacetate, lithium salts of acylated amino acids (e.g., lithium N-acetylcysteinate or lithium N-stearoylcysteinate), a lithium salt of poly(lactic acid), a lithium salt of a polysaccharides or derivative thereof, lithium acetylsalicylate, lithium adipate, lithium hyaluronate and derivatives thereof, lithium polyacrylate and derivatives thereof, lithium chondroitin sulfate and derivatives thereof, lithium stearate, lithium linoleate, lithium oleate, lithium taurocholate, lithium cholate, lithium glycocholate, lithium deoxycholate, lithium alginate and derivatives thereof, lithium ascorbate, lithium L-aspartate, lithium benzenesulfonate, lithium benzoate, lithium 4-acetamidobenzoate, lithium (+)-camphorate, lithium camphorsulfonate, lithium (+)-(15)-camphor-10-sulfonate, lithium caprate, lithium caproate, lithium caprylate, lithium cinnamate, lithium citrate, lithium cyclamate, lithium cyclohexanesulfamate, lithium dodecyl sulfate, lithium ethane-1,2-disulfonate, lithium ethanesulfonate, lithium 2-hydroxyethanesulfonate, lithium formate, lithium fumarate, lithium galactarate, lithium gentisate, lithium glucoheptonate, lithium D-gluconate, lithium D-glucuronate, lithium L-glutamate, lithium a-oxoglutarate, lithium glycolate, lithium hippurate, lithium (+)-L-lactate, lithium (±)-DL-lactate, lithium lactobionate, lithium laurate, lithium (-)-L-malate, lithium maleate, lithium malonate, lithium (±)-DL-mandelate, lithium methanesulfonate, lithium naphthalene-2-sulfonate, lithium naphthalene-1,5-disulfonate, lithium 1-hydroxy-2-napthoate, lithium nicotinate, lithium oleate, lithium orotate, lithium oxalate, lithium palmitate, lithium pamoate, lithium L-pyroglutamate, lithium saccharate, lithium salicylate, lithium 4-amino-salicylate, sebacic acid, lithium stearate, lithium succinate, lithium tannate, lithium (+)-L-tartarate, lithium thiocyanate, lithium p-toluenesulfonate, lithium undecylenate, or lithium valerate. In some embodiments, the organic lithium salt for use in these embodiments is lithium (S)-2-alkylthio-2-phenylacetate or lithium (R)-2-alkylthio-2-phenylacetate (e.g., wherein the alkyl is C2-C22 straight chain alkyl, preferably C8-16). See, e.g., International Patent Application Publication No. WO 2009/019385, published February 12, 2009, which is incorporated herein by reference in its entirety.

[00186] In some embodiments, the organic lithium salts used for a lithium treatment comprise the lithium salts of acetic acid, 2,2-dichloroacetic acid, acetylsalicylic acid, acylated amino acids, adipic acid, hyaluronic acid and derivatives thereof, polyacrylic acid and derivatives thereof, chondroitin sulfate and derivatives thereof, poly(lactic acid-co-glycolic
acid), poly(lactic acid), poly(glycolic acid), pegylated lactic acid, stearic acid, linoleic acid, oleic acid, taurocholic acid, cholic acid, glycocholic acid, deoxycholic acid, alginic acid and derivatives thereof, ascorbic acid, L-aspartic acid, benzenesulfonic acid, benzoic acid, 4-acetamidobenzoic acid, (+)-camphoric acid, camphorsulfonic acid, (+)-(15)-camphor-10-sulfonic acid, capric acid, caproic acid, caprylic acid, cinnamic acid, citric acid, cyclamic acid, cyclohexanesulfamic acid, dodecylsulfuric acid, ethane-1,2-disulfonic acid, ethanesulfonic acid, 2-hydroxy-ethanesulfonic acid, formic acid, fumaric acid, galactaric acid, gentisic acid, glucoheptonic acid, D-gluconic acid, D-glucuronic acid, L-glutamic acid, a-oxoglutaric acid, glycolic acid, hippuric acid, (+)-L-lactic acid, (±)-DL-lactic acid, lactobionic acid, lauric acid, maleic acid, (-)-L-malic acid, malonic acid, (±)-DL-mandelic acid, methanesulfonic acid, naphthalene-2-sulfonic acid, naphthalene-1,5-disulfonic acid, 1-hydroxy-2-naphthoic acid, nicotinic acid, oleic acid, orotic acid, oxalic acid, palmitic acid, pamoic acid, L-pyroglutamic acid, saccharic acid, salicylic acid, 4-amino-salicylic acid, sebacic acid, stearic acid, succinic acid, tannic acid, (+)-L-tartaric acid, thiocyanic acid, p-toluenesulfonic acid, undecylenic acid, or valeric acid. Other organic lithium salts for use in these embodiments is the lithium salt of (S)-2-alkylthio-2-phenylacetic acid or the lithium salt of (R)-2-alkylthio-2-phenylacetic acid (e.g., wherein the alkyl is C2-C22 straight chain alkyl, preferably C8-16). See, e.g., International Patent Application Publication No. WO 2009/019385, published February 12, 2009, which is incorporated herein by reference in its entirety.

5.1.2.1 SUSTAINED RELEASE ORGANIC LITHIUM SALTS

[00187] In some embodiments, the organic lithium salt can be modified to create sustained release lithium salts. Due to the size of the lithium ion, it is possible that the residence time of ion at the treatment site will be short. In efforts to generate sustained release lithium salts, the hydrophobicity of the salt can be enhanced and made "lipid-like," to, for example, lower the rate of ionization of the salt into lithium ions. For example, lithium chloride has a much faster rate of ionizing into lithium ions, than lithium stearate or lithium orotate. In that regard, the lithium salt can be that of a cholesterol derivative, or a long chain fatty acids or alcohols. Lipid complexed lithium salts of size less than 10 microns can also be effectively targeted to the skin pores or hair follicles and "tethered" to the sebaceous glands, by hydrophobic-hydrophobic interactions.
In some embodiments, the organic lithium salt can be in the form of complexes with anionic compounds or anionic poly(amino acids) and other polymers. The complexes can be neutral, wherein all of the negative charges of the complexation agent are balanced by equimolar concentrations of Li ions. The complexes can be negatively charged, with Lithium ions bound to an anionic polymer. The complexes can be in the form of nano-complexes, or micro-complexes, small enough to be targeted to the skin pores or hair follicles. If the complexes are targeted to the dermis, the charged nature of the complexes will "tether" the complexes to the positively charged collagen. This mode of tethering holds the Li ions at the site of delivery, thereby hindering fast in-vivo clearance. Examples of negatively charged polymers that can be used in this application are poly(acrylates) and its copolymers and derivatives thereof, hyaluronic acid and its derivatives, alginate and its derivatives, etc. In one variation, the anionic lithium complexes formed as described above can be further complexed with a cationic polymer such as chitosan, or polyethylimine form cell-permeable delivery systems.

In some embodiments, particularly for administration of the lithium formulation to the skin, the salt can be that of a fatty acid, e.g., lithium stearate, thereby promoting absorption through skin tissues and extraction into the lipid compartments of the skin. In another example, the lithium salt of sebacic acid can be administered to the skin for higher absorption and targeting into structures of the skin, such as hair follicles.

5.1.3 INORGANIC LITHIUM SALTS

In some embodiments, the lithium salts are inorganic lithium salts. Inorganic lithium salts for use in these embodiments include halide salts, such as lithium bromide, lithium chloride, lithium fluoride, or lithium iodide. In one embodiment, the inorganic lithium salt is lithium fluoride. In another embodiment, the inorganic lithium salt is lithium iodide. In certain embodiments, the lithium salts do not comprise lithium chloride. Other inorganic lithium salts for use in these embodiments include lithium borate, lithium carbonate, lithium nitrate, lithium perchlorate, lithium phosphate, or lithium sulfate.

In some embodiments, the inorganic lithium salts used for a lithium treatment comprise the lithium salts of boric acid, hydrobromic acid, hydrochloric acid, hydrofluoric acid, hydroiodic acid, nitric acid, perchloric acid, phosphoric acid, or sulfuric acid.

In another embodiment, the inorganic lithium salt for use in the embodiments described herein is lithium carbonate.
5.2 LITHIUM FORMULATIONS AND MODES OF DELIVERY

5.2.1 LITHIUM FORMULATIONS


[00194] Suitable excipients are well known to those skilled in the art, and non-limiting examples of suitable excipients are provided herein. Whether a particular excipient is suitable for incorporation into a pharmaceutical composition or dosage form depends on a variety of factors well known in the art, including, but not limited to, the method of administration. For example, forms for topical administration such as a cream may contain excipients not suited for use in transdermal or intravenous administration. The suitability of a particular excipient depends on the specific active ingredients in the dosage form. Exemplary, non-limiting, pharmaceutically acceptable carriers for use in the lithium formulations described herein are the cosmetically acceptable vehicles provided in International Patent Application Publication No. WO 2005/120451, which is incorporated herein by reference in its entirety.

[00195] The lithium compounds suitable for use in a lithium treatment may be formulated to include an appropriate aqueous vehicle, including, but not limited to, water, saline, physiological saline or buffered saline (*e.g.*, phosphate buffered saline (PBS)), sodium chloride for injection, Ringers for injection, isotonic dextrose for injection, sterile water for injection, dextrose lactated Ringers for injection, sodium bicarbonate, or albumin for
injection. Suitable non-aqueous vehicles include, but are not limited to, fixed oils of vegetable origin, castor oil, corn oil, cottonseed oil, olive oil, peanut oil, peppermint oil, safflower oil, sesame oil, soybean oil, hydrogenated vegetable oils, hydrogenated soybean oil, and medium-chain triglycerides of coconut oil, lanolin oil, lanolin alcohol, linoleic acid, linolenic acid and palm seed oil. Suitable water-miscible vehicles include, but are not limited to, ethanol, wool alcohol, 1,3-butanediol, liquid polyethylene glycol (e.g., polyethylene glycol 300 and polyethylene glycol 400), propylene glycol, glycerin, N-methyl-2-pyrrolidone (NMP), N,N-dimethylacetamide (DMA), and dimethyl sulfoxide (DMSO). In one embodiment, the water-miscible vehicle is not DMSO.

[00196] The lithium compounds for use in the methods disclosed herein may also be formulated with one or more of the following additional agents. Suitable anti-microbial agents or preservatives include, but are not limited to, alkyl esters of p-hydroxybenzoic acid, hydantoins derivatives, propionate salts, phenols, cresols, mercurials, phenoxethanol, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoates, thimerosal, benzalkonium chloride (e.g., benzethonium chloride), butyl, methyl- and propyl-parabens, sorbic acid, and any of a variety of quaternary ammonium compounds. Suitable isotonic agents include, but are not limited to, sodium chloride, glycerin, and dextrose. Suitable buffering agents include, but are not limited to, phosphate, glutamate and citrate. Suitable antioxidants are those as described herein, including ascorbate, bisulfite and sodium metabisulfite. Suitable local anesthetics include, but are not limited to, procaine hydrochloride, lidocaine and salts thereof, benzocaine and salts thereof and novacaine and salts thereof, and may be used with epinephrine. Suitable suspending and dispersing agents include but are not limited to sodium carboxymethylcellulose (CMC), hydroxypropyl methylcellulose (HPMC), polyvinyl alcohol (PVA), and polyvinylpyrrolidone (PVP). Suitable emulsifying agents include but are not limited to, including polyoxyethylene sorbitan monolaurate, polyoxyethylene sorbitan monooleate 80, and triethanolamine oleate. Suitable sequestering or chelating agents include, but are not limited to, EDTA. Suitable pH adjusting agents include, but are not limited to, sodium hydroxide, hydrochloric acid, citric acid, and lactic acid. Suitable complexing agents include, but are not limited to, cyclodextrins, including α-cyclodextrin, β-cyclodextrin, hydroxypropyl -P-cyclodextrin, sulfobutylether- β-cyclodextrin, and sulfobutylether 7-P-cyclodextrin (CAPTISOL ®, CyDex, Lenexa, KS).
Soothing preparations, e.g., for topical administration, may contain sodium bicarbonate (baking soda), and coal tar based products. Formulations may also optionally contain a sunscreen or other skin protectant, or a waterproofing agent.

A product for application to skin may additionally be formulated so that it has easy rinsing, minimal skin/eye irritation, no damage to existing skin or hair, has a thick and/or creamy feel, pleasant fragrance, low toxicity, good biodegradability, and, for application to skin with hair, a slightly acidic pH (pH less than 7), since a basic environment weakens the hair by breaking the disulfide bonds in hair keratin.

In particular embodiments, commercially available preparations of lithium can be used, such as, e.g., lithium carbonate; lithium gluconate - for example 8% lithium gluconate (Lithioderm<sup>TM</sup>), which is approved for the treatment of seborrheic dermatitis (see, e.g., Dreno and Moyse, 2002, Eur J Dermatol 12:549-552; Dreno et al., 2007, Ann Dermatol Venereol 134:347-351 (abstract); and Ballanger et al., 2008, Arch Dermatol Res 300:215-223, each of which is incorporated by reference herein in its entirety); lithium succinate (see, e.g., Langtry et al., 1996, Clinical and Experimental Dermatology 22:216-219; and Cuelenaere et al., 1992, Dermatology 184:194-197, each of which is incorporated by reference herein in its entirety); and or 8% lithium succinate with 0.05% zinc sulfate (marketed in the U.K. as Efalith; see, e.g., Efalith Multicenter Trial Group, 1992, J Am Acad Dermatol 26:452-457, which is incorporated by reference herein in its entirety). In a preferred embodiment, a topical preparation of lithium carbonate is used. In some embodiments, a preparation of lithium or lithium salt comprises an anionic polymer (such as, e.g., crosslinked polyacrylic acid), which may form a gel. For example, a preparation provided in the examples of Section 6 below may be used.

5.2.2 MODIFIED RELEASE FORMS

The lithium compounds for use in a lithium treatment can be formulated as a modified release dosage form. As used herein, the term "modified release" refers to a dosage form in which the rate or place of release of the lithium or other active ingredient(s) is different from that of an immediate dosage form when administered by the same route. Modified release dosage forms include, but are not limited to, delayed-, extended-, prolonged-, sustained-, pulsatile-, controlled-, accelerated- and fast-, targeted-, programmed-release, and gastric retention dosage forms. The compositions in modified release dosage forms can be prepared using a variety of modified release devices and methods known to those skilled in the art, including, but not limited to, matrix controlled release devices,
osmotic controlled release devices, multiparticulate controlled release devices, ion-exchange resins, enteric coatings, multilayered coatings, microspheres, liposomes, and combinations thereof. The release rate of the active ingredient(s) can also be modified by varying the particle sizes and polymorphism of the active ingredient(s). In some embodiments, the controlled release is achieved by using an adjuvant that causes a depot effect, i.e., that causes an active agent or antigen to be released slowly, leading to prolonged exposure to a target cell or tissue (e.g., cells of the follicle, an infected skin or wound, or, in the case of immunostimulatory adjuvants, prolonged exposure to the immune system).

5.2.2.1 MATRIX CONTROLLED RELEASE DEVICES


[00203] In certain embodiments, the modified release dosage form is formulated using an erodible matrix device, which is water-swellable, erodible, or soluble polymers, including, but not limited to, synthetic polymers, and naturally occurring polymers and derivatives, such as polysaccharides and proteins. Materials useful in forming an erodible matrix include, but are not limited to, chitin, chitosan, dextran, and pullulan; gum agar, gum arabic, gum karaya, locust bean gum, gum tragacanth, carrageenan, gum ghatti, guar gum, xanthan gum, and scleroglucan; starches, such as dextrin and maltodextrin; hydrophilic colloids, such as pectin; phosphatides, such as lecithin; alginates; propylene glycol alginate; gelatin; collagen; celluloses, such as ethyl cellulose (EC), methylethyl cellulose (MEC), carboxymethyl cellulose (CMC), CMEC, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC), cellulose acetate (CA), cellulose propionate (CP), cellulose butyrate (CB), cellulose acetate butyrate (CAB), CAP, CAT, hydroxypropyl methyl cellulose (HPMC), HPMCP, HPMCAS, hydroxypropyl methyl cellulose acetate trimellitate (HPMCAT), and ethyl hydroxyethyl cellulose (EHEC); polyvinyl pyrrolidone; polyvinyl alcohol; polyvinyl acetate; glycerol fatty acid esters; polyacrylamide; polyacrylic acid; copolymers of ethacrylic acid or methacrylic acid (EUDRAGIT®, Rohm America, Inc., Piscataway, NJ); poly(2-hydroxyethyl-methacrylate); poly(lactides; copolymers of L-glutamic acid and ethyl-L-glutamate; degradable lactic acid-glycolic acid copolymers; poly-D(-)-3-hydroxybutyric acid; and other acrylic acid derivatives, such as homopolymers and copolymers of butylmethacrylate, methyl methacrylate, ethyl methacrylate, ethylacrylate, (2-dimethylaminoethyl)methacrylate, and (trimethylaminoethyl)methacrylate chloride.

[00204] In certain embodiments, the compositions are formulated with a non-erodible matrix device. The active ingredient(s) is dissolved or dispersed in an inert matrix and is released primarily by diffusion through the inert matrix once administered. Materials suitable for use as a non-erodible matrix device include, but are not limited to, insoluble plastics, such as polyethylene, polypropylene, polyisoprene, polyisobutylene, polybutadiene, polymethylmethacrylate, polybutylmethacrylate, chlorinated polyethylene, polyvinylchloride, methyl acrylate-methyl methacrylate copolymers, ethylene-vinyl acetate copolymers, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, vinyl chloride...
copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubbers, epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, ethylene/vinylxyloxyethanol copolymer, polyvinyl chloride, plasticized nylon, plasticized polyethylene terephthalate, natural rubber, silicone rubbers, polydimethylsiloxanes, and silicone carbonate copolymers; hydrophilic polymers, such as ethyl cellulose, cellulose acetate, crospovidone, and cross-linked partially hydrolyzed polyvinyl acetate; and fatty compounds, such as carnauba wax, microcrystalline wax, and triglycerides.

[00205] In a matrix controlled release system, the desired release kinetics can be controlled, for example, via the polymer type employed, the polymer viscosity, the particle sizes of the polymer and/or the active ingredient(s), the ratio of the active ingredient(s) versus the polymer, and other excipients or carriers in the compositions.

[00206] The modified release dosage forms can be prepared by methods known to those skilled in the art, including direct compression, dry or wet granulation followed by compression, and melt-granulation followed by compression.

5.2.2.2 OSMOTIC CONTROLLED RELEASE DEVICES

[00207] The modified release dosage form can be fabricated using an osmotic controlled release device, including, but not limited to, one-chamber system, two-chamber system, asymmetric membrane technology (AMT), and extruding core system (ECS). In general, such devices have at least two components: (a) a core which contains an active ingredient; and (b) a semipermeable membrane with at least one delivery port, which encapsulates the core. The semipermeable membrane controls the influx of water to the core from an aqueous environment of use so as to cause drug release by extrusion through the delivery port(s).

[00208] In addition to the active ingredient(s), the core of the osmotic device optionally includes an osmotic agent, which creates a driving force for transport of water from the environment of use into the core of the device. One class of osmotic agents is water-swellable hydrophilic polymers, which are also referred to as "osmopolymers" and "hydrogels." Suitable water-swellable hydrophilic polymers as osmotic agents include, but are not limited to, hydrophilic vinyl and acrylic polymers, polysaccharides such as calcium alginate, polyethylene oxide (PEO), polyethylene glycol (PEG), polypropylene glycol (PPG), poly(2-hydroxyethyl methacrylate), poly(acrylic) acid, poly(methacrylic) acid, polyvinylpyrrolidone (PVP), crosslinked PVP, polyvinyl alcohol (PVA), PVA/PVP copolymers, PVA/PVP copolymers with hydrophobic monomers such as methyl methacrylate
and vinyl acetate, hydrophilic polyurethanes containing large PEO blocks, sodium
croscarmellose, carrageenan, hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC),
hydroxypropyl methyl cellulose (HPMC), carboxymethyl cellulose (CMC) and carboxyethyl,
cellulose (CEC), sodium alginate, polycarbophil, gelatin, xanthan gum, and sodium starch
glycolate.

[00209] The other class of osmotic agents is osmogens, which are capable of imbibing
water to affect an osmotic pressure gradient across the barrier of the surrounding coating.
Suitable osmogens include, but are not limited to, inorganic salts, such as magnesium sulfate,
magnesium chloride, calcium chloride, sodium chloride, lithium chloride, potassium sulfate,
potassium phosphates, sodium carbonate, sodium sulfite, lithium sulfate, potassium chloride,
and sodium sulfate; sugars, such as dextrose, fructose, glucose, inositol, lactose, maltose,
mannitol, raffinose, sorbitol, sucrose, trehalose, and xylitol; organic acids, such as ascorbic
acid, benzoic acid, fumaric acid, citric acid, maleic acid, sebacic acid, sorbic acid, adipic acid,
edetic acid, glutamic acid, p-toluenesulfonic acid, succinic acid, and tartaric acid; urea; and
mixtures thereof.

[00210] Osmotic agents of different dissolution rates can be employed to influence how
rapidly the active ingredient(s) is initially delivered from the dosage form. For example,
amorphous sugars, such as MANNOGEM™ EZ (SPI Pharma, Lewes, DE) can be used to
provide faster delivery during the first couple of hours to promptly produce the desired
therapeutic effect, and gradually and continually release of the remaining amount to maintain
the desired level of therapeutic or prophylactic effect over an extended period of time. In this
case, the active ingredient(s) is released at such a rate to replace the amount of the active
ingredient metabolized and excreted.

[00211] The core can also include a wide variety of other excipients and carriers as
described herein to enhance the performance of the dosage form or to promote stability or
processing.

[00212] Materials useful in forming the semipermeable membrane include various grades
of acrylics, vinyls, ethers, polyamides, polyesters, and cellulosic derivatives that are water-
permeable and water-insoluble at physiologically relevant pHs, or are susceptible to being
rendered water-insoluble by chemical alteration, such as crosslinking. Examples of suitable
polymers useful in forming the coating, include plasticized, unplasticized, and reinforced
cellulose acetate (CA), cellulose diacetate, cellulose triacetate, CA propionate, cellulose
nitrate, cellulose acetate butyrate (CAB), CA ethyl carbamate, CAP, CA methyl carbamate,
CA succinate, cellulose acetate trimellitate (CAT), CA dimethylaminoacetate, CA ethyl
carbonate, CA chloroacetate, CA ethyl oxalate, CA methyl sulfonate, CA butyl sulfonate, CA p-toluene sulfonate, agar acetate, amylose triacetate, beta glucan acetate, beta glucan triacetate, acetaldehyde dimethyl acetate, triacetate of locust bean gum, hydroxylated ethylene-vinylacetate, EC, PEG, PPG, PEG/PPG copolymers, PVP, HEC, HPC, CMC, CMEC, HPMC, HPMCP, HPMCAS, HPMCAT, poly(acrylic) acids and esters and poly-(methacrylic) acids and esters and copolymers thereof, starch, dextran, dextrin, chitosan, collagen, gelatin, polyalkenes, polyethers, polysulphones, polyethersulphones, polystyrenes, polyvinyl halides, polyvinyl esters and ethers, natural waxes, and synthetic waxes.

A semipermeable membrane can also be a hydrophobic microporous membrane, wherein the pores are substantially filled with a gas and are not wetted by the aqueous medium but are permeable to water vapor, as disclosed in U.S. Pat. No. 5,798,119. Such hydrophobic but water-vapor permeable membrane are typically composed of hydrophobic polymers such as polyalkenes, polyethylene, polypropylene, polytetrafluoroethylene, polyacrylic acid derivatives, polyethers, polysulphones, polyethersulphones, polystyrenes, polyvinyl halides, polyvinylidene fluoride, polyvinyl esters and ethers, natural waxes, and synthetic waxes.

The delivery port(s) on the semipermeable membrane can be formed post-coating by mechanical or laser drilling. Delivery port(s) can also be formed in situ by erosion of a plug of water-soluble material or by rupture of a thinner portion of the membrane over an indentation in the core. In addition, delivery ports can be formed during coating process, as in the case of asymmetric membrane coatings of the type disclosed in U.S. Pat. Nos. 5,612,059 and 5,698,220.

The total amount of the active ingredient(s) released and the release rate can substantially be modulated via the thickness and porosity of the semipermeable membrane, the composition of the core, and the number, size, and position of the delivery ports.

An osmotic controlled-release dosage form can further comprise additional conventional excipients or carriers as described herein to promote performance or processing of the formulation. The osmotic controlled-release dosage forms can be prepared according to conventional methods and techniques known to those skilled in the art. See Remington: The Science and Practice of Pharmacy, supra; Santus and Baker, J. Controlled Release 1995, 35, 1-21; Verma et al, Drug Development and Industrial Pharmacy 2000, 26, 695-708; and Verma et al., J. Controlled Release 2002, 79, 7-27.

In certain embodiments, the compositions are formulated as AMT controlled-release dosage form, which comprises an asymmetric osmotic membrane that coats a core
comprising the active ingredient(s) and other pharmaceutically acceptable excipients or carriers. See, U.S. Patent No. 5,612,059 and International Publication No. WO 2002/17918. The AMT controlled-release dosage forms can be prepared according to conventional methods and techniques known to those skilled in the art, including direct compression, dry granulation, wet granulation, and a dip-coating method. In certain embodiments, the compositions are formulated as ESC controlled-release dosage form, which comprises an osmotic membrane that coats a core comprising the active ingredient(s), a hydroxylethyl cellulose, and other pharmaceutically acceptable excipients or carriers.

5.2.2.3 IN SITU GELLING DRUG DELIVERY SYSTEMS

[00218] In one embodiment, the lithium-containing compound can be loaded into a polymeric solution that consists of a water-soluble polymer that is a solution at room temperature (20-25°C) and below, but gels at physiological temperatures of 32-37°C. In one application the lithium-containing solution can be cooled to 2-8°C to impart a soothing effect, while being sprayed as a liquid spray on the tissue surface. Once sprayed on, the lithium-loaded solution will thicken into a gel, releasing the lithium-containing compound slowly over time. Examples of these thermo-gelling polymers are poly(isopropyl acrylamide), poly(EO)x-(PO)y-(EO)x and poly(PO)x-(EO)y-(PO)x, wherein EO=ethylene oxide and PO=propylene oxide. Other examples include, but are not limited to, PLA-PEO-PLA polymers, wherein PLA=polyactic acid, PEO=polyethylene oxide, poly(sebacic anhydride)-poly(ethylene oxide)-poly(sebacic anhydride) and poly(stearate)-poly(ethylene oxide)-poly(stearate). In a variation of the idea, the lithium-loaded solution can be injected as a liquid, to form an in situ depot within the tissue. In another variation of the concept, the lithium-loaded solution can be delivered as a solution, which can flow into orifices of the tissue, such as hair follicles, and then, form a gel to release lithium for cutaneous microbial infections, follicle-associated conditions, such as MPHL, folliculitis, or another condition described herein. The temperature and time of gelation can be correlated to the concentration of the polymers and the length of the polymer blocks that constitute the polymers.

5.2.2.4 MULTIPARTICULATE CONTROLLED RELEASE DEVICES

[00219] A modified release dosage form can be fabricated as a multiparticulate controlled release device, which comprises a multiplicity of particles, granules, or pellets, ranging from about 10 µm to about 3 mm, about 50 µm to about 2.5 mm, or from about 100 µm to about 1

[00220] Other excipients or carriers as described herein can be blended with the compositions to aid in processing and forming the multiparticulates. The resulting particles can themselves constitute the multiparticulate device or can be coated by various film-forming materials, such as enteric polymers, water-swellable, and water-soluble polymers. The multiparticulates can be further processed as a capsule or a tablet.

### 5.2.3 TARGETED DELIVERY

[00221] The lithium compounds for use herein may be formulated with a carrier that delivers the lithium to the site of action, for example, an infected wound, or a follicle in a particular tissue. Such targeted delivery may be preferable in formulations for systemic administration, in order to reduce side effects associated with lithium therapy and/or ensure that the lithium reaches only the infected wound or tissue, or follicles of particular tissues. The carrier may be an aptamer targeted to a particular protein or cell type in the infected wound or tissue, or the follicle, an antibody or antigen-binding fragment thereof, a virus, virus-like particle, virosome, liposome, micelle, microsphere, nanoparticle, or any other suitable compound.

[00222] Compositions for use in the methods provided herein can also be formulated to be targeted to a particular tissue, follicle, or other area of the body of the subject to be treated, including liposome-, resealed erythrocyte-, and antibody-based delivery systems. Examples include, but are not limited to, those disclosed in U.S. Pat. Nos. 5,709,874; 5,759,542; 5,840,674; 5,900,252; 5,972,366; 5,985,307; 6,004,534; 6,039,975; 6,048,736; 6,060,082; 6,071,495; 6,120,751; 6,131,570; 6,139,865; 6,253,872; 6,271,359; 6,274,552; 6,316,652; and 7,169,410.

[00223] In some embodiments, targeting is accomplished by the attachment of specific targeting moieties to the delivery systems containing the drug. Targeting moieties can be in the form of antibodies, aptamers or small molecules that bind to specific proteins expressed in specific tissues. Specific or guided targeting can "channel" the drug only to the specific
tissue type, thus minimizing distribution to all tissues. This concept is especially useful if the drug causes side effects. Microspheres and nanospheres can be utilized, to deliver drugs into infected skin or infected wounds. Entry into infected skin or infected wounds or the hair follicle is governed by the size of the drug-containing spheres, with microspheres of size 0.5-0.7 microns of ideal size for entry. However, out-flux of sebaceous fluid from skin pores or the follicle can result in a short residence time of the delivery systems in infected skin, wound, or tissue, or in the follicle. To minimize this, the surface of the microspheres can be functionalized with moieties that bind to specific surfaces in the follicular orifice to "retain" them at the site. These moieties can be non-specific, such as hydrophobic coatings, or cationic coatings, in order to be bioadhesive to cells within infected skin, wound, or tissue. The moieties can be specific and targeted to certain proteins that are expressed specifically on specific cell membranes. For example, proteins over-expressed on the follicular lymphoma cell surfaces can be targeted by delivery systems that have antibodies or aptamers designed to bind to these proteins. The surface of the delivery systems can also be functionalized with cell-penetrating moieties such as cell-permeable peptides, positively charged polymers that bind to anionic cell surfaces.

**5.2.4 LOCAL DELIVERY**

[00224] Common side effects of systemic lithium treatment include muscle tremors, twitching, ataxia, and hypothyroidism. Long term use has been linked to hyperparathyroidism, hypercalcemia (bone loss), hypertension, kidney damage, nephrogenic diabetes insipidus (polyuria and polydipsia), seizures, and weight gain. There also appears to be an increased risk of Ebstein (cardiac) Anomaly in infants born to women taking lithium during the first trimester of pregnancy. In order to circumvent these side effects, the dosage of systemically administered lithium is tightly controlled. In some embodiments, an intermittent lithium treatment or a pulse lithium treatment described herein may have a decreased risk of such side effects because of the intermittent or temporary nature of the treatment. Another way in which such side effects may be circumvented is to deliver the lithium treatment locally to the site of the microbial infection.

[00225] The lithium treatments described herein may be delivered locally to any part of the subject in which treatment or prevention of a microbial infection or reduction of colonization, or wound healing or scar revision, is desired, including, e.g., the head (e.g., the scalp, cheek, chin, lips, ears, ear canals, nose, nostrils, eyelid or eyebrow), neck, abdomen, chest, breast, back, arms, armpits, stomach, genital area, perineum, buttocks, legs, hands, feet,
skin of a subject. In one embodiment, the lithium treatment is applied to an infected wound, or wounded or scarred skin. In one embodiment, lithium treatment is applied to skin infected by microbes. In one embodiment, lithium treatment is applied to skin colonized by microbes. In one embodiment, the lithium treatment is applied before the wound is infected. In one embodiment, the lithium treatment is applied before the skin is wounded or scarred.

[00226] Such local delivery of the lithium treatment can be achieved by topical administration, transdermal, intradermal, subcutaneous (depot effect), or by intramuscular, intravenous and oral routes of delivery in formulations for systemically delivering lithiums, or targeting delivered lithium to desired follicles. Such modes of delivery are discussed *supra*.

### 5.2.5 DELIVERY VIA SCAFFOLDS FOR MODULATING WOUND HEALING

[00227] In some embodiments, treatment of a cutaneous microbial infection or treatment of a cutaneous microbial colonization, or enhancement of wound healing or scar revision in wounded or otherwise integumentally perturbed skin, is accomplished by a lithium treatment described herein in combination with a "scaffold," a pre-designed biomaterial dressing that may serve as a substrate to encourage a step-wise attachment of keratinocytes and epithelial cells to it, such that formation of an organized extra-cellular matrix (ECM) is enhanced in order to promote wound healing. A more detailed description and examples of such scaffolds are disclosed in International Patent Application No. PCT/US2010/048439, which is incorporated herein in its entirety.

[00228] In some embodiments, enhancement of wound healing or scar revision in wounded or otherwise integumentally perturbed skin (such as, e.g., as occurs during scar revision) is accomplished by a lithium treatment described herein in combination with a "scaffold," a pre-designed biomaterial dressing that may serve as a substrate to encourage a step-wise attachment of keratinocytes and epithelial cells to it, such that formation of an organized extra-cellular matrix (ECM) is enhanced in order to promote wound healing. Without being bound by any theory, formation of an organized extracellular matrix leads to less granular epithelialization of the wound and, therefore, less scarring. Furthermore, and also without being bound by any theory, it is thought that the presence of a "scaffold" at the wounded or perturbed site prevents rapid wound contraction, whereupon the edges of the wound contract in a rapid, haphazard manner to produce granular collagen-rich skin devoid of any adnexal structures such as follicles or sweat glands, and rapid wound contraction by
secondary intention almost always results in fibrous tissue that is sub-optimal in temperature regulation, tensile and compressive strength and barrier function.

[00229] The scaffold for use in combination with lithium treatment may be comprised of a mesh of a biocompatible, bioabsorbable material that cells recognize and attach to, preferably with ease. For example, these materials can be collagen type I/III, hyaluronic acid, chitosan, alginates, or combinations and derivatives thereof or any other such material described herein or known in the art. The mesh scaffold may be neutral, or charged. If the mesh is positively charged, it may permit cells (which are negatively charged) to adhere to it more effectively. If the mesh scaffold is negatively charged, it may contain signaling moieties that the cells will recognize and attach to. For example, polymers such as hyaluronic acid are present already in skin, and thus a mesh comprised of this material is thought to be compatible with cells.

[00230] In some embodiments, the scaffold is pre-fabricated with a fine microstructure that is of the dimension of cells, for example, red blood cells that will initially diffuse throughout the scaffold, or epithelial cells and keratinocytes from surrounding tissue. Moreover, it is envisioned that the "epithelial tongue" can move with greater ease and organization by crawling on the scaffold mesh.

[00231] In some embodiments, the mesh scaffold has an "open-cell" structure, with the pores inter-connected, much like an open-celled foam. The open, interconnecting nature of the scaffold may allow free diffusion of oxygen and cells, so that optimal organized wound healing can occur.

[00232] In some embodiments, the mesh scaffold has the capacity to hydrate and remain hydrated throughout the wound healing period. This is useful because, without being bound by any theory, drying out of the wound results in a impermeable granular structure that the keratinocytes cannot "crawl upon."

[00233] In some embodiments, the mesh scaffold has moieties that act as molecular signals to the cells, for example, to aid their proliferation. These moieties include, but are not limited to, peptidoglycans and RGD integrin recognition sequences that encourage cell attachment and subsequent proliferation.

[00234] In some embodiments, the mesh scaffold has incorporated within it one or more active agents, for example, a small molecule, or a nucleic acid, or a protein. In some embodiments, the additional active agent is a protein, such as noggin or WNT, or is a nucleic acid that encodes noggin or WNT. In some embodiments, a small molecule is incorporated into the scaffold, such as, e.g., a GSK inhibitor, BMP inhibitor, or PPAR antagonist.
In some embodiments, the compound incorporated in the mesh scaffold is a compound considered for use in the combination therapies described herein, for example, in Section 5.5. For example, the scaffold may incorporate superoxide dismutase, a free radical quenching molecule that functions in the reduction of inflammation. In other embodiments, compounds are included in the mesh scaffold that alter the kinetics of wound healing, for example, that slow wound healing. Such compounds are known in the art and described elsewhere herein. Other compounds that may be incorporated in the mesh scaffold include growth factors that aid in cell proliferation and tissue regeneration. In some embodiments, the compounds aid in hair follicle migration or hair follicle neogenesis in the wound site.

In some embodiments, the lithium compound itself is incorporated within the mesh scaffold. In some embodiments, the lithium compound is incorporated within one or more layers of a multilayered mesh scaffold. For example, in one embodiment the mesh scaffold contains the lithium compound in alternating layers, which may achieve a pulsatile delivery of lithium. In some embodiments, the lithium compound in incorporated in microspheres in the scaffold, enabling a controlled release of lithium from the scaffold.

Another embodiment, the mesh scaffold can be fibrin gels that additionally contain lithium. A fibrin network is the first scaffold that a cell encounters as it performs its role in healing wounds due to trauma (such as, e.g., acute cutaneous trauma) or other insults to tissue. Unlike the extracellular matrices and basement membranes that are formed by collagen, laminin and proteoglycans, which assemble slowly in an ordered manner, the fibrin network (the "scab") assemble rapidly by a modified polycondensation reaction from fibrinogen, an abundant constituent of blood plasma, as soon as the protease thrombin is activated in the clotting cascade—the result is a three-dimensional network of branching fibers. What is envisioned is a fibrin delivery matrix containing lithium, fibrinogen and thrombin, that "gels" in-situ. One issue that is encountered is the ability of lithium to diffuse through the fibrin "scab" - making the drug part of the scab solves this issue.

In another embodiment, the mesh scaffold is a synthetic biodegradable dressing and lithium delivery system that also acts as a "sponge" and absorbs the exudates/bloods from a wound. These exudates intercalating with the synthetic scaffold contain an abundance of fibrinogen, thrombin, fibronectin, cell adhesion proteins, growth factors and hyaluronic acid, all of which create an integrated structure that is an attractive matrix for cell attachment/differentiation and delivery of lithium. The release rate of lithium can be modulated by varying the composition of polymers that comprise the synthetic scaffold, or sponge. For example, a synthetic scaffold fabricated out of poly(lactide)-co-(glycolide) (PLG) and
poly(lactide) (PLA) can be developed to have varied release profiles of lithium. Changing
the ratio of PLA to PLG will change the release profile of the lithium from the scaffold. Other
polymers that can utilized to generate synthetic scaffolds are chitosan, carrageenan, alginate,
poly(vinyl alcohol), poly(ethylene oxide) (PEO), poly(ethylene oxide)-co-poly(propylene
oxide)-co-poly(ethylene oxide) (PEO-PPO-PEO), poly(acrylates) and poly(vinyl pyrrolidone)
(PVP). By varying the composition of polymers, the rate of lithium release from the
formulation (e.g., scaffold or sponge) can be controlled, so that it takes anywhere from 2
hours to 30 days for most (e.g., 80% or more, 85% or more, 90%> or more, 95% or more, 98%>
or more, or 100%) of the lithium ion to be released. In some embodiments, most of the
lithium is released from the formulation within 2 hours, within 4 hours, within 8 hours, within
16 hours, within 24 hours, within 36 hours, within 48 hours, within 3 days, within 5 days,
within 7 days, within 10 days, within 14 days, within 30 days, or within 2 months or more.

[00239] In some embodiments, the mesh scaffold releases the aforementioned compounds
in a timed release manner, acting as a controlled release formulation such as described in
Section 5.2 above. For example, the compounds may be bound to the mesh scaffold, and are
then released at a sustained release manner as a result of de-binding kinetics from the mesh.
In some embodiments, the compound may be bound to a polymer, which is then incorporated
to the mesh scaffold, and which may allow the compound to diffuse from the mesh at a slow
rate, resulting in sustained release.

[00240] In some embodiments, the mesh scaffold is extruded as a gel, with certain
components of the gel precipitating out to form a mesh in situ. Alternatively, in some
embodiments, the in situ mesh can be sprayed on the wounded or otherwise perturbed
surface, such as tissue that has been extensively burned. A large area can be covered in this
manner.

[00241] In some embodiments, the mesh scaffold is pre-fabricated as a dressing or a wrap,
to cover large areas of wounded tissue. In some such embodiments, the mesh scaffold can be
cut to size to fit the size of the wound to present a compatible surface for favorable
movement of the epithelial tongue.

[00242] In some embodiments, the scaffold is prepared by melt spinning, electrospinning,
micromachining, weaving, or other methods known in the art in which open cell foams are
fabricated. Using starting materials that are United States Pharmacopeia (USP)-approved, the
mesh scaffold can be fabricated by these methods, with the optional incorporation of
additional compound(s) (which are optionally sterilized), then sterilized by gentle ethylene
oxide sterilization. In some embodiments, the additional compounds are sterilized, and then added to the sterile mesh scaffold.

[00243] In a particular embodiment, a combinatorial strategy that uses a biodegradable scaffold combined with administration of a lithium formulation described herein (alone or in combination with another treatment, such as described in Section 5.5) is applied, which may result in the in situ generation of embryonic stem cells or recruitment of cells required for wound healing following wounding. This approach may be used together with a form of integumental perturbation described in Section 5.5 (e.g., dermabrasion accomplished by a standard dermabrader or a laser, deep full-thickness excision (as for deep burns) accomplished by a bulk ablative laser) or integumental perturbation by acute wounds, chronic wounds, or wounds generated for the purpose of scar revision. While not being bound by any theory of how the invention works, such integumental perturbation in combination with a scaffold that administers drug results in the in situ generation of stem cells or recruitment of other cells required for the wound healing process and may facilitate more effective wound healing with little or no scarring.

5.2.5.1 BIODEGRADABLE PROPERTIES OF THE SCAFFOLD

[00244] In one embodiment, the scaffold is biodegradable. Placement of a 3-dimensional biodegradable scaffold in the wound assists the attachment, growth and differentiation of cells. Historically, tissue repair has been by autologous cell/tissue transplantation—however, autografts are associated with donor site morbidity and limited availability. An alternative is allografts, but these are susceptible to immune responses and also carry the risk of disease transfer. Thus, tissue engineering has emerged as an interdisciplinary field that makes use of biomaterials, cells and factors either alone, or in combination to restore tissues. The tissue engineering strategy generally involves isolation of healthy cells from a patient, followed by their expansion in vitro. These expanded cells are then seeded onto three-dimensional biodegradable frameworks that provide structural support for the cells and allow cellular infiltration, attachment, proliferation and growth ultimately leading to new tissue. In a sense, natural wound healing utilizes a "scaffold" as well—the fibrin clot. A fibrin network is the natural network that forms rapidly due to a polycondensation reaction from fibrinogen, an abundant constituent of blood plasma, as soon as the protease thrombin is activated in the clotting cascade. The fibrin clot then forms a three-dimensional network for cells to attach, for re-epithelialization.
In some embodiments, the biodegradability of the scaffold is modulated. Ideally, the biodegradability of the scaffold should be matched to the formation of the new epithelium due to wound healing. One skilled in the art would know how to measure whether a synthetic matrix is biodegradable. For example, biodegradability can be measured ex vivo in implants or using rats or another animal model, by histological and HPLC analysis. In one embodiment, biodegradability by hydrolysis can be assessed. In such an embodiment, the scaffold structure of choice is incubated in phosphate buffered saline, pH 7.4 and 37 °C. For degradation by enzymolysis, the incubation buffer includes enzymes. The scaffolds are weighed prior to incubation. The scaffolds are retrieved two-at-a-time at predetermined time points and dried in a vacuum oven. The scaffolds are weighed at each time point and a plot of weight versus time is generated to develop the rate of biodegradability. In one embodiment, the biodegradability of the scaffold matrix is modulated to coincide with the healing process, and can be modulated by changing the composition of polymers utilized to fabricate the mesh. For example, a percentage of polyethylene glycol (PEG) can be included in a composition with PLG to increase biodegradation (for example, see ASTM E1279 - 89, 2008, Standard Test Method for Biodegradation By a Shake-Flask Die-Away Method).

### 5.2.5.2 BIOMIMETIC PROPERTIES OF THE SCAFFOLD

Biodegradable synthetic matrices can be created to mimic the extra-cellular micro-environment for the enhanced cellular attachment necessary for tissue regeneration. In some embodiments, cell-recognition motifs such as RGD peptides may be incorporated to encourage cells to attach themselves to the scaffold.

One skilled in the art would know how to measure whether the biodegradable synthetic matrix has biomimetic properties. For example, in one embodiment, the biomimetic nature of the scaffold is judged on the basis of the content of the mesh and resultant intercalating fibrin.

### 5.2.5.3 PHYSICAL PROPERTIES OF THE SCAFFOLD

The properties of the synthetic scaffold are dependent upon the three-dimensional geometry, matching of the modulus of the matrix with the tissue type and the porosity. It has been shown that the differentiation process can be modulated if the modulus of the tissue type is matched with the modulus of the scaffold.

One skilled in the art would know how to measure whether the biodegradable synthetic matrix has optimal physical properties. For example, in one embodiment, the
modulus of the scaffold is matched with the modulus of the tissue type. In general, the compressive modulus of a scaffold or hydrogel can be measured by a standard Instron instrument (e.g., using the TA Instruments DMA Q800).

5.2.5.4 BIOCOMPATIBILITY OF THE SCAFFOLD

[00250] Further, the micro-environment created by the cells is optimally highly biocompatible to the cells present at the site, namely keratinocytes and stem cells derived from the dermal papilla. In one embodiment, this can be accomplished through the use of hydrophilic components that can absorb water. Use of hydrophobic components such as petrolatum is likely to be occlusive and prevent rapid cell proliferation.

[00251] One skilled in the art would know how to measure whether the biodegradable synthetic matrix is biocompatible. For example, in one embodiment, the scaffold is incubated with human foreskin fibroblasts (HFF) in vitro and the scaffold is considered to be biocompatible if the cells maintain their shape and attach appropriately. See, e.g., the following reference for studies on the biocompatibility of materials: Altankov et al., 1996, Journal of Biomedical Materials Research Part A; 30:385-391, which is incorporated by reference herein in its entirety.

5.2.5.5 OXYGEN PERMEABILITY OF THE SCAFFOLD

[00252] In some embodiments, the biodegradable scaffold is permeable to water, nutrients, oxygen and growth factors, enabling easy exchange of nutrients between tissues and cells (see, e.g., ASTM D39857). In some embodiments, a non-occlusive, non-permeable barrier is avoided.

5.2.5.6 UTILITY OF THE SCAFFOLD IN DEEP WOUNDS

[00253] In one embodiment, the scaffold is used to "fill" a deep wound, as is common in a deep burn, to provide a matrix for the cells to attach, grow and differentiate - existence of the scaffold will likely minimize the scar formation normally observed in deep, large-area wounds.

5.2.5.7 COMBINED BIOLOGICAL/SYNTHETIC MESH

[00254] In another embodiment, a loose, dry, highly porous network or scaffold or mesh is placed in the bleeding site of the wound to gently absorb the blood and the cell adhesion proteins released at the site, as a result of wounding. This will result in creation of a highly
rich environment that consists of a combination of a 3-dimensional scaffold combined with fibrinogen and thrombin, which will ultimately result in a highly biocompatible hydrogel suitable for cell attachment and growth. In some embodiment, inclusion of blood components and cell adhesion proteins into the network is critical for establishment of the ECM (extracellular matrix) necessary to form continuous tissue in-growth, particularly in the case of large-area and deep wounds.

[00255] A dry scaffold has the added advantage of absorbing the blood at the wound site. Thus, a person's own blood components can be used to create a combined synthetic/natural ECM. In practical terms, the scaffold has an added advantage of serving as a blood absorbing gauze.

[00256] In another embodiment, the scaffold has cell-recognition motifs, such as RGD peptides, to recruit cells to the site and attachment, thereof. Once attached, cells will proliferate. Without being bound by any theory, it is hypothesized that the primary attachment of cells to the scaffold is a critical step to prevent premature cell death.

[00257] In one embodiment, a dry, sterile biodegradable scaffold is placed onto the freshly formed wound. The properties of the scaffold will be such that it will transform into an adherent hydrogel upon water absorption.

5.2.5.8 FABRICATING AND APPLYING THE SCAFFOLD

[00258] Methods that may be employed to fabricate the scaffold are known in the art, and include electrospinning, micromachining, and others. Nano-fiber meshes fabricated by electrospinning, hydrogel imprint technologies have been utilized to create three-dimensional microstructures that match the supramolecular architecture of the tissue type. In situ forming scaffolds are also contemplated.

[00259] In some embodiments, the active agents (e.g., lithium alone or in a combination described herein) are administered using an active agent-containing spray-on hydrogel. In one such embodiment, after placement of the biodegradable scaffold, the active agent is sprayed on the tissue. The active agent (or combination of active agents, e.g., lithium and another stem cell signaling agent) may be incorporated into a spray-on hydrogel that will be sprayed on as a liquid, but which transforms into a hydrogel after it is sprayed on the tissue. This will be especially useful if the area of the wound is large and uniform coverage is needed.

[00260] In some embodiments, the active agent-containing spray-on hydrogel is applied on the wound site, forming a cross-linked hydrogel that releases active agent over the time
period of healing or a shorter or longer time period, as necessary. Depending upon the release characteristics that are required, the active agent will either be incorporated in micro-encapsulates or nano-encapsulates and suspended into the pre-hydrogel solution. The active agent can also be dissolved into the pre-hydrogel solution. The "pre-hydrogel" solution is defined as the solution that will be sprayed on the tissue and which also contains the active agent.

[00261] In some embodiments, the active agent is contained within microspheres that can be positively charged to rapidly bind themselves to the negatively charged collagen present in the dermis. Binding the microspheres to the dermis renders the active agent-releasing moiety immobile at the site.

[00262] In a variation of the foregoing embodiments, the wound may be covered with a breathable, non-occlusive spray-on hydrogel to cover the wound from infection during healing.

5.2.6 **MODES OF ADMINISTRATION**

[00263] The intermittent lithium treatments or a pulse lithium treatment can be provided by administration of the lithium compound (or combination treatments, discussed in Section 5.5 *infra*) in forms suitable for topical (e.g., applied directly to the skin, transdermal, or intradermal), subcutaneous, intramuscular, intravenous or by other parenteral means, oral administration, sublingual administration, or buccal administration. In some embodiments, the topical (e.g., applied directly to the skin, transdermal, or intradermal) administration is accomplished with the use of a mechanical device, such as, e.g., an iontophoretic device. The lithium compounds (or combination treatment) can also be formulated as modified release dosage forms, including delayed-, extended-, prolonged-, sustained-, pulsatile-, controlled-, accelerated-, fast-, targetted-, programmed-release, and gastric retention dosage forms. These dosage forms can be prepared according to conventional methods and techniques known to those skilled in the art (see, Rathbone *et al*., eds., 2008, *Remington: The Science and Practice of Pharmacy*, supra; *Modified-Release Drug Delivery Technology*, 2nd ed., New York, NY: Marcel Dekker, Inc.). A lithium treatment can be administered by a health care practitioner or by the subject. In some embodiments, the subject administers the lithium treatment to him or herself.
5.2.6.1 TOPICAL ADMINISTRATION

[00264] In the embodiments described in the subsections that follow, lithium can be applied topically, e.g., as a cream, gel, ointment, or other form for topical administration as described in Section 5.2 supra. Topical lithium may be administered to wounded or unwounded skin.

[00265] Provided herein are pharmaceutical compositions formulated for topical administration, comprising a source of lithium ions formulated into aqueous formulations (e.g., hydrogels), ointments, or creams (e.g., emulsions) for topical administration.

[00266] In some embodiments, the formulation of the pharmaceutical composition for topical administration is varied in order to control the rate of lithium release. This may be accomplished by, for example, varying the molecular fluidity of the carrier, without changing its hydrophobicity, such as by varying the petrolatum to mineral oil ratio. In one embodiment, the pharmaceutical formulation is an ointment, comprising a source of lithium ions, petrolatum, mineral oil, and lanolin alcohol. Exemplary formulations prepared in accordance with such embodiments are provided in Example 7 below.

[00267] In another embodiment, Li+ release can be modulated by varying the hydrophobic/ hydrophilic ratio of the formulation, for example, by preparing a petrolatum/water emulsion. Exemplary formulations prepared in accordance with such embodiments are provided in Example 8 below.

[00268] Sources of lithium ions for use in the foregoing pharmaceutical compositions include, but are not limited to, lithium carbonate, lithium citrate, lithium gluconate, lithium chloride, lithium succinate, or lithium hydroxide. In one embodiment, the source of lithium ions is lithium carbonate. In another embodiment, the source of lithium ions is lithium citrate. In another embodiment, the source of lithium ions is lithium hydroxide. In certain embodiments, regardless of the source of lithium ions, the topical formulation comprises 2.74 mg Li+/gram.

[00269] In other embodiments, a salt form of lithium, such as but not limited to lithium carbonate, lithium citrate, lithium chloride, and lithium succinate, is formulated into an aqueous formulation for topical administration. In one embodiment, the aqueous formulation is an aqueous hydrogel, comprising a source of lithium ions, Carbopol 980, methyl paraben, propyl paraben, propylene glycol, glycerine, and water. Exemplary formulations prepared in accordance with such embodiments are provided in Example 9 below.
In another embodiment, an aqueous lithium formulation is generated using lithium hydroxide. Lithium hydroxide is listed as an inactive ingredient in the FDA database, and is available in pharmaceutical grade form as lithium hydroxide, monohydrate. Formulations containing lithium hydroxide may be desirable because they lack a counterion to Li+. For example, formulations containing lithium gluconate are expected to have equimolar concentrations of ionized, cationic Li+ and its counterion, the gluconate anion, whereas when lithium hydroxide is dissolved, it ionizes into the hydroxyl anion and Li+.

One challenge, however, to generating lithium formulations using lithium hydroxide is that the starting material, lithium hydroxide, is a very strong base and as such is corrosive. This presents an obstacle to the formulation of lithium hydroxide, particularly into forms for topical administration, for example, emulsions. In the present invention, these challenges have been overcome by varying the formulation protocol such that hydrogels and emulsions were successfully generated using lithium hydroxide. Thus, in one embodiment, a hydrogel formulation comprises lithium hydroxide monohydrate, citric acid, CMC, methyl paraben, propyl paraben, allantoin, alginate, and water. In another embodiment, an emulsion formulation comprises lithium hydroxide monohydrate.

Lithium hydroxide hydrogels containing different amounts of Li+ may be generated in accordance with the following table, as described in more detail in Example 13 below.

### Formulations of lithium hydroxide at 0.34 mg Li+/g, 2.74 mg Li+/g, 5.48 mg Li+/g and 10.73 mg Li+/g

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>0.34 mg Li+/g</th>
<th>2.74 mg Li+/g</th>
<th>5.48 mg Li+/g</th>
<th>10.73 mg Li+/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g %</td>
<td>g %</td>
<td>g %</td>
<td>g %</td>
</tr>
<tr>
<td>Lithium Hydroxide monohydrate</td>
<td>0.041 0.206</td>
<td>0.332 1.660</td>
<td>0.662 3.310</td>
<td>1.326 6.630</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>1.600 8.000</td>
<td>1.600 8.000</td>
<td>1.600 8.000</td>
<td>1.600 8.000</td>
</tr>
<tr>
<td>CMC</td>
<td>0.400 2.000</td>
<td>0.400 2.000</td>
<td>0.400 2.000</td>
<td>0.400 2.000</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.021 0.104</td>
<td>0.021 0.104</td>
<td>0.021 0.104</td>
<td>0.021 0.104</td>
</tr>
<tr>
<td>Propyl Paraben</td>
<td>0.010 0.048</td>
<td>0.010 0.048</td>
<td>0.010 0.048</td>
<td>0.010 0.048</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>15.792 78.962</td>
<td>15.502 77.508</td>
<td>15.172 75.858</td>
<td>14.508 72.538</td>
</tr>
<tr>
<td>10% NaOH</td>
<td>PH adjust</td>
<td>PH adjust</td>
<td>PH adjust</td>
<td>PH adjust</td>
</tr>
<tr>
<td>Allantoin</td>
<td>0.032 0.160</td>
<td>0.032 0.160</td>
<td>0.032 0.160</td>
<td>0.032 0.160</td>
</tr>
</tbody>
</table>
[00273] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising carboxymethylcellulose, allantoin, alginate, glycerol, citric acid, distilled water and lithium carbonate. In an embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and 1% to 15% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1%, carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and 1.46% lithium carbonate.

Concentrations of citric acid may be varied from 5%-10% w/w. Concentrations of lithium carbonate can be varied from 1%-15%. Concentrations of glycerol may be varied from 1%-10%. Boric acid may be used instead of citric acid, in concentrations 5-10% w/w.

Hydrochloric acid may be used in conjunction with citric acid or boric acid, for the purpose of adjusting pH to a physiologically acceptable formulation for wounds. Hyaluronic acid at concentrations 0.1-2% may be used instead of carboxymethylcellulose (CMC). Other polymers to be used instead of CMC may be hydroxyethyl cellulose (0.1-2%), hydroxypropylmethyl cellulose (0.1%), Xanthan Gum (0.1-1%), Guar Gum (0.1-1%).

[00274] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising carboxymethylcellulose, allantoin, alginate, glycerol, citric acid, distilled water, mupirocin and lithium carbonate. In a further embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 0.5% to 3% mupirocin. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin, and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1%
carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 1.46% lithium carbonate.

[00275] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 5% mupirocin and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 0.5% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 1.46% lithium carbonate.

[00276] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin and 1.46% lithium carbonate.

[00277] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol,
5% citric acid, distilled water, 3% mupirocin, and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 3% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 3% mupirocin and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 3% mupirocin and 1.46% lithium carbonate.

In a preferred embodiment, topical administration is to the skin, either to the skin surface, transdermally, or intradermally. Topical administration can be with or without occlusion with a bandage or other type of dressing. In some embodiments, topical administration is to orifices or mucosa, or conjunctival, intracorneal, intraocular, ophthalmic, auricular, nasal, vaginal, urethral, respiratory, and rectal administration. The formulation used for topical administration can be designed to retain the lithium in the skin or to deliver a dose of lithium systematically. In some embodiments, topical administration of a lithium compound is combined with another treatment described herein, such as, but not limited to, a technique of integumental perturbation or an antimicrobial agent.

Dosage forms that are suitable for topical administration for preferably local but also possible systemic effect, include emulsions, solutions, suspensions, creams, gels, hydrogels, ointments, dusting powders, dressings, elixirs, lotions, suspensions, tinctures, pastes, powders, crystals, foams, films, aerosols, irrigations, sprays, suppositories, sticks, bars, ointments, sutures, bandages, wound dressings, microdermabrasion or dermabrasion particles, drops, and transdermal or dermal patches. The topical formulations can also comprise micro- and nano-sized capsules, liposomes, micelles, microspheres, microparticles, nanosystems, e.g., nanoparticles, nano-coacervates and mixtures thereof. See, e.g., International Patent Application Publication Nos. WO 2005/107710, published November 17, 2005, and WO 2005/020940, published March 10, 2005, each of which is incorporated herein by reference in its entirety. In one embodiment, the nano-sized delivery matrix is fabricated through a well-defined process, such as a process to produce lithium encapsulated in a polymer. In another embodiment, the lithium-releasing compound is spontaneously assembled in aqueous solutions, such as in liposomes and micelles. In some embodiments,
the formulation for topical administration is a shampoo or other hair product, tanning product or sun protectant, skin lotion, or cosmetic.

[00280] In certain embodiments, a selected formulation will penetrate into the skin. In certain embodiments, the selected formulation will penetrate into the skin and reach the hair follicle. Thus, in some embodiments, the stratum corneum and/or epidermis have been or are removed by a method of integumental perturbation described herein (including by wounding or scar revision procedure, by laser, or by dermabrasion or microdermabrasion, which is a less vigorous form of dermabrasion), permitting application of the dosage form for topical administration directly into the exposed dermis. In some embodiments, the formulation for topical administration will be lipid-based, so that it will penetrate the stratum corneum. In some embodiments, the formulation for topical administration will contain a skin penetrant substance, such as, e.g., propylene glycol or transcutol. See, e.g., International Patent Application Publication No. WO 2004/103353, published December 2, 2004, which is incorporated herein by reference in its entirety. The ability to penetrate into the skin can be tested using any method known in the art, such as, e.g., the method described in International Patent Application Publication No. WO 2005/107710, which is incorporated herein by reference in its entirety. In one embodiment, a formulation in ointment form comprises one or more of the following ingredients: wool alcohol (acetylated lanolin alcohol), hard paraffin, white soft paraffin, liquid paraffin, and water. See, e.g., Langtry et al., supra. In some embodiments, the selected formulation is inconspicuous when applied to the skin, for example, is colorless, odorless, quickly-absorbing, etc. In some embodiments, the selected formulation is applied on the skin surface as a solution, which can crosslink into a hydrogel within a few minutes, thus creating a biocompatible dressing. In one application, the hydrogel may be biodegradable. In another embodiment, the solution will absorb into the skin and crosslink into depots releasing drug. In another embodiment, the lithium ion will be used to crosslink the polymer, with release of the lithium ion controlled by the rate of degradation of the hydrogel.

[00281] Pharmaceutically acceptable carriers and excipients suitable for use in topical formulations include, but are not limited to, aqueous vehicles, water-miscible vehicles, non-aqueous vehicles, anti-microbial agents or preservatives against the growth of microorganisms, stabilizers, solubility enhancers, isotonic agents, buffering agents, antioxidants, local anesthetics, suspending and dispersing agents, wetting or emulsifying agents, complexing agents, sequestering or chelating agents, penetration enhancers, cryoprotectants, lyoprotectants, thickening agents, and inert gases.
Forms for topical administration can also be in the form of ointments, creams, and gels. Suitable ointment vehicles include oleaginous or hydrocarbon vehicles, including lard, benzoinated lard, olive oil, cottonseed oil, mineral oil and other oils, white petrolatum, paraffins; emulsifiable or absorption vehicles, such as hydrophilic petrolatum, hydroxystearin sulfate, and anhydrous lanolin; water-removable vehicles, such as hydrophilic ointment; water-soluble ointment vehicles, including polyethylene glycols of varying molecular weight; emulsion vehicles, either water-in-oil (W/O) emulsions or oil-in-water (O/W) emulsions, including cetyl alcohol, glyceryl monostearate, lanolin, wool alcohol (acylated lanolin alcohol), and stearic acid (see, Remington: The Science and Practice of Pharmacy, supra). These vehicles are emollient but generally require addition of antioxidants and preservatives.

Suitable cream base can be oil-in-water or water-in-oil. Suitable cream vehicles may be water-washable, and contain an oil phase, an emulsifier, and an aqueous phase. The oil phase is also called the "internal" phase, which is generally comprised of petrolatum and a fatty alcohol such as cetyl or stearyl alcohol. The aqueous phase usually, although not necessarily, exceeds the oil phase in volume, and generally contains a humectant. The emulsifier in a cream formulation may be a nonionic, anionic, cationic, or amphoteric surfactant.

Gels are semisol, suspension-type systems. Single-phase gels contain organic macromolecules distributed substantially uniformly throughout the liquid carrier. Suitable gelling agents include, but are not limited to, crosslinked acrylic acid polymers, such as carbomers, carboxypolyalkylenes, and CARBOPOL®; hydrophilic polymers, such as polyethylene oxides, polyoxyethylene-polyoxypropylene copolymers, and polyvinylalcohol; cellulcosic polymers, such as hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methylcellulose, hydroxypropyl methylcellulose phthalate, and methylcellulose; gums, such as tragacanth and xanthan gum; sodium alginate; and gelatin. In order to prepare a uniform gel, dispersing agents such as alcohol or glycerin can be added, or the gelling agent can be dispersed by trituration, mechanical mixing, and/or stirring.

In particular embodiments, commercially available preparations of lithium can be used for topical administration in the methods described herein. These include, e.g., lithium carbonate, lithium gluconate, e.g., 8% lithium gluconate (Lithioderm™), approved for the treatment of seborrheic dermatitis (see, e.g., Dreno and Moyse, 2002, Eur J Dermatol 12:549-552; Dreno et al, 2007, Ann Dermatol Venereol 134:347-351 (abstract); and Ballanger et al., 2008, Arch Dermatol Res 300:215-223, each of which is incorporated by reference herein in its entirety); 8% lithium succinate (see, e.g., Langtry et al, 1996, Clinical and Experimental
Dermatology 22:216-219; and Cuelenaere et al., 1992, Dermatology 184:194-197, each of which is incorporated by reference herein in its entirety; or 8% lithium succinate with 0.05% zinc sulfate (marketed in the U.K. as Efalith; see, e.g., Efalith Multicenter Trial Group, 1992, J Am Acad Dermatol 26:452-457, which is incorporated by reference herein in its entirety).

[00286] Other means of topical administration of lithium compounds are also contemplated. Each of these methods of topical administration may be used alone to administer lithium compounds or in combination with one or more other treatments as described in Section 5.5 infra.

[00287] In some embodiments, topical administration is by electrical current, ultrasound, laser light, or mechanical disruption or integumental perturbation. These include electroporation, RF ablation, laserporation, laser ablation (fractional or non-fractional), non-ablative use of a laser, iontophoresis, phonophoresis, sonophoresis, ultrasound poration, or using a device that accomplishes skin abrasion, or microneedle or needle-free injection, such as topical spray or POWDERJECT™ (Chiron Corp., Emeryville, CA), BIOJECT™ (Bioject Medical Technologies Inc., Tualatin, OR), or JetPee™ (from TavTech, Tel Aviv, Israel), which uses supersonically accelerated saline to remove epidermis. Means of topical administration that can be used in accordance with the methods described herein are known in the art and are described in, e.g., U.S. Patent Nos. 5,957,895, 5,250,023, 6,306,119, 6,726,693, and 6,764,493, and International Patent Application Publication Nos. WO 2009/061349, WO 1999/003521, WO 1996/017648, and WO 1998/01 1937, each of which is incorporated herein by reference in its entirety.

[00288] In some embodiments, the device for topical administration of lithium compounds is an automatic injection device worn continuously but delivers lithium intermittently. In some embodiments, the device for topical administration of lithium compounds is an automatic injection device that is inconspicuous, for example, can be worn without undue discomfort under clothes, in the hair, under a hairpiece, etc. In some embodiments, a device for administration of the intermittent lithium treatment or a pulse lithium treatment delivers the lithium at a controlled depth in the skin, but entry into the circulation is minimized. In some embodiments, a device for administration of the intermittent lithium treatment or a pulse lithium treatment delivers the lithium at a controlled depth in the skin so that it reaches hair follicles, but entry into the circulation is minimized.

[00289] Other methods for administration of the lithium compounds described herein, used alone or in combination with a delivery device described below or in combination with other treatments described herein (e.g., in combination with integumental perturbation methods
such as dermabrasion, laser treatment, or partial thickness or full thickness excision) include use of a transdermal particle injection system, such as, e.g., a "gene gun." Such systems typically accelerate drug or drug particles to supersonic velocities and "shoot" a narrow stream of drug through the stratum corneum. In some embodiments, the stratum corneum and epidermis is previously removed using a method of integumental perturbation (or by integumental perturbation as a result of wounding) described herein, and thus the required delivery pressures and velocities can be reduced. This reduction reduces the required complexity of the firing mechanisms. In some embodiments, a narrow firing stream is used, particularly to accomplish systemic delivery. In other embodiments, the particle injection system administers the lithium compound over a broad area of skin. An exemplary particle delivery device compatible with broad-based skin delivery (in some embodiments, for use in conjunction with integumental perturbation, wherein the surface of skin to which drug is administered corresponds to the perturbed area) includes a low pressure / low velocity firing mechanism with a spray nozzle designed to deliver to a broad area. For example, a single-shot device that delivers to a 25-cm² area could be fired or used multiple times on the scalp or other skin surface until the entire area is treated.

[00290] In another embodiment, a dry particle spraying mechanism similar to an airbrush or miniature grit-blaster can be used to "paint" drug or drug particles onto the infected perturbed, wounded, or scarred area. In some embodiments, the stratum corneum and epidermis are already removed, e.g., by a method of integumental perturbation (e.g., wounding) described herein, and thus permits effective use of the mechanism using lowered pressure and velocity requirements to achieve dermal delivery.

[00291] In another embodiment, the lithium compound (and/or additional drug) is present in an aqueous suspension, permitting use of standard aerosol spray can technology to deliver the lithium compound to the desired skin area.

[00292] Specific embodiments of modes of administration using a device that combines integumental perturbation and lithium compound delivery follow. An advantage of using such a device is that it offers a convenient one step process for administration of the lithium compound.

[00293] In one embodiment, dermabrasion (e.g., using a mechanical device, including microdermabrasion devices that can be used to dermabrade, or alumina-, silica- or ice-based dermabrasion (as described by Webber, U.S. 6,764,493; U.S. 6,726,693; and U.S. 6,306,119) is customized to include a drug particle delivery feature using methods readily known in the art. As the device fires ablation particles at the skin, it could also fire smaller drug particles
that would simultaneously embed in the exposed dermis. Alternatively, via an internal valve control, the device could switch over to firing drug particles once it is determined that adequate skin disruption has occurred. See, International Patent Application Publication No. WO 2009/061349, which is incorporated herein by reference in its entirety.

[00294] In another embodiment, a standard dermabrasion device can be modified to incorporate any of the devices described above, e.g., a spraying/painting device. In one embodiment, a spray nozzle is located behind the dermabrasion wheel such that drug is sprayed into the dermis as it is exposed by the wheel. Alternatively, the dermabrasion device, via internal controls, could turn off the abrasion wheel once it is determined that adequate skin disruption has occurred, and switch on the drug spray to convert to drug painting mode.

[00295] In one embodiment, a pulsed dye laser (585-595 nm) is combined with drug spraying either before or without skin perturbation, in conjunction with skin perturbation, or following skin perturbation.

[00296] In another embodiment, a non-fractional C0₂ or Erbium-YAG laser is combined with drug spraying either without or before skin disruption, in conjunction with skin disruption, or following skin disruption.

[00297] In another embodiment, the lithium compound (and/or additional drug) can be administered using a two-chamber sprayer device, wherein the lithium compound (and/or additional drug) is dispersed, solubilized, or emulsified in a liquid contained in one of the chambers. The other chamber of the device would contain a liquid that is capable of reacting with the drug-containing liquid in the first chamber, to form a physically crosslinked hydrogel or a covalently linked hydrogel. When co-eluted/or sprayed together, the liquids can react and form a drug-containing bioadhesive hydrogel to deliver the lithium compound (and/or additional drug) to the desired area. In an embodiment, the drug-containing hydrogel will have additional features of supporting cell attachment and proliferation. In another embodiment, the lithium compound (and/or additional drug) will be sprayed as a dry powder that is adherent to the underlying tissue.

[00298] In another embodiment, a fractional non-ablative laser (e.g., an Erbium-YAG laser used at 1540-1550 nm) is combined with drug spraying either before or without skin perturbation, in conjunction with skin perturbation, or following skin perturbation.

[00299] In another embodiment, a fractional ablative laser (e.g., an Erbium-YAG laser used at 2940 nm or a C0₂ laser used at 10,600 nm) is combined with drug spraying either before or without skin perturbation, in conjunction with skin perturbation, or following skin perturbation.
In another embodiment, fractional ablative laser treatment of the skin (e.g., an Erbium-YAG laser used at 2940 nm or a CO₂ laser used at 10,600 nm) is combined with lithium compound delivery. For example, by invoking inkjet technology, a fractional laser could be combined with a precise delivery means such that as the laser forms a hole in the skin, the inkjet-like delivery component could fill that same hole with drug. One of skill in the art would appreciate that adequate integrated hardware and software controls are required such that the laser ablation and drug delivery are properly timed resulting in each newly formed hole being properly filled with drug. In another embodiment, fractional ablative laser treatment of the skin (e.g., an Erbium-YAG laser used at 2940 nm or a CO₂ laser used at 10,600 nm) is combined with lithium compound delivery. For example, by invoking inkjet technology, use of a non-ablative, fractional laser could be combined with a precise delivery means such that as the laser forms a hole in the skin, the inkjet-like delivery component could fill that same hole with drug. One of skill in the art would appreciate that adequate integrated hardware and software controls are required such that the laser treatment and drug delivery are properly timed resulting in each newly formed hole being properly filled with drug.

In some embodiments, topical administration comprises administration of lithium-containing particles. The particles can be delivered to the skin in combination with any of the means above and described elsewhere infra. Additionally, the particles can be designed for intermittent or pulse delivery of lithium. In one embodiment, particles with different release properties are be delivered simultaneously to achieve pulse delivery.

In another embodiment, topical administration comprises administration of a lithium-containing formulation that is delivered through channels that are created by the use of needling or micro-needle technology. The formulation can be, e.g., a liquid, a gel or a dry spray. In another variation, topical administration may be through delivery of a lithium-containing formulation through hollow needles.

In another embodiment, topical administration comprises administration of a lithium-containing formulation that is delivered into the skin by an iontophoretic patch. In one example of this embodiment, a patch can be developed in which the lithium-containing formulation is incorporated.

In another embodiment, the lithium compound (and/or additional drug) will be sprayed as a dry powder that is adherent to the underlying tissue.

In another embodiment, topical administration comprises administration of a lithium-containing formulation that is incorporated into micro-needle shaped biodegradable polymers. In one such embodiment, the biodegradable microneedles penetrate the targeted
skin tissue, and are optionally left in place to deliver the lithium ions in a sustained fashion over time.

5.2.6.2 LITHIUM CARBONATE FOR TOPICAL ADMINISTRATION

[00306] Provided herein are compositions comprising lithium carbonate formulated for topical administration. The lithium carbonate compositions may additionally comprise a pharmaceutically acceptable carrier or excipient, such as described in this section or Section 5.2 supra. The lithium carbonate compositions may also comprise one or more additional ingredients, such as described in Section 5.5. Lithium carbonate compositions provided herein may be formulated as aqueous compositions, emulsions, creams, gels (e.g., a hydrogel), etc. In some embodiments, a lithium carbonate composition described herein is occlusive. In other embodiments, the lithium carbonate composition is non-occlusive. The lithium carbonate compositions described herein may be administered via any topical means of delivery known in the art. In particular embodiments, the lithium carbonate composition is administered as part of an article of manufacture, such as a bandage or other wound dressing, such as described in Section 5.2 supra. In particular embodiments, the lithium carbonate composition is administered using a drug delivery system, such as described in Section 5.1 infra.

[00307] In some embodiments, a topical formulation of lithium comprises 0.10%-0.25%, 0.25%-0.75%, 0.75%-1.5%, 1.5%-3%, 3%-4.5%, or 4.5-6% or more (w/w) lithium carbonate.

[00308] Lithium carbonate is a well characterized active pharmaceutical ingredient (API), with numerous cost effective cGMP sources. Lithium carbonate is approved in the United States as an oral medication for the treatment of bipolar disorder and has an extensively characterized safety profile in humans. Systemic lithium exposure from the topical use of lithium carbonate is anticipated to be significantly lower than the levels from oral dosing.

[00309] Lithium carbonate is highly stable as bulk substance, lacking known degradation products as determined by forced degradation studies under stressed conditions of heat, humidity, acid/base and oxidation. Lithium carbonate products are inert to near and far UV irradiation and lack a photo-reactive chromophore. Thus, in certain embodiments, a lithium carbonate composition described herein will not need to be refrigerated and, optionally, will have a shelf-life of 24 months or longer. The stability of a lithium carbonate composition described herein may be determined using methods known in the art, as summarized, for example, in the following table.
Some challenges to generating lithium formulations using lithium carbonate as the salt form may be that the manufacturing process generates carbon dioxide gas, and dissolved carbonic acid from the carbonate moiety may impair stability of the formulation. In the present invention, these formulation challenges have been overcome, permitting the successful generation of various compositions for topical administration, including hydrogels and creams (such as emulsions), using lithium carbonate, as described in this section and the examples of Section 6.

**Lithium Carbonate Hydrogels**

In a particular embodiment, the lithium carbonate composition formulated for topical administration is in the form of a hydrogel. In certain embodiments, the lithium carbonate hydrogel contains from 0.18% lithium carbonate (which contains 0.34 mg Li+/g gel) to 5.66% lithium carbonate (10.64 mg Li+/g gel). However, the invention is not to be so limited, and other variations with higher percentages of lithium carbonate can be prepared.

In a particular embodiment, a lithium carbonate hydrogel has the following composition (referred to herein as "lithium carbonate hydrogel Formulation A"): lithium carbonate at a chosen strength (0.18%>5.66 % w/w; or 0.34 mg Li+/g -10.64 mg Li+/g ), glycerol (10.4%), carboxymethyl cellulose (2% w/w), allantoin (0.16%), sodium alginate (0.12%), methyl paraben (0.1%), propyl paraben (0.048%), water (Q.S.), and sodium hydroxide (pH adjusted to 6.5-7.5). In one embodiment, lithium carbonate hydrogel Formulation A contains 0.18% w/w lithium carbonate (0.34 mg Li+/g gel). In another embodiment, lithium carbonate hydrogel Formulation A contains 1.46% w/w lithium carbonate (2.74 mg Li+/g gel). In another embodiment, lithium carbonate hydrogel Formulation A contains 2.91% w/w lithium carbonate (5.48 mg Li+/g gel). In another embodiment, lithium carbonate hydrogel Formulation A contains 5.66% w/w lithium carbonate (10.64 mg Li+/g gel). Methods for formulating lithium carbonate hydrogel Formulation A at different Li+ strengths are described in detail in Example 10 below. These methods may be adapted to generate other lithium carbonate hydrogel formulations using methods known in the art and described herein.
In certain embodiments, a lithium carbonate hydrogel contains approximately 75%, 80%, 85%, 90%, or 95% water. In a particular embodiment, the lithium carbonate hydrogel contains 90% water. Preferably, the lithium carbonate hydrogel has one or more or all of the following characteristics: is transparent, odorless, colorless, has a viscosity (at 25 °C) of, e.g., 2,000-10,000 cP, 2,000-8,000 cP, or 6,000-10,000 cP (measured using, for example, a rheometer), has assay and dose uniformity (which can be measured by, e.g., flame photometry or atomic adsorption spectrometry (AAS)), has an emollient "smooth-feel" texture, could be easily applied to skin, readily spreads over a surface, has minimal migration to surrounding sites, has minimal run off, has a neutral pH (e.g., pH 6.5-7.5), is sterile, is stable for an extended period (e.g., 1 week or more, 2 weeks or more, 4 weeks or more, 8 weeks or more, 12 weeks or more, 4 months or more, 6 months or more, 1 year or more, or 2 years or more) at one or more temperature conditions (e.g., 4 °C, 25 °C and 40 °C) with respect to, for example, strength, viscosity, and homogeneity. In one embodiment, the hydrogel is stable at room temperature for up to 4 weeks or more. In one embodiment, the hydrogel is stable at room temperature for up to 8 weeks or more. In one embodiment, the hydrogel is stable at 4 °C for up to 6 months or more. In one embodiment, the hydrogel is stable at 4 °C for up to 1 year or more. In certain embodiments, a lithium carbonate hydrogel is prepared with the excipients and an amount of active ingredient chosen to contribute to one or more of the foregoing or following attributes, which may be desirable for a topical formulation for use in the methods described herein: viscosity (e.g., imparted by carboxymethyl cellulose), surface wetting ability and prevention of "dry-out" (e.g., imparted by glycerol), preservative effectiveness (e.g., imparted by parabens, such as methyl or propyl parabens, although in certain embodiments, a paraben-free formulation may also be generated), maintenance of pH, stability (e.g., imparted by altering the strength of surfactants used in the hydrogel) and pharmacokinetic properties (such as rate of Li+ release from the formulation, and peak and trough concentrations in skin and blood). In embodiments where the formulation is for administration to skin that is wounded or that may be wounded, excipients that are wound compatible, contribute to sterility, wound healing, and/or aid in cell attachment and/or proliferation may be included, such as, e.g., allantoin or sodium alginate.

In some embodiments, the lithium carbonate hydrogel is formulated so that it releases Li+ at varying rates. Release rate of Li+ may be modified by one or more of the following: incorporating the formulation into different scaffolds, such as described in Section 5.2 supra, modifying the concentration of lithium carbonate in the formulation, or modifying the types and concentrations of excipients. In some embodiments, most or all of the Li+ is
released from the formulation within 2 hours, within 4 hours, within 8 hours, within 10 hours, within 12 hours, within 16 hours, within 24 hours, within 36 hours, within 48 hours, within 3 days, within 5 days, within 7 days, within 10 days, within 14 days, within 30 days, or within 2 months or more. In a specific embodiment, most or all of the Li+ is released from a lithium carbonate hydrogel described herein within 12 hours. In one embodiment, all of the Li+ is released from the hydrogel within 12 hours. In another embodiment, most or all of the Li+ is released from a lithium carbonate hydrogel described herein within 24 hours. In one embodiment, the formulation is an "immediate release" formulation, i.e., releases 90-100% of Li+ within the first day of administration. In another embodiment, the formulation is an "Intermediate Release" formulation, i.e., releases 90-100% of Li+ within 1 to 3 days of administration. In another embodiment, the formulation is a "Sustained Release" formulation, i.e., releases 90-100% of Li+ within 3 to 7 days of administration.

Lithium Carbonate Creams

In another particular embodiment, the lithium carbonate composition formulated for topical administration is in the form of a cream. In one embodiment, the lithium carbonate cream is an oil/water emulsion. In certain embodiments, the lithium carbonate cream contains from 0.18% lithium carbonate (which contains 0.34 mg Li+/g) to 5.66% lithium carbonate (10.64 mg Li+/g). In a specific embodiment, the cream contains at least 3.38 mg/g Li+, equivalent to 1.80% w/w lithium carbonate. However, the invention is not to be so limited, and other variations with lower or higher percentages of lithium carbonate can be prepared.

In certain embodiments, a lithium carbonate cream contains approximately 75%, 80%, 85%, 90%, or 95% water. In certain embodiments, the lithium carbonate cream (e.g., dispersion, suspension, colloid or emulsion) has one or more or all of the following characteristics: is odorless, colorless upon application to the skin, has a viscosity (at 25 °C) of, e.g., 2,000-10,000 cP, 2,000-8,000 cP, or 6,000-10,000 cP (measured using, for example, a rheometer), has assay and dose uniformity (which can be measured by, e.g., flame photometry or atomic adsorption spectrometry (AAS)), has an emollient "smooth-feel" texture, could be easily applied to skin, readily spreads over a surface, has minimal migration to surrounding sites, has minimal run off, has a neutral pH (e.g., pH 6.5-7.5), is sterile, is stable for an extended period (e.g., 1 week or more, 2 weeks or more, 4 weeks or more, 8 weeks or more, 12 weeks or more, 4 months or more, 6 months or more, 1 year or more, or 2 years or more) at one or more temperature conditions (e.g., 4 °C, 25 °C and 40 °C) with respect to, for example, strength, viscosity, and homogeneity. In one embodiment, the cream
is stable at room temperature for up to 4 weeks or more. In one embodiment, the cream is stable at room temperature for up to 8 weeks or more. In one embodiment, the cream is stable at 4 °C for up to 6 months or more. In one embodiment, the cream is stable at 4 °C for up to 1 year or more. In certain embodiments, a lithium carbonate cream is prepared with the excipients and an amount of active ingredient chosen to contribute to one or more of the foregoings or following attributes, which may be desirable for a topical formulation for use in the methods described herein: viscosity, surface wetting ability and prevention of "dry-out," preservative effectiveness, maintenance of pH, stability (e.g., imparted by altering the strength of surfactants used in the cream), and pharmacokinetic properties (such as rate of Li+ release from the formulation, and peak and trough concentrations in skin and blood). In embodiments where the formulation is for administration to skin that is wounded or that may be wounded, excipients that are wound compatible, contribute to wound healing, and/or aid in cell attachment and/or proliferation may be included, such as, e.g., allantoin or sodium alginate.

[00317] The rate of Li+ release from the cream may be modified by one or more of the following: incorporating the formulation into different scaffolds, such as described in Section 5.2 supra, modifying the concentration of lithium carbonate in the formulation, or modifying the types and concentrations of excipients. For example, in one embodiment, the rate of Li+ release from the cream may be decreased by decreasing the concentration of hydrophilic polymers in the cream. In some embodiments, the rate of Li+ release from the cream may be altered by varying the concentration of cetearyl alcohol, lanolin alcohol, or by varying the types of aqueous or non-aqueous carrier(s), and preferably non-aqueous carrier(s) (e.g., silicone, mineral oil, petrolatum, etc.), used.

[00318] In some embodiments, most or all of the Li+ is released from the formulation within 2 hours, within 4 hours, within 8 hours, within 10 hours, within 12 hours, within 16 hours, within 24 hours, within 36 hours, within 48 hours, within 3 days, within 5 days, within 7 days, within 10 days, within 14 days, within 30 days, or within 2 months or more. In a specific embodiment, most or all of the Li+ is released from a lithium carbonate cream described herein within 10 hours. In one embodiment, all of the Li+ is released from the cream within 10 hours. In another embodiment, most or all of the Li+ is released from a lithium carbonate cream described herein within 24 hours. In one embodiment, the formulation is an "immediate release" formulation, i.e., releases 90-100% of Li+ within the first day of administration. In another embodiment, the formulation is an "Intermediate Release" formulation, i.e., releases 90-100% of Li+ within 1 to 3 days of administration. In
another embodiment, the formulation is a "Sustained Release" formulation, *i.e.*, releases 90-100% of Li+ within 3 to 7 days of administration.

[00319] In a specific embodiment, the lithium carbonate cream is an immediate release formulation. Such a formulation may be generated using a two-phase system: (i) an aqueous phase for dissolving lithium carbonate and hydrophilic excipients and (ii) a non-aqueous phase for dissolving hydrophobic polymers. In an exemplary embodiment, the cream is a water-in-oil emulsion, which acts not only as a biocompatible skin emollient, but also as a delivery system for Li+.

[00320] In an exemplary embodiment, an immediate release lithium carbonate cream is generated as follows, as described in greater detail in Section 6.12.1 *infra*:
PHASE I: 24 G total

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Carbonate</td>
<td>3</td>
<td>0.72</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>5</td>
<td>1.2</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>3</td>
<td>0.72</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.2</td>
<td>0.048</td>
</tr>
<tr>
<td>Deionized water</td>
<td>88.8</td>
<td>21.312</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>24 G</td>
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</table>

PHASE II: 16 G total

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<tr>
<th>Ingredient</th>
<th>%</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetearyl Alcohol</td>
<td>10</td>
<td>1.6</td>
</tr>
<tr>
<td>S25 (Mixture of Silicon 350 CST, Silicon 12,500 CST, 25:75)</td>
<td>67.8</td>
<td>10.848</td>
</tr>
<tr>
<td>Span 80</td>
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<td>0.032</td>
</tr>
<tr>
<td>Lanolin Alcohol</td>
<td>20</td>
<td>3.2</td>
</tr>
<tr>
<td>Emulsifier 10</td>
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<td>0.32</td>
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<td>Total</td>
<td></td>
<td>16 G</td>
</tr>
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</table>

FINAL PHASE: 40.1 G total

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<tr>
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<tbody>
<tr>
<td>Lithium Carbonate</td>
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<td>0.72</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>3.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>1.8</td>
<td>0.72</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.13</td>
<td>0.05</td>
</tr>
<tr>
<td>Deionized water</td>
<td>53.27</td>
<td>21.37</td>
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<tr>
<td>Cetearyl Alcohol</td>
<td>4.03</td>
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</tr>
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<td>S25 (Mixture of Silicon 350 CST, Silicon 12,500 CST, 25:75)</td>
<td>27.02</td>
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<td>Span 80</td>
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<tr>
<td>Lanolin Alcohol</td>
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<tr>
<td>Emulsifier 10</td>
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<td>Total</td>
<td>100</td>
<td>40.11</td>
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</table>

[00321] This method may be adapted to generate other lithium carbonate cream immediate release formulations using methods known in the art and described herein.

[00322] In another embodiment, the lithium carbonate cream is an intermediate release formulation. In one embodiment, the intermediate release cream formulation is an emulsion prepared by homogenization of two phases, as described, e.g., for the immediate release cream formulation above. In an exemplary embodiment, an intermediate release lithium carbonate cream is generated as follows, as described in greater detail in Section 6.12.2 infra:
<table>
<thead>
<tr>
<th>Step 1</th>
<th>Phase 1</th>
<th>Total wt for 24g</th>
<th>Actual amount</th>
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<tr>
<td></td>
<td>%</td>
<td>mg/g</td>
<td>(g)</td>
</tr>
<tr>
<td>Lithium Carbonate</td>
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<td>30</td>
<td>0.72</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>5</td>
<td>50</td>
<td>1.2</td>
</tr>
<tr>
<td>Carbopol 980</td>
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<td>30</td>
<td>0.72</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.2</td>
<td>2</td>
<td>0.048</td>
</tr>
<tr>
<td>10% NaOH use to neutralize to 6.5</td>
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<td></td>
<td>0</td>
</tr>
<tr>
<td>Deionized Water</td>
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<td>888</td>
<td>21.312</td>
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</table>

<table>
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<tr>
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<tbody>
<tr>
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<tr>
<td>Cetearyl alcohol</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>Drakeol 350 Mineral Oil</td>
<td>61.8</td>
<td>618</td>
</tr>
<tr>
<td>Span 80</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>Lanolin alcohol</td>
<td>16</td>
<td>160</td>
</tr>
<tr>
<td>Emulsifier 10</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 3</th>
<th>Mix &amp; Homogenize</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<tr>
<td>Final Composition</td>
<td>%</td>
</tr>
<tr>
<td>Lithium Carbonate</td>
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<tr>
<td>Citric Acid</td>
<td>3</td>
</tr>
<tr>
<td>Carbopol 980</td>
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</tr>
<tr>
<td>Tween 20</td>
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</tr>
<tr>
<td>Deionized Water</td>
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<tr>
<td>Cetearyl alcohol</td>
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<tr>
<td>D350 Mineral Oil</td>
<td>24.72</td>
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<tr>
<td>Span 80</td>
<td>0.08</td>
</tr>
<tr>
<td>Lanolin alcohol</td>
<td>6.4</td>
</tr>
</tbody>
</table>
[00323] In another embodiment, the lithium carbonate cream is a sustained release formulation. In one embodiment, the sustained release cream formulation is prepared by homogenization of two phases (an aqueous phase and a non-aqueous phase), as described, *e.g.*, for the immediate and intermediate release cream formulations above, but by decreasing the concentration of hydrophilic polymers in the non-aqueous phase. In an exemplary embodiment, a sustained release lithium carbonate cream is generated as follows, as described in greater detail in Section 6.12.3 *infra*:

<table>
<thead>
<tr>
<th>Emulsifier</th>
<th>10</th>
<th>0.8</th>
<th>8</th>
<th>0.32</th>
<th>0.82</th>
</tr>
</thead>
<tbody>
<tr>
<td>total</td>
<td></td>
<td>40.10</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Step 1 | Phase 1 | | Total wt for 24G | Actual amount |
|--------|---------| | (g) | (g) |
|        | % | mg/g |
| Lithium Carbonate | 3 | 30 | 0.72 | 0.72 |
| Citric Acid | 5 | 50 | 1.2 | 1.20 |
| Carbopol 980 | 3 | 30 | 0.72 | 0.72 |
| Tween 20 | 0.2 | 2 | 0.048 | 0.05 |
| Deionized Water | 88.8 | 888 | 21.312 | 21.32 |

| Step 2 | Phase II | | |
|--------|---------| | 16 g |
|        | % | mg/g |
| Lecithin | 10 | 100 | 1.6 | 1.60 |
| D350 Mineral Oil:Silicone 350 | 67.8 | 678 | 10.848 | 10.92 |
| Span 80 | 0.2 | 2 | 0.032 | 0.04 |
| Lanolin alcohol | 20 | 200 | 3.2 | 3.28 |

<table>
<thead>
<tr>
<th>Step 3</th>
<th>Mix &amp; Homogenize</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Final Composition</td>
<td>%</td>
<td>mg/g</td>
</tr>
<tr>
<td>Lithium Carbonate</td>
<td>1.8</td>
<td>18</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>1.8</td>
<td>18</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.12</td>
<td>1.2</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>53.28</td>
<td>532.8</td>
</tr>
<tr>
<td>Lecithin</td>
<td>4</td>
<td>40</td>
</tr>
</tbody>
</table>
The foregoing lithium carbonate formulations for topical administration may be administered in accordance with any embodiments described herein. For example, in specific embodiments, a 50 kg patient is administered a single droplet of a lithium carbonate hydrogel described herein - approximately 0.1 ml of 0.10%–0.25%, 0.25%–0.75%, 0.75%–1.5%, 1.5%–3%, 3%–4.5%, or 4.5–6% (w/w) lithium carbonate - at 3 sites, twice daily. In some embodiments, the lithium carbonate hydrogel is administered once daily. In some embodiments, the lithium carbonate hydrogel is administered twice daily. In some embodiments of a twice daily treatment regimen, doses are administered 6 hours apart, or 7 hours apart, or 8 hours apart, or 9 hours apart, or 10 hours apart, or 11 hours apart, or 12 hours apart. In a particular embodiment, the doses are administered 7 to 8 hours apart.

In other embodiments, a 50 kg patient is administered a single droplet of a lithium carbonate cream described herein - approximately 0.1 ml of 0.10%–0.25%, 0.25%–0.75%, 0.75%–1.5%, 1.5%–3%, 3%–4.5%, or 4.5–6% (w/w) lithium carbonate - at 3 sites, twice daily. In some embodiments, the lithium carbonate cream is administered once daily. In some embodiments, the lithium carbonate cream is administered twice daily. In some embodiments of a twice daily treatment regimen, doses are administered 6 hours apart, or 7 hours apart, or 8 hours apart, or 9 hours apart, or 10 hours apart, or 11 hours apart, or 12 hours apart. In a particular embodiment, the doses are administered 7 to 8 hours apart.

### 5.2.6.3 PARENTERAL ADMINISTRATION

Administration can be parenterally by injection, infusion, or implantation, for local or systemic administration. Parenteral administration, as used herein, includes intravenous, intra-arterial, intraperitoneal, intrathecal, intraventricular, intraurethral, intrasternal, intracranial, intramuscular, intrasynovial, intravesical, and subcutaneous administration. Compositions for parenteral administration can be formulated in any dosage forms that are suitable for parenteral administration, including solutions, suspensions, emulsions, micelles, liposomes, microspheres, nanosystems, and solid forms suitable for solutions or suspensions in liquid prior to injection. Such dosage forms can be prepared according to conventional methods known to those skilled in the art of pharmaceutical
science (see, Remington: The Science and Practice of Pharmacy, supra). Compositions intended for parenteral administration can include one or more pharmaceutically acceptable carriers and excipients, including, but not limited to, aqueous vehicles, water-miscible vehicles, non-aqueous vehicles, anti-microbial agents or preservatives against the growth of microorganisms, stabilizers, solubility enhancers, isotonic agents, buffering agents, antioxidants, local anesthetics, suspending and dispersing agents, wetting or emulsifying agents, complexing agents, sequestering or chelating agents, cryoprotectants, lyoprotectants, thickening agents, pH adjusting agents, and inert gases. All such compositions must be sterile, as known in the art. The compositions for parenteral administration can be formulated as a suspension, solid, semi-solid, or thixotropic liquid, for administration as an implanted depot. In one embodiment, the compositions are dispersed in a solid inner matrix, which is surrounded by an outer polymeric membrane that is insoluble in body fluids but allows the active ingredient in the pharmaceutical compositions diffuse through. Suitable inner matrixes include, but are not limited to, polymethylmethacrylate, polybutyl-methacrylate, plasticized or unplasticized polyvinylchloride, plasticized nylon, plasticized polyethylene terephthalate, natural rubber, polyisoprene, polyisobutylene, polybutadiene, polyethylene, ethylene-vinyl acetate copolymers, silicone rubbers, polydimethylsiloxanes, silicone carbonate copolymers, hydrophilic polymers, such as hydrogels of esters of acrylic and methacrylic acid, collagen, cross-linked polyvinyl alcohol, and cross-linked partially hydrolyzed polyvinyl acetate. Suitable outer polymeric membranes include but are not limited to, polyethylene, polypropylene, ethylene/propylene copolymers, ethylene/ethyl acrylate copolymers, ethylene/vinyl acetate copolymers, silicone rubbers, polydimethyl siloxanes, neoprene rubber, chlorinated polyethylene, polyvinylchloride, vinyl chloride copolymers with vinyl acetate, vinylidene chloride, ethylene and propylene, ionomer polyethylene terephthalate, butyl rubber epichlorohydrin rubbers, ethylene/vinyl alcohol copolymer, ethylene/vinyl acetate/vinyl alcohol terpolymer, and ethylene/vinylxyethanol copolymer.

5.2.6.4 ORAL ADMINISTRATION

Suitable oral dosage forms include, but are not limited to, tablets, fastmelts, chewable tablets, capsules, pills, strips, troches, lozenges, pastilles, cachets, pellets, medicated chewing gum, bulk powders, effervescant or non-effervescent powders or...
granules, oral mists, solutions, emulsions, suspensions, wafers, sprinkles, elixirs, and syrups. In addition to the active ingredient(s), the pharmaceutical compositions can contain one or more pharmaceutically acceptable carriers or excipients, including, but not limited to, binders, fillers, diluents, disintegrants, wetting agents, lubricants, glidants, coloring agents, dye-migration inhibitors, sweetening agents, flavoring agents, emulsifying agents, suspending and dispersing agents, preservatives, solvents, non-aqueous liquids, organic acids, and sources of carbon dioxide. Compositions for oral administration can be also provided in the forms of liposomes, micelles, microspheres, or nanosystems. Micellar dosage forms can be prepared as described in U.S. Pat. No. 6,350,458.

[00328] In particular embodiments, oral formulations approved for treating mood disorders, e.g., lithium carbonate (Li$_2$CO$_3$), sold under several trade names, lithium citrate (Li$_3$C$_6$H$_5$O$_7$), lithium sulfate (Li$_2$SO$_4$), lithium aspartate, or lithium orotate, may be administered in accordance with the methods described herein.

### 5.2.6.5 EX VIVO DELIVERY

[00329] The lithium treatments described herein may also be administered to skin-derived cells or skin tissue ex vivo. For example, a lithium treatment may be used to enhance the re-association of dissociated hair follicle cells into follicles and their growth and expansion in culture for their implantation into fresh wounds and scar revisions. Thus, in some embodiments, hair follicles promoted by lithium treatments are added to the wound before, at the time of, and/or subsequent to, either acute wounding or, more typically, during the wounding that is induced in scar revision. With these methods, traditional approaches to scar revision, such as human skin transplantation, can be efficiently replaced with transplantation of follicular units or other smaller appendage structures from skin. Thus, hair follicles can be introduced to the wound by migration or de novo hair follicle neogenesis, or by transplanting one or more of the following skin elements: full skin (xeno-; autologous human), follicular units, dissociated cells (donor dominance; recipient effects), ex vivo-expanded skin and/or follicular units, or human skin equivalents in vivo (universal donors). Engineered human skin, or human skin equivalents, can also be used for hair follicle neogenesis and scar revision platforms.

[00330] Human skin equivalents can be grown and assembled in vitro, with the advantage that they can be grown to theoretically to any size/shape; can be comprised of different types of cells, including keratinocytes (hair follicle derived and non-hair follicle derived), dermal cells (hair follicle derived and non-hair follicle derived), other cell types (e.g., mesenchymal
stem cells); can contain cells that are genetically modified to include, e.g., markers or "inducible" signaling molecules; provide an unlimited and uniform source of human cells; from normal skin based on histology and marker studies; are generally devoid of skin appendages; and can be wounded and show similar wound healing events as in vivo.

5.3 **DRUG SPRAYING DEVICE**

[00331] An example of a device that can be used to deliver the therapeutic compound to the skin site is depicted in Figures 37-41. The device or drug sprayer 2 includes a control unit or generator 4, a foot switch 6, a hand piece 8, and a power module 10.

[00332] The control unit 4 is the interface between the foot switch 6, the hand piece 8, and the power module 10. It serves as the central point of connectivity and provides a user with a means to power the system on or off, load/eject a drug cartridge into/from the hand piece 8, and select the drug delivery speed. To control all of this functionality, the control unit 4 includes at least one circuit board that controls operation of the hand piece 8 via embedded software.

[00333] As can be seen in Figure 38, the control unit 4 comprises a housing 12 that includes a hand piece connection port 14, a load/eject button 16, a means 18 to control the drug delivery or spray speed, a means 20 to display the drug delivery speed, an on/off switch 22, and a handle 24. In the embodiment depicted in Figure 38, the means 18 to control the drug delivery speed includes a pair of up/down buttons and the means 20 to display the drug delivery speed comprises 8 discreet LEDs (light-emitting diodes) that light up to indicate the drug delivery speed. Examples of materials that can be used for the load/eject button 16, the up/down buttons 18, and the on/off switch 22 include, but are not limited to, elastomeric materials such as silicon rubber, plastics, and metals. The housing 12 can be made from an injection molded thermoplastic material such as, for example, acrylonitrile butadiene styrene.

[00334] Figures 39A and 39B depict an embodiment of the drug sprayer's hand piece 8. The hand piece 8 comprises a housing 26, a drive motor 28, a universal joint 30 and at least one plunger 32. Attached to the end of the hand piece 8 is a drug cartridge 34 that can either be disposable or reusable. The hand piece's housing 26 can be made from an injection molded thermoplastic material such as, for example, acrylonitrile butadiene styrene. It will be readily apparent to those skilled in the art that other materials may be used to construct the hand piece's housing 26.

[00335] Some therapeutic compounds may quickly become unstable after their components are mixed or some may have a short shelf life unless they are refrigerated. Thus,
in order to keep these compounds stable and increase shelf life, the components of the compounds are isolated from each other until the compounds are ready to be administered when they are mixed together forming, for example, a gel, controlled release, drug delivery matrix. Prior devices, such as those described in U.S. Patent No. 4,381,778, U.S. Patent No. 4,689,042, U.S. Patent No. 5,122,117, and U.S. Patent No. 5,423,752, the entirety of each are expressly incorporated herein by reference thereto, have been developed to store drug components separately and then mix the components prior to being dispensed.

[00336] Figures 40 and 41 depict embodiments of a drug cartridge having two separate chambers that keep the drug components isolated until the therapeutic compound is to be dispensed. Figures 40A-40D depict a drug cartridge 40 that contains two liquid components and its associated hand piece 42. The drug cartridge 40 includes a housing 44 having a front end 46, a back end 48, a nozzle 50, a static mixer 52 having a mixing chamber 54 and two piercing elements 56 that extend from the back end 48 thereof and which are in fluid communication with the mixing chamber 54, two liquid component chambers 58, 60, a first liquid component 62 stored in the first component chamber 58, a second liquid component 64 stored in the second component chamber 60, and a piston 66 inserted into the back end 48 of each component chamber 58, 60 to rearwardly confine each liquid component 62, 64 within its respective component chamber 58, 60. The pistons 66 form an airtight seal with the interior walls of their respective component chambers 58, 60. To seal off the front end of each chamber 58, 60, a pierceable seal 68 is included. Thus, when the drug cartridge 40 is attached to the hand piece 42, the piercing elements 56 penetrate the pierceable seals 68 of the first and second component chambers 58, 60, thereby forming a fluid connection between the static mixer 52 and the first and second component chambers 58, 60. In order to promote mixing of the two liquid components 62, 64, the mixing chamber 54 includes mixing elements 70 therein. For example, these mixing elements 70 can be pathways or channels formed in the interior walls of the mixing chamber 54 or can be mixing vanes that cause the liquid components 62, 64 to swirl as they travel through the mixing chamber 54 resulting in turbulent fluid flow, thereby mixing the liquid components 62, 64 together.

[00337] To use the two liquid component drug cartridge 40 with the drug sprayer 2, as can be seen in Figures 40B-40D, a user inserts the drug cartridge 40 into the front end 72 of the hand piece 42. When inserted, detents 74 on the drug cartridge 40 engage detents 76 on the hand piece 42 and lock the drug cartridge 40 and the hand piece 42 together. As shown in Figures 40C and 40D, when the drug cartridge 40 is fully inserted into the hand piece 42, the connecting portions 78 of each plunger 80 engage a corresponding cavity 82 in the pistons.
66, forming a press-fit connection between the two. Thus, when the plungers 80 move, the pistons 66 move in a corresponding manner.

[00338] When a user desires to dispense the therapeutic compound, the user activates the hand piece 42 via the control unit 4. Activation of the hand piece 42 in turn energizes the drive motor 28, which acts through a universal joint (see Figures 39A and 39B) to move or drive the plungers 80 towards the front end 46 of the drug cartridge 40. Thus, as can be seen in Figure 40D, as the plungers 80 move in the direction indicated by arrow 83, the pistons 66 move into the component chambers 58, 60 in a corresponding manner, forcing each separate liquid component 62, 64 through the piercing elements 56 and into the mixing chamber 54. As the liquid components 62, 64 travel through the mixing chamber 54, the turbulent flow created therein causes the liquid components 62, 64 to mix with each other. The newly mixed components then exit the nozzle 50 as the mixed therapeutic compound 81.

[00339] In another embodiment, the drug sprayer 2 can be used with a drug cartridge 84 that contains a liquid component 86 and a solid component 88. As depicted in Figures 41A-41E, the drug cartridge 84 includes a housing 90 having a front end 92, a back end 94, a lower chamber 96, a nozzle 98, a liquid component chamber 100 that houses the liquid component 86, a solid component chamber 102 that houses the solid component 88, a first piston 104 inserted into the back end of the liquid component chamber 100 to rearwardly confine the liquid component 86 therein, and a second piston 106 inserted into the back end of the solid component chamber 102 to rearwardly confine the solid component 88 therein. The first and second pistons 104, 106 form an airtight seal with the interior walls of the liquid and solid component chambers 100, 102.

[00340] The front end 92 of the liquid component chamber 100 includes a first one-way or check valve 108 that confines the liquid component 86. The front end 92 of the solid component chamber 102 does not include a check valve. Instead, a second check valve 110 is included at the back end of the of the lower chamber 96. An example of such a one-way valve that can be used with the present drug cartridge 84 is a duck bill valve. This configuration of the first and second check valves 108, 110 allows the liquid component 86 and the solid component 88 to be stored separate from each other and also closes the front ends of the liquid and solid component chambers 100, 102 forming a sealed volume of air between the first and second pistons 104, 106 and the first and second check valves 108, 110.

[00341] To use the drug cartridge 84 containing a liquid component 86 and a solid component 88 with the drug sprayer 2, as can be seen in Figures 41A-41E, a user inserts the drug cartridge 84 into the front end 112 of the hand piece 114. When inserted, detents 116 on
the drug cartridge 84 engage detents 118 on the hand piece 114 and thereby lock the drug cartridge 84 and the hand piece 114 together. In contrast to the liquid-liquid drug cartridge 40 discussed above, the hand piece 114 for use with the liquid-solid drug cartridge 84 includes a single plunger 120, which is inserted into the solid component chamber 102. As can be seen in Figures 41B-41E, when the drug cartridge 84 is fully inserted into the hand piece 114, the connecting portion 122 of the plunger 120, engages a corresponding cavity 124 in the second piston 106 forming a press-fit connection between the two. Thus, when the plunger 120 moves, the second piston 106 moves in a corresponding manner.

When a user desires to dispense the therapeutic compound, the user activates the hand piece 114 via the control unit 4. Activation of the hand piece 114 in turn energizes the drive motor 28, which acts through a universal joint 30 (see Figures 39A and 39B) to move the plunger 120. Initially, the plunger 120 is retracted in the direction shown by arrow 126 in Figure 41D causing the second piston 106 to move in a corresponding manner. Because of the air tight seals created by the first and second pistons 104, 106 and the first and second check valves 108, 110, as can be seen in Figure 41D, as the second piston 106 retracts, negative pressure or suction is created in the solid component chamber 102. This negative pressure or suction causes the first piston 104 to move in the direction of arrow 128 forcing the liquid component 86 through the first check valve 108 and into the liquid component chamber 102. As the liquid component 86 enters the solid component chamber 102, turbulent fluid flow is created, which operates to mix the liquid and solid components 86, 88 together. The negative pressure within the closed system acts to keep the second check valve 110 closed.

After the liquid and solid components 86, 88 are mixed together in the solid component chamber 102, a user can dispense the mixed drug by reversing the direction of travel of the plunger 120 as shown by arrow 130 in Figure 41E. This forces the mixed therapeutic compound 132 through the second check valve 110, into the lower chamber 96, and out through the nozzle 98. Pressure acting on the front end of the first check valve 108, forces the first check valve 108 to remain closed during the dispensing operation.

It will be readily apparent to those skilled in the art that the amount of the liquid component 86 that is added to the solid component 88 and, hence, the concentration of the mixed drug, can be controlled by adjusting the distance that the plunger 120 is retracted before reversing the direction of travel of the plunger 120 and dispensing the mixed drug. Therefore, for example, the further back that the plunger 120 is retracted, the more liquid
component 86 enters into the solid component chamber 102 and thus, the more dilute the therapeutic compound becomes.

[00345] In certain embodiments, the first liquid component 62 is a solution comprising a lithium salt and the second liquid component 64 is a polymeric solution that comprises a water-soluble polymer that is a solution at room temperature (20-25°C) and below, but gels at physiological temperatures of 32-37°C. The lithium concentration in the lithium solution can be at least 1.2 times, 1.4 times, 1.6 times, 1.8 times, 2 times, 2.2 times, 2.4 times, 2.6 times, 2.8 times, 3 times, 4 times, or at least 5 times the concentration of the final concentration. The lithium solution can be a water-based solution.

[00346] In certain embodiments, the liquid component 86 is a polymeric solution that comprises a water-soluble polymer that is a solution at room temperature (20-25°C) and below, but gels at physiological temperatures of 32-37°C and the solid component 88 comprises a lithium salt as described in Section 5.1. In a more specific embodiment, the lithium salt is an organic lithium salt such as, e.g., lithium gluconate (see Section 5.1).

[00347] In certain embodiments, either the drug spraying device, the drug cartridge, or both may be manufactured as a disposable. In certain embodiments, the drug spraying device may be altered so that it is battery powered.

[00348] As will be readily apparent to those skilled in the art, the components of the present device can be modified to dispense a therapeutic compound that comprises more than two components that need to be mixed together prior to dispensing.

[00349] In certain embodiments, a device for spraying a therapeutic compound comprises:

(A) a control unit;
(B) a foot piece;
(C) a power module;
(D) a hand piece comprising:
   (i) a housing;
   (ii) a first plunger having a first connecting portion; and
   (iii) a second plunger having a second connecting portion; and
(E) a drug cartridge comprising:
   (i) a housing;
   (ii) a first chamber containing a first liquid component, wherein the first liquid component is rearwardly confined by a first piston having a first cavity formed in an end thereof;
   (iii) a second chamber containing a second liquid component, wherein the second liquid component is rearwardly confined by a second piston having a second cavity formed in an end thereof;
   (iv) a static mixer; and
   (v) a nozzle;
(F) wherein the first connecting portion engages the first cavity such that movement of the first plunger moves the first piston within the first chamber in a corresponding manner, and

(G) wherein the second connecting portion engages the second cavity such that movement of the second plunger moves the second piston within the second chamber in a corresponding manner.

[00350] In certain embodiments, a device for spraying a therapeutic compound comprises:

(A) a control unit;
(B) a foot piece,
(C) a power module;
(D) a hand piece including a housing and a plunger having a connecting portion; and
(E) a drug cartridge comprising:
   (i) a drug cartridge housing having a front end and a back end;
   (ii) a first chamber containing a liquid component, wherein the liquid component is confined at a first end by a first piston and at a second end by a first one-way valve;
   (iii) a second chamber containing a solid component, wherein the solid component is confined at a first end by a second piston having a cavity formed in an end thereof and at a second end by the first one-way valve and a second one-way valve;
   (iv) a bottom chamber; and
   (v) a nozzle;

(F) wherein the connecting portion engages the cavity in the second piston such that movement of the plunger moves the second piston within the second chamber in a corresponding manner, and

(G) wherein movement of the piston away from the front end of the drug cartridge housing creates negative pressure within the second chamber, and

(H) wherein the negative pressure created in the second chamber pulls the liquid component through the first one-way valve into second chamber.

[00351] In certain embodiments, a drug cartridge for use in a device for spraying a therapeutic compound comprises:

(A) a housing having a front end and a back end;
(B) a first chamber containing a liquid component, wherein the liquid component is confined at a first end by a first piston and at a second end by a first one-way valve;
(C) a second chamber containing a solid component, wherein the solid component is confined at a first end by a second piston having a cavity formed in an end thereof and at a second end by the first one-way valve and a second one-way valve;
(D) a bottom chamber; and
(E) a nozzle;

(F) wherein movement of the second piston away from the front end of the housing creates negative pressure within the second chamber, and

(G) wherein the negative pressure created in the second chamber pulls the liquid component through the first one-way valve into second chamber.
DELIVERY OF CROSS-LINKED MICROSPHERES

[00352] In certain embodiments, the drug spraying device disclosed herein enables the sustained release of ionized lithium (Li+), without the use of highly hydrophobic, occlusive matrices. In particular, the drug spraying device enables the delivery of ionized lithium in microspheres (e.g., PLG microspheres) such that the microspheres stay at the wound site for a prolonged period of time and are not cleared rapidly by phagocytosis. A prolonged period of time can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at least 20 days.

[00353] Without being bound by theory, the drug spraying device enables the administration of lithium microspheres to the tissue such that the microspheres are sequestered to the wound surface by an in-situ crosslinking hydrogel that will form molecular bonds with the tissue surface. An in-situ crosslinking hydrogel cannot be "rubbed" off like an ointment or a cream. The microspheres (that contain the lithium salt) will be sequestered in the hydrogel, releasing Li+ in a sustained manner. Thus, the issue of phagocytosis of the microspheres is overcome.

[00354] In certain embodiments, to deliver cross-linked microspheres, the drug spraying device disclosed herein comprises two chambers. The component of the first chamber comprises a polymer macromonomer (Polymer 1) (a polymer that can further crosslink with another component) and microspheres containing a lithium salt. The component of the second chamber comprises another polymer macromonomer (Polymer 2) that is capable of reacting with Polymer 1.

[00355] In certain embodiments, to deliver cross-linked microspheres, the solid component (e.g., solid component 88 described above) comprises a polymer macromonomer (Polymer 1) (a polymer that can further crosslink with another component) and microspheres containing a lithium salt. The liquid component (e.g., liquid component 86 described above) comprises another polymer macromonomer (Polymer 2) that is capable of reacting with Polymer 1. Polymer 2 does not contain hydrolytically labile linkages and is stable in water.

[00356] Illustrative embodiments of the use of the presently disclosed drug spraying device to deliver cross-linked microspheres to a wound site are set forth in Example 14.

DELIVERY OF CROSS-LINKED BIODEGRADABLE SCAFFOLD

[00357] In certain embodiments, the drug spraying device disclosed herein enables the sustained release of ionized lithium (Li+) and uptake by the skin through a scab. In
particular, the drug spraying device enables the delivery of ionized lithium such that the delivery system is incorporated into the scab. This can be accomplished by placing a lithium containing thin, gauze-like, pliable biodegradable scaffold on the fresh wound. The material properties of the scaffold will be adjusted such the gauze is able to absorb the blood and other exudates from the wound. In certain, more specific embodiments, the biodegradable scaffold has high content of void space, to absorb blood, fibrin and fibrinogen. Without being bound by theory, this incorporation of the scaffold into the fibrin clot during its formation, results in its incorporation into the fibrous network, also called a scab, after it solidifies. After placement of the drug-containing biodegradable scaffold into the wound, an in-situ crosslinking hydrogel may be applied on top to cover the entire site as a wound dressing.

[00358] In certain embodiments, the solid component (e.g., solid component 88 described above) comprises a polymer macromonomer (Polymer 1) (a polymer that can further crosslink with another component) and the liquid component (e.g., liquid component 86 described above) comprises another polymer macromonomer (Polymer 2) that is capable of reacting with Polymer 1. Polymer 2 does not contain hydrolytically labile linkages and is stable in water. Mixing of these two components yields a cross-linking hydrogel that is applied to the wound. The cross-linking hydrogel is applied together with a biodegradable scaffold that comprises lithium. The biodegradable scaffold can be in the form a pliable, gauze-like material that is a blend of PLG polymers. Other polymers may be added to the main component (PLG) to impart attributes such as biodegradability, pliability, etc. In a specific embodiment, Lithium Carbonate can be incorporated in the biodegradable scaffold. In certain embodiments, the cross-linking hydrogel is applied to the wound before the scaffold is applied; the cross-linking hydrogel is applied to the wound at the same time when the scaffold is applied; the cross-linking hydrogel is applied to the wound after the scaffold is applied.

[00359] In certain embodiments, the biodegradable scaffold has an "open-cell" structure that would allow cells to attach themselves, differentiate and proliferate. The scaffold can have other components such as RGD peptides, etc. incorporated to encourage cell attachment. The scaffold can have bioadhesive attributes to keep it "in place."

[00360] Illustrative embodiments of the use of the presently disclosed drug spraying device to deliver cross-linked biodegradable scaffold to a wound site are set forth in Example 15.
DELIVERY OF DRUG COMBINATIONS

[00361] In certain embodiments, the drug spraying device disclosed herein enables the concurrent delivery of two or more drugs with different solubility properties and/or physical/chemical incompatibilities (such as different excipient requirements; binding and/or reaction of the two or more drugs with each other).

[00362] In certain embodiments, the first liquid component (e.g., liquid component 62 described above) is a first formulated drug and the second liquid component (e.g., liquid component 64 described above) is a second formulated drug. In certain other embodiments, the presently disclosed drug spray device can be engaged for spraying each drug separately. For example, an alcoholic solution (±drug) can be used to first "prepare" the wound by thorough cleansing, followed by spraying a lithium formulation as disclosed herein. In even other embodiments, both chambers could contain the same drug, but in different forms and formulated differently to achieve different release profiles. For example, the first liquid component (e.g., liquid component 62 described above) could contain micronized lithium carbonate suspended in a FDA-approved liquid excipient and the second liquid component (e.g., liquid component 64 described above) can be a dissolved ionized lithium in an aqueous sprayable gel. Co-spraying both forms of lithium provides instantly-bioavailable, ionized Li+ and a sustained form of Li+ made available as the micronized lithium carbonate dissolves.

[00363] Illustrative embodiments of the use of the presently disclosed drug spraying device to deliver cross-linked biodegradable scaffold to a wound site are set forth in Example 16.

CLEANSING AND DRUG DELIVERY

[00364] In certain embodiments, the drug spraying device disclosed herein enables the cleansing and administration of one or more drugs with one single device. In these embodiments, the contents of each chamber could be sprayed separately. Once chamber can contain the cleansing solution; the liquid in the other chamber contains lithium. Any wound-cleansing solution known to the skilled artisan can be used with these embodiments.

[00365] Illustrative embodiments of the use of the presently disclosed drug spraying device to deliver cross-linked biodegradable scaffold to a wound site are set forth in Example 17.

[00366] It will be evident to the skilled artisan that while the drug delivery devices described above may be preferred for delivery of the cross-linked microspheres, cross-linked...
biodegradable scaffold, drug combinations, and drug delivery with a cleansing solution described above, their delivery - to wounded or unwounded skin - may be accomplished using any method or device described herein or known in the art.

[00367] In an embodiment, a drug spraying device is used to administer a combination treatment comprising a lithium compound described herein and chlorhexidine. In an embodiment, a combination treatment comprising a lithium compound described herein and chlorhexidine is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*.

[00368] In an embodiment, a drug spraying device is used to administer a combination treatment comprising a lithium compound described herein and iodine. In an embodiment, a combination treatment comprising a lithium compound described herein and iodine is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*.

[00369] In an embodiment, a drug spraying device is used to administer a combination treatment comprising a lithium compound described herein and povidone-iodine. In an embodiment, a combination treatment comprising a lithium compound described herein and povidone-iodine is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*.

### 5.4 LITHIUM TREATMENT REGIMENS

[00370] In the embodiments described herein, the lithium compound or formulation thereof can be administered topically, subcutaneously, orally, etc. Regardless of the route of administration used for lithium ion delivery, the dosing regimen should be adjusted to achieve peak concentrations of lithium in the target skin area of at least about 0.1 mM to 10 mM, and/or peak concentrations of lithium in the blood (serum or plasma samples) of at least about 1 mM (these values are sometimes referred to herein as the "target concentration"). It is noted that, with regard to the concentrations of lithium (including its concentration in formulations, in tissue, in serum, etc., and as a salt form, as an ionized atom in solution, etc.) described herein, since ionized lithium is a monovalent cation, the concentration of lithium expressed in millimolar units (mM) is equal to its concentration expressed in milliequivalents (mEq), as is sometimes used in the art (*i.e.*, 1 mM Li+ = 1 mEq Li+). The peak concentration of lithium can be established by taking samples when peak concentrations are achieved and assaying them for lithium content using techniques well known to those skilled in the art (*see, e.g.*, the examples of Sections 6 to 9 and the techniques described therein; *see* also Wood *et*
al., 1986, Neuropharmacology 25:1285-1288; and Smith, 1978, Acta Pharmacol et Toxicol 43:51-54, each of which is incorporated herein by reference in its entirety). For example, when using oral formulations, samples can be taken when peak blood concentrations are typically achieved - for example, within 1 to 2 hours for standard release formulations, and 4-5 hours for sustained release formulations. The peak concentration times for other formulations, including topical preparations, can be determined for the particular formulation used, and sampling can be adjusted accordingly.

[00371] In some embodiments, the target concentration of lithium should be maintained in the skin and/or blood for at least 1 day; at least 2 days; at least 3 days; at least 4 days; at least 5 days; at least 6 days; at least 7 days; at least 10 days; at least 12 days; at least 14 days; at least 16 days; at least 19 days; or at least 21 days; and, in certain embodiments, not more than 21 days. This can be accomplished using, e.g., repeated applications of the lithium compound or a single application of a sustained release or extended release lithium formulation. Either the single pulse protocol or the intermittent treatments can be used to achieve the target concentration of lithium for the shorter maintenance periods (i.e., for at least 1, 2 or 3 days). Maintenance periods longer than 3 days may require repeated application of intermittent lithium treatments or a single pulse protocol. In some embodiments, it is preferable to allow the concentration of lithium to decline between dosages, in order to achieve a pulsatile effect.

[00372] In some embodiments, topical administration of a lithium compound is preferred over oral or subcutaneous administration. Depending on the formulation used, a topically administered lithium compound may achieve a higher concentration of lithium in skin than in the blood, thereby reducing the risk of toxicity associated with elevated blood levels of lithium. Conversely, and depending on the formulation used, a subcutaneously or orally administered lithium compound may be preferred in order to achieve a controlled release of lithium from the blood to the skin.

[00373] Regardless of the route of administration, care should be taken to avoid toxicity. In this regard, lithium doses should be adjusted on the basis of the blood concentration (serum or plasma) drawn (by convention) 12 or 24 hours after the last dose of the lithium compound; this trough blood concentration should be maintained below 2 mM Li+ and preferably, below about 1.5 mM Li+. In some embodiments, the steady state blood concentration of lithium should not exceed a maximum of 1.5 mM to 2 mM. The relatively stable and characteristic pharmacokinetics of the lithium ion in individual patients makes it possible to predict dosage requirements for that individual based on the results of
administration of a single test dose, followed by a skin and/or blood sample assay (plasma or serum) at the peak concentration time; followed by blood sample assays to monitor toxicity at the 12 hour or 24 hour trough concentration; and 24 hours later (when lithium is generally eliminated) which serves as the control value. Once the dose is established for a patient, routine monitoring for toxicity is recommended. For a review of the pharmacokinetics and monitoring of lithium concentrations, see Amdisen, 1980, Ther. Drug. Monit. 2:73-83; Goodman & Gilman, 1980, "The Pharmacological Basis of Therapeutics" at pp. 430-434; Grandjean & Aubry, 2009, CNS Drugs 23:331-349; and the APA Practice Guideline for the Treatment of Patients with Bipolar Disorder, Second Edition, 2002, each of which is incorporated by reference herein in its entirety.

[00374] In some embodiments, a trough concentration of lithium in the skin of no less than 0.01 mM to 0.05 mM is preferred. In some embodiments, a trough concentration of lithium in the skin of 0.05 mM to 0.1 mM is preferred. In some embodiments, a trough concentration of lithium in the skin of less than 1 mM is preferred. In some embodiments, a trough concentration of lithium in the skin of less than 3 mM is preferred. In some embodiments, lithium concentrations at trough can be increased by twice daily dosing, or more frequent dosing. In such embodiments, topical administration of a lithium compound is preferred. In this regard, a pulsatile effect is achieved by the multiple dosing, but the trough concentrations do not decline as much as when once daily dosing is used. In some embodiments, a trough skin concentration of lithium is maintained at 0.25 mM or higher, for example from 0.25 mM to 0.5 mM or 0.5 mM to 0.75 mM. In some embodiments, the trough concentration is maintained at approximately 0.6 mM to 1.4 mM lithium. In some such embodiments, a trough skin concentration is maintained at 1 mM to 3 mM lithium. In some such embodiments, the trough skin concentration is maintained at less than 0.5 mM, or less than 0.75 mM, or less than 1 mM, or less than 2 mM, or less than 3 mM of lithium.

[00375] In some embodiments, a peak concentration of lithium in the skin of no more than 0.1 mM to 10 mM is preferred. In some embodiments, a peak concentration of lithium in the skin of at least about 1 mM is preferred. In some embodiments, an average concentration of lithium in the skin of about 3 to 6 mM is preferred. In some embodiments, an average concentration of lithium in the skin of about 4 to 5 mM is preferred.

[00376] In specific embodiments, an effective amount of a lithium compound is administered such that the target concentration of lithium ions in plasma or serum, as measured 30 minutes to 1 hour after the lithium treatment, is 0.10-0.20 µM, 0.20-0.50 µM, 0.50-1.0 µM, 1.0-5.0 µM, 5.0-10 µM, 10-20 µM, 20-50 µM, 50-100 µM, 100-500 µM, 0.1-
0.5 mM, 0.5-1.0 mM, 1.0 mM-2.0 mM, 2.0-2.5 mM, 2.5-3.0 mM, 3.0-4.0 mM, 4.0 mM-5.0 mM, 5.0-7.0 mM, or 7.0 mM or greater. In some embodiments, an effective amount of lithium is administered such that the plasma or serum lithium ion concentration measured either 8 hours, 16 hours, 1 day, 1 week, 2 weeks, or 1 month after the lithium treatment, is 0.1 to 0.5 µM, 0.1 to 1.0 µM, 0.5 to 1.0 µM, 0.5 to 1.5 µM, 1 to 10 µM, 10 to 50 µM, 50 to 100 µM, 100 to 150 µM, 150 to 200 µM, 250 to 300 µM, 100 to 250 µM, 100 to 500 µM, 200 to 400 µM, 500 to 1000 µM; or 1000 to less than 1500 µM. In one embodiment, the plasma or serum lithium concentration reaches at least 1 µM. In one embodiment, the plasma or serum lithium concentration reaches at least 100 µM. In one embodiment, the plasma or serum lithium concentration does not exceed 1 mM. In another embodiments, the plasma or serum concentration of lithium does not exceed 1.5 mM. Serum lithium concentration may be measured using any technique known in the art, such as described in Sampson et al., 1992, Trace Elements in Medicine 9:7-8.

[00377] In specific embodiments, an amount of a lithium compound is administered such that the target concentration of lithium in the skin is 0.01 to 0.05 µM, 0.05 to 0.1 µM, 0.1 to 0.5 µM, 0.1 to 1 µM, 0.5 to 1.0 µM, 1.0 to 1.5 µM, 1 to 2.5 µM, 1 to 5 µM, 5 to 10 µM, 10 to 50 µM, 50 to 100 µM, 100 to 150 µM, 150 to 200 µM, 250 to 300 µM, 100 to 250 µM, 100 to 500 µM, 200 to 400 µM, 500 to 1000 µM, 1 to 10 mM, 1 to 5 mM, 5 to 10 mM, 10 to 100 mM, 100 to 200 mM, or 500 to 1000 mM. In some embodiments, the concentration of lithium achieved in the skin is greater than 0.1 mM. In some embodiments, the concentration of lithium achieved in the skin is greater than 1.0 mM. In some embodiments, the concentration of lithium achieved in the skin is greater than 1.5 mM. In one embodiment, the amount of lithium achieved in the skin is approximately 1 mM to 5 mM. In one embodiment, the amount of lithium achieved in the skin is approximately 5 mM to 10 mM. In one embodiment, the amount of lithium achieved in the skin is approximately 100 to 200 mM. In one embodiment, the amount of lithium achieved in the skin does not exceed 5 mM. In one embodiment, the amount of lithium achieved in the skin does not exceed 10 mM. In one embodiment, the amount of lithium achieved in the skin does not exceed 50 mM. In some embodiments, an amount of lithium is administered such that the concentration of lithium delivered to the stratum corneum is 0.1 to 0.5 mM, 0.5 to 1 mM, 1 to 10 mM, 10 to 100 mM, 100 to 200 mM, or 500 to 1000 mM. In some embodiments, the concentration of lithium delivered to the stratum corneum is greater than 1.5 mM. In one embodiment, the amount of lithium achieved in the stratum corneum is approximately 100 to 200 mM. In one
embodiment, the amount of lithium achieved in the stratum corneum does not exceed 5 mM. In one embodiment, the amount of lithium achieved in the stratum corneum does not exceed 10 mM. One of skill in the art would be able to measure lithium concentrations in skin using techniques known in the art, for example, mass spectroscopy, e.g., inductively coupled plasma mass spectroscopy (ICP-MS). For example, the concentration of lithium in skin can be measured using the method provided in the example of Section 6 below or equivalent methods.

In other embodiments, the lithium concentration is measured in the hair shaft using techniques known in the art, e.g., Tsanaclis & Wicks, 2007, Forensic Science Intl. 176: 19-22, which is incorporated by reference herein in its entirety.

Specific, non-limiting, formulations of lithium for topical, subcutaneous, and oral administration are provided in Sections 5.4.1-5.4.3 below.

5.4.1  **TOPICAL FORMS FOR ADMINISTRATION**

In the embodiments described in the subsections that follow, lithium can be applied topically, e.g., as a cream, gel, ointment, or other form for topical administration as described in Section 5.2 *supra*. Topical lithium may be administered to wounded or unwounded skin.

In some embodiments, the lithium formulation for topical administration (e.g., gel, cream, ointment, salve, etc.) comprises lithium (or monovalent lithium salt) at a concentration of 50 mM, 75 mM, 100 mM, 125 mM, 150 mM, 175 mM, 200 mM, 250 mM, 300 mM, 350 mM, 400 mM, 450 mM, 500 mM, 550 mM, 600 mM, 650 mM, 700 mM, 750 mM, 800 mM, 900 mM, 1 M, 1.1 M, or 1.2 M, or more. As used herein, a monovalent lithium salt (e.g., lithium gluconate, lithium chloride, lithium stearate, lithium orotate, etc.) refers to a salt form of lithium in which there is one lithium cation for each anion of the salt. A divalent lithium salt (e.g., in some embodiments, lithium succinate, lithium carbonate) refers to a salt form of lithium in which there are two lithium cations for each anion of the salt. A trivalent lithium salt (e.g., in some embodiments, lithium citrate), refers to a salt form of lithium in which there are three lithium cations for each anion of the salt. In some embodiments, a lithium formulation comprising lithium (or monovalent lithium salt) at a concentration in the range of 50 mM to 200 mM is chosen for use in the embodiments described herein. In some embodiments, a lithium formulation comprising lithium (or monovalent lithium salt) at a concentration in the range of 200 mM to 400 mM is used. In some embodiments, a lithium formulation comprising lithium (or monovalent lithium salt) at a concentration in the range of...
400 mM to 600 mM is used. In some embodiments, a lithium formulation comprising lithium (or monovalent lithium salt) at a concentration in the range of 600 mM to 800 mM is used. The concentration of lithium in a particular topical lithium formulation to deliver the intended dose of lithium will depend on the release properties of the lithium ion, the hydrophobicity of the lithium salt form, the partition coefficient of the lithium salt form, etc.

[00382] Lithium formulations comprising the foregoing lithium (or monovalent lithium salt) concentrations may be achieved using, for example, a formulation comprising, w/w, lithium ions at a concentration of 0.10% lithium, 0.15% lithium, 0.20% lithium, 0.25% lithium, 0.30% lithium, 0.35% lithium, 0.40% lithium, 0.45% lithium, 0.50% lithium, 0.55% lithium, 0.60% lithium, 0.65% lithium, 0.70% lithium, 0.75% lithium, 0.80% lithium, 0.85% lithium, 0.90% lithium, 0.95% lithium. In some embodiments, the form of lithium for topical administration comprises, w/w, 0.1% to 0.5%> lithium ions, 0.2%> to 0.5%> lithium ions, 0.5%> to 1% lithium ions, or more.

[00383] The amount of a salt form of lithium to generate a topical lithium formulation with one of the aforementioned concentrations of lithium ion is readily deducible by one of ordinary skill in the art, and depends upon several factors including, e.g., the valency of the salt form, the stability of the salt form, the ability of the salt form to release the lithium ion, the hydrophobicity or hydrophilicity, etc. For example, Lithioderm (Labcatat) comprises 8% lithium gluconate, which corresponds to 0.275% lithium ion (i.e., 274.8 mg Li+/100 g gel). It is noted that a formulation of topical 8% lithium gluconate, w/w, contains approximately 80 mg/ml lithium gluconate, which is approximately 400 mM lithium gluconate (and, thus, 400 mM lithium ion). Thus, in some exemplary embodiments, a formulation for topical administration comprises a salt form of lithium (e.g., lithium carbonate or other form described in Section 5.1 above) at a concentration, w/w, of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 15%, 16%, 18%, 20%, or more. In some embodiments, a salt form of lithium for topical administration comprises, w/w, 1% to 2% lithium salt (e.g., lithium carbonate or other form described in Section 5.1 above), 2% to 5% lithium salt, 5% to 10% lithium salt, 10% to 15% lithium salt, 15% to 20% lithium salt, 20% to 25% lithium salt, or 25% to 50% lithium salt. In one embodiment, the form of lithium for topical administration is 1% to 20% w/w lithium salt.

[00384] In a specific embodiment, a formulation for topical administration comprises lithium carbonate at a concentration of 1.46% w/w. In another embodiment, a formulation for topical administration comprises lithium carbonate at a concentration of 2.91% w/w. In
yet another embodiment, a formulation for topical administration comprises lithium carbonate at a concentration of 5.66% w/w.

[00385] In some embodiments, a topical formulation of lithium comprises 0.25%-0.75%, 0.75%-1.5%, or 1.5%-3%, 3%-6%, or 8%-16%, or more lithium carbonate (w/w). In some embodiments, a topical formulation of lithium comprises 1%-4% lithium gluconate (w/w). In some embodiments, a topical formulation of lithium comprises 4%-8% lithium gluconate (w/w). In some embodiments, a topical formulation of lithium comprises 8%-16% or more lithium gluconate (w/w). In some embodiments, a topical formulation of lithium comprises 0.2%-1%, or 1%-5%, or more lithium chloride (w/w). In some embodiments, a topical formulation of lithium comprises 0.5%-2%, or 2%-4%, or 4%-8%, or 8%-16%, or more lithium succinate (w/w). In some embodiments, a topical formulation of lithium comprises 0.5%-6%, 6%-12%, or 12%-25%, or more lithium stearate (w/w). In some embodiments, a topical formulation of lithium comprises 1%-4%, 4%-8%, or 8%-16%, or more lithium orotate (w/w). In certain other embodiments, the topical lithium formulation comprises 0.10%-0.25%, 0.25%-0.75%, 0.75%-1.5%, 1.5%-3%, 3%-4.5%, or 4.5%-6%, or more lithium carbonate (w/w). In some embodiments, a topical formulation of lithium comprises 0.25%-1.5%, 1.5%-3.0%, or 3%-6%, or more 8% lithium citrate (w/w).

[00386] In an exemplary embodiment, a 50 kg patient is administered a single droplet approximately 0.1 mL of 8% (w/w) lithium gluconate at 3 sites, twice daily. This corresponds to approximately 8 mg lithium gluconate (0.274 mg Li+) per site, i.e., 0.16 mg/kg lithium gluconate (0.005 mg/kg Li+) per site. Over three sites twice daily, this corresponds to approximately 0.96 mg/kg lithium gluconate (0.033 mg/kg Li+) per day. Thus, in some embodiments, a patient (e.g., a 50 kg patient) is administered about 30-50 mg, about 50-75 mg, or about 75-100 mg topical lithium gluconate/day, which is equivalent to about 1-1.7 mg, 1.7-2.2 mg, or 2.2-3.5 mg, respectively, Li+/day.

[00387] In some embodiments, a patient is administered topical lithium carbonate that is equivalent to about 2.74 mg, 5.48 mg, 10.73 mg, or 20 mg respectively, Li+/gram. In some embodiments, a patient is administered topical lithium carbonate that is equivalent to about 1-3 mg, 1.5-3 mg, 2-3 mg, 2.4-2.8 mg, 2.6-2.8 mg or 2.65-2.75 mg Li+/gram. In some embodiments, a patient is administered topical lithium carbonate that is equivalent to about 2-8 mg, 3-7 mg, 4-6 mg, 5-6 mg, 5.2-5.6 mg, 5.3-5.5 mg, or 5.4-5.5 mg Li+/gram. In some embodiments, a patient is administered topical lithium carbonate that is equivalent to about 8-12 mg, 9-11 mg, 10-11 mg, 10.2-10.9 mg, 10.3-10.8 mg, 10.5-10.75 mg, or 10.70-10.76 mg Li+/gram. In some embodiments, a patient is administered topical lithium carbonate that is
equivalent to about 18-22 mg, 19-21 mg, 19.5-20.5 mg, 19.6-20.4 mg, 19.7-20.3 mg, 19.8-20.2 mg, or 19.9-20.1 mg Li+/gram.

[00388] In some embodiments, a topical lithium formulation is administered once daily. In some embodiments, a topical lithium formulation is administered twice daily. In some embodiments of a twice daily treatment regimen, doses are administered 6 hours apart, or 7 hours apart, or 8 hours apart, or 9 hours apart, or 10 hours apart, or 11 hours apart, or 12 hours apart. In a particular embodiment, the doses are administered 7 to 8 hours apart.

[00389] In some embodiments when lithium is administered topically, an amount of lithium is administered such that the peak lithium concentration in skin is between 0.01 mM and 0.05 mM, 0.05 mM and 0.1 mM, 0.1 mM and 0.5 mM or between 0.5 mM and 10 mM, for example, between 0.1 and 0.5 mM, 0.5 mM and 1 mM, 1 mM and 2 mM, between 2 mM and 5 mM, 5 mM to 10 mM, or 10 mM to 50 mM. In some such embodiments, the peak lithium concentration in blood may be one or more orders of magnitude lower than the peak concentration in skin (for example, 0.001 mM to 0.01 mM, 0.01 mM to 0.1 mM, or 0.1 mM to 0.5 mM, 0.5 mM to 1.0 mM, or 1.0 mM to 10 mM). In some such embodiments, the steady state blood concentration of lithium should not exceed a maximum of 1.5 mM to 2 mM.

5.4.2 SUBCUTANEOUS FORMS FOR ADMINISTRATION

[00390] In some embodiments, a formulation of lithium described herein (by non-limiting e.g., lithium carbonate, lithium gluconate, lithium chloride, lithium succinate, lithium citrate, lithium stearate, lithium orotate, etc.) is administered subcutaneously, to either wounded or unwounded skin.

[00391] In some embodiments, the form of lithium for subcutaneous administration is administered at a dose comprising 0.001 mg lithium ion per kg of patient weight. In some embodiments, the dose is 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, 0.010 mg/kg, 0.020 mg/kg, 0.025 mg/kg, 0.050 mg/kg, 0.075 mg/kg, 0.10 mg/kg, 0.15 mg/kg, 0.20 mg/kg, 0.25 mg/kg, 0.30 mg/kg, 0.40 mg/kg, 0.50 mg/kg, 0.75 mg/kg, 1 mg/kg, 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, 4.5 mg/kg, 5 mg/kg, 5.5 mg/kg, 6 mg/kg, 6.5 mg/kg, 7 mg/kg, 7.5 mg/kg, 8 mg/kg, 8.5 mg/kg, 9 mg/kg, 9.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, or 50 mg/kg or more of lithium ions. In some embodiments, the dose does not exceed 50 mg/kg. The lower ranges of dosages may be preferably used for bolus dosing. For a controlled release (e.g., a delayed release or a sustained release) dosage form, the maximum dosage that may be administered at any one
time may vary depending on the release kinetics of the lithium and the concentration of efficacy of the formulation.

[00392] The concentration of a salt form of lithium required to generate a subcutaneously administered formulation that delivers lithium ions at one of the aforementioned dosages is readily deducible by one of ordinary skill in the art, and depends upon several factors including, *e.g.*, the valency of the salt form, the stability of the salt form, the ability of the salt form to release the lithium ion, the hydrophobicity or hydrophilicity, etc. For example, to achieve an equivalent dosage of lithium ions, a formulation comprising lithium carbonate may be subcutaneously administered at a dosage of approximately 10 mg lithium carbonate per kg of patient weight (mg/kg), 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 50 mg/kg, 75 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, 250 mg/kg, 300 mg/kg, 350 mg/kg, 400 mg/kg, 450 mg/kg, 500 mg/kg, 550 mg/kg, 600 mg/kg, 650 mg/kg, 700 mg/kg, 750 mg/kg, 800 mg/kg, 850 mg/kg, 900 mg/kg, 950 mg/kg, or 1000 mg/kg. In some embodiments, the formulation for subcutaneous administration contains a dose of 10 mg/kg to 50 mg/kg, 50 mg/kg to 100 mg/kg, 100 mg/kg to 200 mg/kg, 200 mg/kg to 400 mg/kg, 400 mg/kg to 600 mg/kg, or 100 mg/kg to 600 mg/kg of lithium carbonate. In one embodiment, the formulation for subcutaneous administration contains a dose in the range of 30 mg/kg to 150 mg/kg lithium carbonate. In one embodiment, the formulation for subcutaneous administration contains a dose in the range of about 30 mg/kg to 300 mg/kg lithium carbonate. In one embodiment, the dose for subcutaneous administration does not exceed 300 mg/kg lithium carbonate. In another embodiment, the dose for subcutaneous administration does not exceed 600 mg/kg lithium carbonate. The lower ranges of dosages may be preferably used for bolus dosing. For a controlled release (*e.g.*, a delayed release or a sustained release) dosage form, the maximum dosage that may be administered at any one time may vary depending on the release kinetics of the lithium and the concentration of efficacy of the formulation.

[00393] In some embodiments, the lithium formulation is administered subcutaneously once daily. In some embodiments, the lithium formulation is administered subcutaneously twice daily. In some embodiments of a twice daily treatment regimen, doses are administered 6 hours apart, or 7 hours apart, or 8 hours apart, or 9 hours apart, or 10 hours apart, or 11 hours apart, or 12 hours apart. In a particular embodiment, the doses are administered 7 to 8 hours apart.

[00394] In some embodiments when lithium is administered subcutaneously (*for example, once daily, although smaller doses may be administered more than once daily*), an amount of
lithium is administered such that the peak lithium concentration in skin is between 0.1 µM and 0.2 µM, 0.2 µM and 0.5 µM, 0.5 and 1 µM, 1 µM and 2 µM, 2 µM to 10 µM, 10 µM to 100 µM, 100 µM to 500 µM, 500 µM to 1000 µM. These peak values will depend on the lithium release properties of the formulation, the hydrophobicity of the lithium salt form, the partition coefficient of the lithium salt form, etc. In some embodiments, the peak concentration in skin is 0.2 µM to 1.5 µM lithium. In some embodiments, the peak concentration in skin should not exceed 1 µM or 1.5 µM lithium. In some embodiments, the peak concentration in skin is 0.2 µM to 100 µM lithium. In some embodiments, the peak concentration in skin is 100 µM to 1000 µM lithium. In some such embodiments, the peak lithium concentration in blood may be several orders of magnitude higher, for example, 0.1 mM to 0.5 mM, 0.5 mM to 1.1 mM, 1.1 to 1.5 mM, 1.5 mM to 5 mM, 5 mM to 10 mM, 10 mM to 50 mM, or 50 mM to 100 mM. These peak values will depend on the lithium release properties of the formulation, the hydrophobicity of the lithium salt form, the partition coefficient of the lithium salt form, etc. In some such embodiments, the steady state blood concentration of lithium should not exceed a maximum of 1.5 mM to 2 mM.

5.4.3 **ORAL FORMS FOR ADMINISTRATION**

[00395] In some embodiments, a formulation of lithium described herein (by non-limiting e.g., lithium gluconate, lithium chloride, lithium succinate, lithium carbonate, lithium citrate, lithium stearate, lithium orotate, etc.) is administered orally, for example, once daily, or twice daily as determined by the medical practitioner and in accordance with Section 5.4 above.

[00396] In some embodiments, an oral formulation comprising of 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.5 mM, 0.6 mM, 0.7 mM, 0.8 mM, 0.9 mM, 1 mM, 1.1 mM, 1.2 mM, 1.3 mM, 1.4 mM, 1.5 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM, or more, but preferably less than 10 mM, of lithium ions (or monovalent lithium salt) is administered. In some embodiments, an oral formulation comprising lithium ions or a monovalent lithium salt in the range of 0.1 to 0.5 mM, 0.4 to 0.6 mM, 0.5 to 1 mM, 0.6 to 1.2 mM, or 1 to 1.5 mM, is administered.

[00397] Administration of the foregoing amounts of lithium may be achieved by oral administration of a lithium formulation at a dosage comprising 0.001 mg lithium ion per kg of patient weight. In some embodiments, the dose is 0.001 mg/kg, 0.002 mg/kg, 0.003 mg/kg, 0.004 mg/kg, 0.005 mg/kg, 0.006 mg/kg, 0.007 mg/kg, 0.008 mg/kg, 0.009 mg/kg, 0.010 mg/kg, 0.020 mg/kg, 0.025 mg/kg, 0.050 mg/kg, 0.075 mg/kg, 0.10 mg/kg, 0.15 mg/kg, 0.20 mg/kg, 0.25 mg/kg, 0.30 mg/kg, 0.40 mg/kg, 0.50 mg/kg, 0.75 mg/kg, 1 mg/kg,
1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, 4.5 mg/kg, 5 mg/kg, 5.5 mg/kg, 6 mg/kg, 6.5 mg/kg, 7 mg/kg, 7.5 mg/kg, 8 mg/kg, 8.5 mg/kg, 9 mg/kg, 9.5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, or 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, or 50 mg/kg or more of lithium ions. In some embodiments, the dose does not exceed 50 mg/kg Li+.

For a controlled release (e.g., a delayed release or a sustained release) dosage form, the maximum dosage that may be administered at any one time may vary depending on the release kinetics of the lithium and the concentration of efficacy of the formulation.

[00398] The concentration of a salt form of lithium required to generate an orally administered formulation that delivers lithium ions at one of the aforementioned dosages is readily deducible by one of ordinary skill in the art, and depends upon several factors including, e.g., the valency of the salt form, the stability of the salt form, the ability of the salt form to release the lithium ion, the hydrophobicity or hydrophilicity, etc. For example, to achieve an equivalent dosage of Li+, a formulation comprising lithium carbonate, which is a divalent lithium salt (e.g., trade names Eskalith CR, Eskalith, Lithobid), may be orally administered at a dosage of approximately 2 mg lithium carbonate per kg of patient weight (mg/kg), 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 75 mg/kg, 100 mg/kg, 125 mg/kg, 150 mg/kg, 175 mg/kg, 200 mg/kg, or 250 mg/kg or more is administered. In some embodiments, the oral formulation contains a dose of 2 mg/kg to 10 mg/kg, 10 mg/kg to 25 mg/kg, 25 mg/kg to 50 mg/kg, 50 mg/kg to 100 mg/kg, 100 mg/kg to 200 mg/kg, or 200 mg/kg to 500 mg/kg of lithium carbonate. In one embodiment, the oral formulation contains a dose in the range of 5 mg/kg to 100 mg/kg lithium carbonate. In one embodiment, the oral formulation contains a dose in the range of about 5 mg/kg to 50 mg/kg lithium carbonate. In one embodiment, the oral formulation contains a dose in the range of about 10 mg/kg to 100 mg/kg lithium carbonate. In one embodiment, the oral formulation contains a dose that does not exceed 300 mg/kg lithium carbonate.

For a controlled release (e.g., a delayed release or a sustained release) dosage form, the maximum dosage that may be administered at any one time may vary depending on the release kinetics of the lithium and the concentration of efficacy of the formulation.

[00399] In some embodiments when the lithium formulation is for oral administration (for example, for once daily administration, although smaller doses may be administered more than once daily), an amount of lithium compound is administered such that the peak lithium concentration in skin is between 0.1 µM and 0.2 µM, 0.2 µM and 0.5 µM, 0.5 and 1 µM, 1 µM and 2 µM, 2 µM to 10 µM, 10 µM to 100 µM, 100 µM to 500 µM, 500 µM to 1000 µM. These peak values will depend on the lithium release properties of the formulation, the
hydrophobicity of the lithium salt form, the partition coefficient of the lithium salt form, etc. In some embodiments, the peak concentration in skin is 0.2 μM to 1.5 μM lithium. In some embodiments, the peak concentration in skin should not exceed 1 μM or 1.5 μM lithium. In some embodiments, the peak concentration in skin is 10 μM to 100 μM lithium. In some embodiments, the peak concentration in skin is 100 μM to 1000 μM lithium. In some such embodiments, the peak lithium concentration in blood may be several orders of magnitude higher, for example, 0.1 mM to 0.5 mM, or 0.5 mM to 1.1 mM, 1.1 to 1.5 mM, 1.5 mM to 5 mM, 5 mM to 10 mM, 10 mM to 50 mM, or 50 mM to 100 mM. These peak values will depend on the lithium release properties of the formulation, the hydrophobicity of the lithium salt form, the partition coefficient of the lithium salt form, etc. In some such embodiments, the steady state blood concentration of lithium should not exceed a maximum of 1.5 mM to 2 mM.

5.4.4 PULSE TREATMENT

[00400] A pulse lithium treatment can be administered one time, or multiple times at intervals of time. It is understood that the precise dosage and duration of treatment may vary with the age, weight, and condition of the patient being treated, and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test or diagnostic data. It is further understood that for any particular individual, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations. For example, in the treatment of bipolar disorder, therapeutically useful amounts of lithium (~0.4 to 1.2 mM) are only slightly lower than toxic amounts (>1.5 mM), so the skilled practitioner knows that the blood levels of lithium must be carefully monitored during treatment to avoid toxicity.

[00401] In some embodiments, a pulse lithium treatment is administered at the time of integumental perturbation. In some embodiments, a pulse lithium treatment is administered following integumental perturbation. In some embodiments, a pulse lithium treatment is administered immediately following integumental perturbation. In one embodiment, a pulse lithium treatment is begun 1 week prior to wound closure (e.g., day 4-7 following integumental perturbation). In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered before scab formation. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to
formation of a scab, the pulse lithium treatment is administered during scab formation. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered periscab detachment. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered immediately after scab detachment. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered 1 hour after scab detachment. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered up to 6 hours after scab detachment. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered 6-12 hours after scab detachment. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered 12-18 hours after scab detachment. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered 1 day after scab detachment. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered 2 days after scab detachment. In one embodiment, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered 3 days after scab detachment. In some embodiments, in which a pulse lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is administered within 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, or 3 weeks after integumental perturbation.

[00402] In one embodiment, the pulse lithium treatment is administered at the time of integumental perturbation, or immediately after, and then maintained for 3 or 4 or 5 or 6 or 7 days thereafter (in some embodiments, a scab forms during this time). In some embodiments, a pulse lithium treatment is administered as soon as the scab falls off and maintained for 3 or 4
or 5 or 6 or 7 days. In one embodiment, the pulse lithium treatment is administered at the time of integumental perturbation and then maintained for 7 or 10 or 12 or 14 days thereafter (in some embodiments, a scab forms during this time). In some embodiments, a pulse lithium treatment is administered as soon as the scab falls off and maintained for 7 or 10 or 12 or 14 days. In one embodiment, the pulse lithium treatment is administered at the time of integumental perturbation and then maintained for 19 or 21 days thereafter (in some embodiments, a scab forms during this time). In some embodiments, a pulse lithium treatment is administered as soon as the scab falls off and maintained for 19 or 21 days. In some embodiments, the pulse lithium treatment is administered in order to modulate the neoepidermis that forms underneath the scab. In some such embodiments, the pulse lithium treatment is administered at the time of integumental perturbation and is maintained up to some time after scab falls off, for example, between 5 - 14 days or more (e.g., up to 2 weeks, or 3 weeks, or 4 weeks or more) following integumental perturbation. In some embodiments, the pulse lithium treatment is administered beginning 1 week prior to wound closure (e.g., 4-7 days after integumental perturbation), and continued for 5 - 14 days or more. In some embodiments, the pulse lithium treatment is administered beginning at the time the scab falls off (e.g., 11-14 days after integumental perturbation), for 5 - 14 days or more.

[00403] In some embodiments, the course of treatment with lithium is short, for example, limited to a few days just following scab detachment, or even continued only for as long as the scab is still attached. In some embodiments, the course of treatment with lithium is longer, for example, exceeding 2 weeks. The timing of integumental perturbation and lithium administration is preferably monitored and adjusted so that optimal results are achieved.

[00404] In some embodiments, a pulse treatment is combined with a form of integumental perturbation that does not lead to formation of a scab. In one such embodiment, the pulse lithium treatment is administered at the time of integumental perturbation. In some embodiments, a pulse lithium treatment is administered following integumental perturbation. In some embodiments, in which a pulse lithium treatment is administered following an integumental perturbation that does not lead to formation of a scab, the pulse lithium treatment is administered within 15 minutes of, or 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 18 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 2 weeks, or 3 weeks after integumental perturbation.
5.4.5 INTERMITTENT TREATMENTS

[00405] An intermittent lithium treatment can be administered one time (e.g., using a controlled release formulation), or multiple times at intervals of time. It is understood that the precise dosage and duration of treatment may vary with the age, weight, and condition of the patient being treated, and may be determined empirically using known testing protocols or by extrapolation from in vivo or in vitro test or diagnostic data. It is further understood that for any particular individual, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the formulations.

[00406] In one embodiment, lithium can be administered daily (e.g., once, twice or three times daily) for at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days; and in some embodiments not more than 14 or 15 days, or not more than 3 weeks, or not more than 4 weeks. In some embodiments, lithium can be administered daily (e.g., once, twice or three times daily) for at least 12 days, 14 days, at least 16 days, at least 19 days, or at least 21 days; in some embodiments not more than 21 days. Holidays can be interspersed for at least 1 day, at least 2 days, at least 3 days, at least 4 days, at least 5 days, at least 6 days, at least 7 days; and in some embodiments not more than 14 or 15 days, or not more than 3 weeks, or not more than 4 weeks. In some embodiments, holidays are interspersed for at least 14 days, 21 days, 28 days, or longer.

[00407] In some embodiments, an intermittent lithium treatment is begun at the time of integumental perturbation. In some embodiments, an intermittent lithium treatment is begun following integumental perturbation. In some embodiments, an intermittent lithium treatment is begun immediately following integumental perturbation. In one embodiment, in which an intermittent lithium treatment is begun following an integumental perturbation that leads to formation of a scab, the intermittent lithium treatment is begun before scab formation. In one embodiment, in which an intermittent lithium treatment is begun following an integumental perturbation that leads to formation of a scab, the intermittent lithium treatment is begun during scab formation. In one embodiment, in which an intermittent lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium in the intermittent lithium treatment is periscab detachment. In one embodiment, in which the intermittent lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium is immediately after scab detachment. In one embodiment, in which the intermittent lithium
treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium is up to 6 hours after scab detachment. In one embodiment, in which the intermittent lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium is 6-12 hours after scab detachment. In one embodiment, in which the intermittent lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium is 12-18 hours after scab detachment. In one embodiment, in which the intermittent lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium is 18-24 hours after scab detachment. In one embodiment, in which the intermittent lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium is 1 day after scab detachment. In one embodiment, in which the intermittent lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium is 2 days after scab detachment. In one embodiment, in which the intermittent lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium is 3 days after scab detachment. In one embodiment, in which the intermittent lithium treatment is administered following an integumental perturbation that leads to formation of a scab, the first administration of lithium is administered immediately after scab detachment (e.g., 11-14 days after integumental perturbation), followed by another administration each day for several days to 1 week, or two 2 weeks or more. In some embodiments, in which an intermittent lithium treatment is begun following an integumental perturbation that leads to formation of a scab, the pulse lithium treatment is begun within 3 days, 4 days, 5 days, 6 days, 7 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, or 3 weeks after integumental perturbation.

[00408] In one embodiment, the intermittent lithium treatment is begun at the time of integumental perturbation, or immediately after, and then administered daily (or twice daily) for 3 or 4 or 5 days thereafter (in some embodiments, a scab forms during this time). In some embodiments, the intermittent lithium treatment is begun as soon as the scab falls off, and administered daily for 3 or 4 or 5 days. In one embodiment, the intermittent lithium treatment is begun at the time of integumental perturbation, or immediately after, and then administered daily (or twice daily) for 7 days thereafter (in some embodiments, a scab forms during this time). In some embodiments, the intermittent lithium treatment is begun at the time of integumental perturbation and then maintained for 7 or 10 or 12 or 14 days thereafter.
(in some embodiments, a scab forms during this time). In some embodiments, an intermittent lithium treatment is begun as soon as the scab falls off and maintained for 7 or 10 or 12 or 14 days. In some embodiments, the intermittent lithium treatment is begun at the time of integumental perturbation and then maintained for 19 or 21 days thereafter (in some embodiments, a scab forms during this time). In some embodiments, an intermittent lithium treatment is begun as soon as the scab falls off and maintained for 19 or 21 days. In some embodiments, the intermittent lithium treatment is begun as soon as the scab falls off, and administered daily for 7 days. In one embodiment, the intermittent lithium treatment is begun at the time of integumental perturbation, or immediately after, and then administered daily (or twice daily) for 14 or 15 days thereafter (in some embodiments, a scab forms during this time). In some embodiments, the intermittent lithium treatment is begun 1 week prior to wound closure (e.g., day 4-7 following integumental perturbation), and continued for 5 - 14 days or more. In some embodiments, the intermittent lithium treatment is begun as soon as the scab falls off, and administered daily for 14 or 15 days. In some embodiments, the intermittent lithium treatment is to modulate the neoeipidermis that forms underneath the scab. In some such embodiments, the intermittent lithium treatment is begun at the time of integumental perturbation and is continued with daily dosing up to some time after scab falls off, for example, between 5 - 14 days following integumental perturbation. In some embodiments, the course of treatment with lithium is short, for example, limited to daily doses for a few days just following scab detachment, or even continued only for as long as the scab is still attached. In some embodiments, the course of treatment with lithium is longer, for example, exceeding 2 weeks or 3 weeks or one month or more. The timing of integumental perturbation and lithium administration is preferably monitored and adjusted so that optimal results are achieved.

[00409] In some embodiments, an intermittent lithium treatment is combined with a form of integumental perturbation that does not lead to formation of a scab. In one such embodiment, the intermittent lithium treatment is begun at the time of integumental perturbation. In some embodiments, an intermittent lithium treatment is begun following integumental perturbation. In some embodiments, in which an intermittent lithium treatment is begun following an integumental perturbation that does not lead to formation of a scab, the intermittent lithium treatment is begun within 15 minutes of, or 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, 18 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, or 3 weeks after integumental perturbation.
5.5 COMBINATION TREATMENTS

[00410] Without being bound by any theory, the combination treatments described herein are also used for wound healing and scar revision.

[00411] The lithium treatments described herein may be in combination with other methods, including conventional methods, for treating, reducing or preventing microbial infections or microbial colonizations. The effect that each drug offers could be an additive or synergistic improvement, or a combination of two different pharmacologically defined effects, to achieve the desired end result. The combined modality of treatment could involve alternating treatment of each dosage form or concurrent or simultaneous treatment. Synergism occurs when the combination has an effect that is more than would be expected from merely the additive effect of each element in the combination.

[00412] The lithium treatments described herein may be in combination with other methods, including conventional methods, for wound healing or scar revision. The effect that each modality (e.g., drug or integumental perturbation method) offers could be an additive or synergistic improvement, or a combination of two different (e.g., pharmacologically defined effects), to achieve the desired end result. The combined modality of treatment could involve alternating each treatment or concurrent or simultaneous treatment. Synergism occurs when the combination has an effect that is more than would be expected from merely the additive effect of each element in the combination.

[00413] In certain embodiments, a lithium treatment described herein may be in combination with other methods, including conventional methods, for (i) treating, reducing or preventing microbial infections or microbial colonizations; and (ii) wounding healing or scar revision.

[00414] The lithium treatments described herein may be in combination with any additional treatment(s) described or incorporated by reference herein or determined to be appropriate by the medical practitioner. The amount of an additional treatment(s) will depend on the desired effect and the additional compound or treatment method that is selected. Dosages and regimens for administering such additional treatment(s) are the dosages and regimens commonly in use, which can be easily determined by consulting, for example, product labels or physicians’ guides, such as the Physicians’ Desk Reference ("PDR") (e.g., 63rd edition, 2009, Montvale, NJ: Physicians’ Desk Reference). Specific examples of treatments for use in combination with the lithium treatments described herein follow.
[00415] In one embodiment, the combination treatment comprises lithium and an additional compound(s) formulated together. The lithium in such formulations may be released concurrently with or separately from the additional compound(s), or may be released and/or delivered to the tissue site with different pharmacokinetics. For example, in some embodiments, one or more of the compounds in the formulation undergoes controlled release, whereas one or more of the other compounds does not. For example, one or more of the compounds in the formulation undergoes sustained release whereas one or more of the other compounds undergoes delayed release.

[00416] In another embodiment, the combination treatment comprises lithium and an additional compound(s) formulated separately. The separate formulations may be administered concurrently, sequentially, or in alternating sequence. For example, the lithium compound may be administered sequentially, or concurrently with another compound to achieve the desired effect of improved wound healing or scar revision.

[00417] In an embodiment, the combination treatment comprises lithium and mupirocin. In a specific embodiment, the combination treatment comprises lithium carbonate and mupirocin. A detailed description of a combination treatment comprising lithium carbonate and mupirocin is provided below. However, in the detailed description of lithium carbonate below, lithium carbonate can be substituted with any other lithium compound, including but not limited to lithium gluconate, lithium succinate, lithium chloride, lithium citrate, a lithium salt of mupirocin, and lithium hydroxide.

[00418] Provided herein is a method for treating a microbial infection or microbial colonization, comprising (a) administering a lithium carbonate composition that delivers an effective amount of lithium ions to a human subject in need thereof; and (b) administering mupirocin to the human subject in need thereof. Also provided herein is a combination of lithium carbonate and mupirocin for use in treating a microbial infection or a microbial colonization. In a specific embodiment, the microbial infection is a bacterial infection. In another specific embodiment, the bacterial infection is caused by Methicillin-resistant Staphylococcus aureus.

[00419] In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to treat or ameliorate one or more of the following symptoms caused by Methicillin-resistant Staphylococcus aureus: boils, abscesses, a sty, carbuncles, impetigo, a rash, fever, chills, low blood pressure, joint pains, furunculosis, septic arthritis, headaches, and shortness of breath. In another embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to treat or ameliorate the
symptoms of one or more of the following conditions caused by Methicillin-resistant *Staphylococcus aureus*: cellulitis, necrotizing fasciitis, osteomyelitis, sepsis, pyomyositis, necrotizing pneumonia, infective endocarditis, bone infections and joint infections.

In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to treat or ameliorate one or more of the following symptoms caused by *Staphylococcus aureus* and/or *Streptococcus pyogenes*: secondarily infected traumatic skin lesions, skin and soft tissue infections, and impetigo.

In one embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to treat, reduce, or prevent a microbial biofilm. In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to treat, reduce, or prevent a microbial biofilm comprising Methicillin-resistant *Staphylococcus aureus*. In an embodiment, the combination treatment comprising lithium carbonate and mupirocin results in 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% reduction of a biofilm.

In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to a subject to prevent, reduce, or eradicate colonization of one or more microbes. As used herein, the term "colonization" refers to presence, growth, and multiplication of an organism without observable clinical symptoms or immune reaction. In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to a subject to prevent, reduce, or eradicate colonization in or on one or more of the following: nares, anterior nares, axillae, chronic wounds, decubitus ulcer surface, perineum, around gastrostomy sites, around tracheostomy sites, in sputum, in urine, or in the bowel. In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to a subject to prevent, reduce, or eradicate nasal colonization. In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to a subject to prevent, reduce, or eradicate colonization of one or more microbes in order to reduce the risk of developing a subsequent infection in that subject. In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to a subject to prevent, reduce, or eradicate colonization of one or more microbes, including but limited to *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, and drug-resistant *Enterococci*. In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to a subject to prevent, reduce, or eradicate colonization in order to reduce the risk of developing a subsequent infection by of one or more microbes, including
but not limited to *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, and drug-resistant *Enterococci*.

[00423] In specific embodiments, colonization that can be prevented or reduced using a combination treatment comprising lithium carbonate and mupirocin is colonization with methicillin-resistant *Staphylococcus aureus* in the nares, axillae, chronic wounds or decubitus ulcer surface, perineum, around gastrostomy and tracheostomy sites, in the sputum or urine. In other embodiments, colonization that can be prevented or reduced using a combination treatment comprising lithium carbonate and mupirocin is colonization with *Enterococci* in the bowel and the female genital tract. In a specific embodiment, colonization that can be prevented or reduced using a combination treatment comprising lithium carbonate and mupirocin is colonization with drug-resistant *Enterococci* in the bowel.

[00424] In an embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to subjects at high risk of methicillin-resistant *Staphylococcus aureus* infection, such as adult patients and health care workers, to prevent infection or reduce the risk of infection with methicillin-resistant *Staphylococcus aureus*. In another embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to adult patients and health care workers for the reduction or eradication of nasal colonization with methicillin-resistant *S. aureus* in a as part of a comprehensive infection control program to reduce the risk of spreading colonization or infection among patients and health care workers and those they come into contact with at high risk of methicillin-resistant *S. aureus* infection during institutional outbreaks of infections with this pathogen. In another embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to adult patients and health care workers for the reduction or eradication of nasal colonization with methicillin-resistant *S. aureus* in a as part of a comprehensive infection control program to reduce the risk of spreading colonization or infection among patients, health care workers, and those they come into contact with at high risk of methicillin-resistant *S. aureus* infection or colonization during community outbreaks of infections with this pathogen (for example, Community-Acquired MRSA).

[00425] In an embodiment, a topical formulation of a combination treatment comprising lithium carbonate and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection or colonization by bacteria including, but not limited to, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Streptococcus pyogenes*, *corynebacterium species*, *enterococci*, *Proteus mirabilis*, group D
streptococci, other gram-positive aerobes, and Bacteroides fragilis. In an embodiment, a topical formulation of a combination treatment comprising lithium carbonate and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection or colonization by fungi. In an embodiment, topical formulations of the lithium treatments described herein are administered as a skin sanitizer and/or hand sanitizer to prevent infection or colonization by fungi and bacteria. In an embodiment, the skin sanitizer formulation and/or hand sanitizer formulation is aqueous based or glycerol based. In another embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.

In an embodiment, the combination treatment comprises separate administration of lithium carbonate and mupirocin. In a more specific embodiment lithium carbonate and mupirocin are formulated separately. The separate formulations of lithium carbonate and mupirocin may be administered concurrently, sequentially, or in alternating sequence. The administration regimens for lithium carbonate and mupirocin can be overlapping or offset.

In one embodiment, lithium carbonate is administered to a subject at reasonably the same time as the mupirocin. This method provides that the two administrations are performed within a time frame of less than one minute to about five minutes, or up to about sixty minutes from each other, for example, at the same doctor's visit. In another embodiment, lithium carbonate and mupirocin are administered at exactly the same time. In yet another embodiment, lithium carbonate and mupirocin are administered in a sequence and within a time interval such that lithium treatment and the other treatment can act together to provide an increased benefit than if they were administered alone.

In an embodiment, mupirocin is administered twice daily for 10 days and lithium carbonate is also administered twice daily for 10 days. In specific embodiment, mupirocin is administered before lithium carbonate. In another specific embodiment, lithium carbonate is administered before mupirocin. The combination of lithium carbonate and mupirocin can be administered according to any of the regimens for combination treatments described in Section 5.5.

In an embodiment, a combination treatment described herein contains 0.01% mupirocin, 0.02% mupirocin, 0.03% mupirocin, 0.04% mupirocin, 0.05% mupirocin, 0.06% mupirocin, 0.07% mupirocin, 0.08% mupirocin, 0.09% mupirocin, 0.1% mupirocin, 0.2% mupirocin, 0.3% mupirocin, 0.4% mupirocin, 0.5% mupirocin, 0.6% mupirocin, 0.7% mupirocin, 0.8% mupirocin, 0.9% mupirocin, 1.0% mupirocin, 1.1% mupirocin, 1.2% mupirocin, 1.3% mupirocin, 1.4% mupirocin, 1.5% mupirocin, 1.6% mupirocin, 1.7% mupirocin, 1.8% mupirocin, 1.9% mupirocin, 2.0% mupirocin, 2.2% mupirocin, 2.3%
mupirocin, 2.4% mupirocin, 2.5% mupirocin, 2.6% mupirocin, 2.7% mupirocin, 2.8%
mupirocin, 2.9% mupirocin, 3.0% mupirocin, 3.1% mupirocin, 3.2% mupirocin, 3.3%
mupirocin, 3.4% mupirocin, 3.5% mupirocin, 3.6% mupirocin, 3.7% mupirocin, 3.8%
mupirocin, 3.9% mupirocin, 4.0% mupirocin, 4.5% mupirocin, 5.0% mupirocin and above.

[00430] In some embodiments a combination treatment described herein contains 0.5% -
2.5%, 0.75% - 2.75%, 1.0% - 3%, 1.25% - 3.25%, 1.5% - 3.5%, 1.75% - 3.75%, or 0.1% -
5% mupirocin. In some embodiments, a combination treatment described herein contains
0.10%-0.25%, 0.25%-0.75%, 0.75%-1.5%, 1.5%-3%, 3%-4.5%, 4.5%-6%, 6%-8% or 8%-16%
lithium carbonate.

[00431] In some embodiments, a combination treatment described herein contains 0.5% -
2.5%, 0.75% - 2.75%, 1.0% - 3%, 1.25% - 3.25%, 1.5% - 3.5%, 1.75% - 3.75%, or 0.1% -
5% mupirocin and 0.10%-0.25%, 0.25%-0.75%, 0.75%-1.5%, 1.5%-3%, 3%-4.5%, or 4.5%-6%,
6%-8% or 8%-16% lithium carbonate. In some embodiments, a combination treatment
described herein contains 1.0% mupirocin and 0.10%-0.25%, 0.25%-0.75%, 0.75%-1.5%,
1.5%-3%, 3%-4.5%, or 4.5%-6%, 6%-8% or 8%-16% lithium carbonate.

[00432] In some embodiments, a combination treatment described herein contains 2.0%
mupirocin and 0.10%-0.25%, 0.25%-0.75%, 0.75%-1.5%, 1.5%-3%, 3%-4.5%, or 4.5%-6%,
6%-8% or 8%-16% lithium carbonate. In some embodiments, a combination treatment
described herein contains 1.0% mupirocin and 0.10%-0.25%, 0.25%-0.75%, 0.75%-1.5%,
1.5%-3%, 3%-4.5%, or 4.5%-6%, 6%-8% or 8%-16% lithium carbonate.

[00433] In an embodiment a combination treatment described herein comprises 1.46%
w/w lithium carbonate (2.74 mg Li+/g), 5.66% w/w lithium carbonate (10.64 mg Li+/g), or
11.5% w/w lithium carbonate (20 mg Li+/g).

[00434] In a specific embodiment, a combination treatment described herein comprises
1.46% w/w lithium carbonate (2.74 mg Li+/g) and 0.5% - 2.5%, 0.75% - 2.75%, 1.0% - 3%,
1.25% - 3.25%, 1.5% - 3.5%, 1.75% - 3.75%, or 0.1% - 5% mupirocin. In another specific
embodiment, a combination treatment described herein comprises 5.66% w/w lithium
carbonate (2.74 mg Li+/g) and 0.5% - 2.5%, 0.75% - 2.75%, 1.0% - 3%, 1.25% - 3.25%,
1.5% - 3.5%, 1.75% - 3.75%, or 0.1% - 5% mupirocin. In another specific embodiment, a
combination treatment described herein comprises 11.5% w/w lithium carbonate (2.74 mg
Li+/g) and 0.5% - 2.5%, 0.75% - 2.75%, 1.0% - 3%, 1.25% - 3.25%, 1.5% - 3.5%, 1.75%-
3.75%, or 0.1% - 5% mupirocin.

[00435] In a specific embodiment, a combination treatment described herein comprises
1.46% w/w lithium carbonate (2.74 mg Li+/g) and 2% mupirocin. In another specific
embodiment, a combination treatment described herein comprises 5.66% w/w lithium
carbonate (2.74 mg Li+/g) and 2% mupirocin. In another specific embodiment, a
combination treatment described herein comprises 11.5% w/w lithium carbonate (2.74 mg Li+/g) and 2% mupirocin.

[00436] Provided herein is a combination of lithium carbonate and mupirocin for use in treating a microbial infection or a microbial colonization prepared to be administered as 1.46% w/w lithium carbonate (2.74 mg Li+/g) and 2% mupirocin. Provided herein is a combination of lithium carbonate and mupirocin for use in treating a microbial infection prepared to be administered as 5.66% w/w lithium carbonate (2.74 mg Li+/g) and 2% mupirocin. Provided herein is a combination of lithium carbonate and mupirocin for use in treating a microbial infection or a microbial colonization prepared to be administered as 11.5% w/w lithium carbonate (2.74 mg Li+/g) and 2% mupirocin.

[00437] Also provided herein are pharmaceutical compositions formulated for topical administration, comprising lithium carbonate, mupirocin, and a pharmaceutically acceptable carrier or excipient. In one embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 0.18%-1 1.5% w/w lithium carbonate, 2% mupirocin, glycerol, carboxymethyl cellulose, allantoin, sodium alginate, methyl paraben, and propyl paraben. In another embodiment, the pharmaceutical composition is formulated as a cream comprising 0.18%-1 1.5% w/w lithium carbonate, 2% mupirocin Citric Acid, Carbopol 980, Tween 20, Cetearyl Alcohol, Silicon 350 CSt, Silicon 12,500 CSt, Span 80, Lanolin Alcohol, and Emulsifier 10. In another embodiment, the pharmaceutical is formulated as a cream comprising 0.18%-1 1.5% w/w lithium carbonate, Citric Acid, Carbopol 980, Tween 20, Cetearyl Alcohol, D350 Mineral Oil, Span 80, Lanolin Alcohol, and Emulsifier 10. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin, and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 2.90% lithium carbonate.
carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 1.46% lithium carbonate. Concentrations of citric acid may be varied from 5%-10% w/w. Concentrations of lithium carbonate can be varied from 1%-15%. Concentrations of glycerol may be varied from 1%-10%. Boric acid may be used instead of citric acid, in concentrations 5-10% w/w. Hydrochloric acid may be used in conjunction with citric acid or boric acid, for the purpose of adjusting pH to a physiologically acceptable formulation for wounds. Hyaluronic acid at concentrations 0.1-2% may be used instead of carboxymethylcellulose (CMC). Other polymers to be used instead of CMC may be hydroxyethyl cellulose (0.1-2%), hydroxypropylmethyl cellulose (0.1%), Xanthan Gum (0.1-1%), Guar Gum (0.1-1%).

[00439] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising carbomethylcellulose, allantoin, alginate, glycerol, citric acid, distilled water, mupirocin and lithium carbonate. In a further embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 0.5% to 3% mupirocin

[00440] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 0.5% mupirocin, and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 0.5% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 0.5% mupirocin and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 2% mupirocin and 1.46% lithium carbonate.

[00441] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin, and 11.5% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin and 5.66% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled
water, 1% mupirocin and 2.90% lithium carbonate. In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 1% mupirocin and 1.46% lithium carbonate.

[00442] In another embodiment, the pharmaceutical composition is formulated as a hydrogel comprising 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water, 3% mupirocin and 2.90% lithium carbonate. In another embodiment, the combination treatment comprises administering both sustained release mupirocin. In a n embodiment, the combination treatment comprises administering both sustained release mupirocin and immediate release mupirocin.

[00443] In an embodiment, the combination treatment is formulated as an "immediate release" formulation, i.e., releases 90-100% of Li+ within the first day of administration. In an exemplary embodiment, a combination treatment is formulated as an immediate release formulation as described in greater detail in Section 6.12.1 infra.

[00444] In another embodiment, the combination treatment is formulated as a "Sustained Release" formulation, i.e., releases 90-100% of Li+ within 3 to 7 days of administration. In an exemplary embodiment, a combination treatment is formulated as a sustained release formulation as described in greater detail in Section 6.12.3 infra.

[00445] In an embodiment, the combination treatment comprises administering immediate release mupirocin and immediate release lithium carbonate. In another embodiment, the combination treatment comprises administering sustained release mupirocin and sustained release lithium carbonate. In another embodiment, the combination treatment comprises administering sustained release mupirocin and immediate release lithium carbonate. In another embodiment, the combination treatment comprises administering immediate release mupirocin and sustained release lithium carbonate.

[00446] In an embodiment, the combination treatment comprises administering both sustained release and immediate release lithium carbonate and immediate release mupirocin. In an embodiment, the combination treatment comprises administering both sustained release...
and immediate release lithium carbonate and sustained release mupirocin. In an embodiment, the combination treatment comprises administering both sustained release and immediate release mupirocin and immediate release lithium carbonate. In an embodiment, the combination treatment comprises administering both sustained release and immediate release mupirocin and sustained release lithium carbonate. In an embodiment, the combination treatment comprises administering both sustained release and immediate release mupirocin and both sustained release and immediate release lithium carbonate.

[00447] In an embodiment, the combination treatment comprising lithium carbonate and mupirocin is bactericidal. In an embodiment, the combination treatment comprising lithium carbonate and mupirocin causes a decrease in bacterial load by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99%. In an embodiment, the combination treatment comprising lithium carbonate and mupirocin inhibits tRNA synthetase activity by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99%. In another embodiment, the combination treatment comprising lithium carbonate and mupirocin inhibits protein synthesis by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99%. In another embodiment, the combination treatment comprising lithium carbonate and mupirocin inhibits DNA synthesis by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99%. In another embodiment, the combination treatment comprising lithium carbonate and mupirocin inhibits cell wall synthesis by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99%. In another embodiment the combination treatment comprising lithium carbonate and mupirocin does not inhibit RNA synthesis, more specifically RNA synthesis is inhibited by at most 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99%. In another embodiment, the combination treatment comprising lithium carbonate and mupirocin inhibits tRNA synthetase, protein synthesis, lipid synthesis, DNA synthesis, and cell wall synthesis by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99%.

[00448] In another embodiment, the combination treatment comprising lithium carbonate and mupirocin inhibits lipid synthesis by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, or 100%.

[00449] In another embodiment, the combination treatment comprising lithium carbonate and mupirocin causes a decrease in bacterial load 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% faster than 2% mupirocin alone. In another embodiment, the
combination treatment comprising lithium carbonate and mupirocin causes a decrease in bacterial load 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% faster than 11.5% lithium carbonate alone.

In another embodiment, the combination treatment comprising lithium carbonate and mupirocin causes a decrease in bacterial load in pathogens selected from the following: gram-positive pathogens, including but not limited to Staphylococcus aureus, methicillin resistant Staphylococcus aureus, Streptococcus pyogenes, and gram-negative bacteria including but not limited to Haemophilus influenzae, Neisseria spp., and Bordetella pertussis.

An article of manufacture, as contemplated by the present invention, comprises a combination of lithium carbonate and mupirocin described herein formulated as wound dressings, which can be in the form of barriers, membranes, or films. Alternatively, a combination of lithium carbonate and mupirocin is added to dressing backings, such as barriers, membranes, or films. A barrier, membrane, or film can be supplied in a variety of standard sizes, which can be further cut and sized to the area being treated. The backing can be a conventional dressing material, such as a bandage or gauze to which lithium carbonate and mupirocin described herein is added or coated on, prior to application to the patient. Alternatively, a combination of lithium carbonate and mupirocin can be formulated as a nasal spray to treat or reduce nasal colonization. Alternatively, a combination of lithium carbonate and mupirocin can be formulated as a barrier, membrane, or film made out of strings, microbeads, microspheres, or microfibrils, or the composition can be formulated as a barrier-forming mat. Alternatively, a combination of lithium carbonate and mupirocin can be formulated as a spray that can be sprayed on to skin or a wound to treat infection, and reduce or eradicate colonization (see Section 5.1 for a detailed description of a spray delivery system). Alternatively, a combination of lithium carbonate and mupirocin can be formulated as a spray that can be sprayed on to skin or a wound as a protective layer (see Section 5.1 for a detailed description of a spray delivery system). A combination of lithium carbonate and mupirocin can also be formulated as a component of a wipe or spray to disinfect surfaces.

In another embodiment, the combination treatment comprises lithium carbonate and a tRNA synthetase inhibitor selected from the following: mupirocin, L-tryptophan, indolmycin, chuangxinmycin, borrelidin, granaticin, furanomycin, ochratoxin A, and cispentacin. In an embodiment, mupirocin and lithium carbonate are co-mixed as a simple cream.

In an embodiment, a combination treatment comprising a lithium compound described herein and chlorhexidine is administered to a subject to prevent, reduce, or
eradicate colonization of methicillin resistant *Staphylococcus aureus*. A combination of a lithium compound described herein and chlorhexidine can be formulated as a hand or body wash, a wound dressing, which can be in the form of barriers, membranes, or films, a gel, a lotion, a cream, an ointment, a surgical scrub, or a spray that can be sprayed on to skin or a wound as a protective layer. In an embodiment, a combination treatment comprising a lithium compound described herein, chlorhexidine, and mupirocin is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*. A combination of a lithium compound described herein, chlorhexidine, and mupirocin can be formulated as a hand or body wash, a wound dressing, which can be in the form of barriers, membranes, or films, a gel, a lotion, a cream, an ointment, a surgical scrub, or a spray that can be sprayed on to skin or a wound as a protective layer.

[00454] In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein and chlorhexidine is administered as a skin sanitizer and/or hand sanitizer to prevent infection by bacteria including, but not limited to, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Propionibacterium acnes, Staphylococcus epidermidis, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloaceae, Streptococcus pyogenes, corynebacterium species, enterococci, Proteus mirabilis, group D streptoccci, other gram-positive aerobes, and Bacteroides fragilis*. In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein and chlorhexidine is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi. In an embodiment, topical formulations of a combination treatment comprising a lithium compound described herein and chlorhexidine is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi and bacteria. In an embodiment, the skin sanitizer formulation and/or hand sanitizer formulation is aqueous based or glycerol based. In another embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.

[00455] In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein, chlorhexidine, and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection by bacteria including, but not limited to, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Propionibacterium acnes, Staphylococcus epidermidis, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloaceae, Streptococcus pyogenes, corynebacterium species, enterococci, Proteus mirabilis, group D streptoccci, other gram-positive aerobes, and Bacteroides fragilis*. In an embodiment, a topical formulation of a combination treatment comprising a
lithium compound described herein, chlorhexidine, and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi. In an embodiment, topical formulations of a combination treatment comprising a lithium compound described herein, chlorhexidine, and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi and bacteria. In an embodiment, the skin sanitizer formulation and/or hand sanitizer formulation is aqueous based or glycerol based. In another embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.

[00456] In an embodiment, a combination treatment comprising a lithium compound described herein and iodine is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*. A combination of a lithium compound described herein and iodine can be formulated as a hand or body wash, a wound dressing, which can be in the form of barriers, membranes, or films, a gel, a lotion, a cream, an ointment, a surgical scrub, or a spray that can be sprayed on to skin or a wound as a protective layer. In an embodiment, a combination treatment comprising a lithium compound described herein, iodine, and mupirocin is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*. A combination of a lithium compound described herein, iodine, and mupirocin can be formulated as a hand or body wash, a wound dressing, which can be in the form of barriers, membranes, or films, a gel, a lotion, a cream, an ointment, a surgical scrub, or a spray that can be sprayed on to skin or a wound as a protective layer.

[00457] In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein and iodine is administered as a skin sanitizer and/or hand sanitizer to prevent infection by bacteria including, but not limited to, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Streptococcus pyogenes*, *corynebacterium species*, enterococci, *Proteus mirabilis*, group D streptococci, *other gram-positive aerobes*, and *Bacteroides fragilis*. In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein and iodine is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi. In an embodiment, topical formulations of a combination treatment comprising a lithium compound described herein and iodine is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi and bacteria. In an embodiment, the skin sanitizer formulation and/or hand sanitizer formulation is aqueous based or glycerol based. In another
embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.

[00458] In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein, iodine, and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection by bacteria including, but not limited to, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Streptococcus pyogenes*, *corynebacterium species*, *enterococci*, *Proteus mirabilis*, group D streptococci, *other gram-positive aerobes*, and *Bacteroides fragilis*. In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein, iodine, and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi. In an embodiment, topical formulations of a combination treatment comprising a lithium compound described herein, iodine, and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi and bacteria. In an embodiment, the skin sanitizer formulation and/or hand sanitizer formulation is aqueous based or glycerol based. In another embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.

[00459] In an embodiment, a combination treatment comprising a lithium compound described herein and povidone-iodine is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*. A combination of a lithium compound described herein and povidone-iodine can be formulated as a hand or body wash, a wound dressing, which can be in the form of barriers, membranes, or films, a gel, a lotion, a cream, an ointment, a surgical scrub, or a spray that can be sprayed on to skin or a wound as a protective layer. In an embodiment, a combination treatment comprising a lithium compound described herein, povidone-iodine, and mupirocin is administered to a subject to prevent, reduce, or eradicate colonization of methicillin resistant *Staphylococcus aureus*. A combination of a lithium compound described herein, povidone-iodine, and mupirocin can be formulated as a hand or body wash, a wound dressing, which can be in the form of barriers, membranes, or films, a gel, a lotion, a cream, an ointment, a surgical scrub, or a spray that can be sprayed on to skin or a wound as a protective layer.

[00460] In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein and povidone-iodine is administered as a skin sanitizer and/or hand sanitizer to prevent infection by bacteria including, but not limited to, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium
acnes, Staphylococcus epidermidis, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae, Streptococcus pyogenes, corynebacterium species, enterococci, Proteus mirabilis, group D streptococci, other gram-positive aerobes, and Bacteroides fragilis. In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein and povidone-iodine is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi. In an embodiment, topical formulations of a combination treatment comprising a lithium compound described herein and povidone-iodine is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi and bacteria. In an embodiment, the skin sanitizer formulation and/or hand sanitizer formulation is aqueous based or glycerol based. In another embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.

[00461] In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein, povidone-iodine, and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection by bacteria including, but not limited to, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Propionibacterium acnes, Staphylococcus epidermidis, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae, Streptococcus pyogenes, corynebacterium species, enterococci, Proteus mirabilis, group D streptococci, other gram-positive aerobes, and Bacteroides fragilis. In an embodiment, a topical formulation of a combination treatment comprising a lithium compound described herein, povidone-iodine, and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi. In an embodiment, topical formulations of a combination treatment comprising a lithium compound described herein, povidone-iodine, and mupirocin is administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi and bacteria. In an embodiment, the skin sanitizer formulation and/or hand sanitizer formulation is aqueous based or glycerol based. In another embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.

[00462] In an embodiment, the combination treatment comprises lithium and an antibacterial agent selected from, e.g., benzyl alcohol, methyl paraben ethanol, isopropanol, glutaraldehyde, formaldehyde, chlorine compounds, iodine compounds, hydrogen peroxide, peracetic acid, ethylene oxide, triclocarban, chlorhexidine, alexidine, triclosan, hexachlorophene, polymeric biguanides, aminoglycoside antibiotics, glycopeptides, amphenicol antibiotics, ansamycin antibiotics, cephalosporins, cephemycins oxazolidinones, penicillins, quinolones, streptogamins, tetracyclins and analogs thereof, ampicilllin,
amoxicillin, ciprofloxacin, gentamycin, kanamycin, neomycin, penicillin G, streptomycin, sulfanilamide, and vancomycin, azithromycin, cefonicid, cefotetan, cephalothin, cephapirin, clortetracycline, clarithromycin, clindamycin, cycloserine, dalfopristin, doxycycline, erythromycin, linezolid, mupirocin, oxytetracycline, quinupristin, rifampin, spectinomycin, and trimethoprim, aminoglycoside antibiotics (e.g., apramycin, arbekacin, bambermycin, butirosin, dibekacin, neomycin, neomycin, undecylenate, netilmicin, paromomycin, ribostamycin, sisomicin, and spectinomycin), amphenicol antibiotics (e.g., azidamfenicol, chloramphenicol, florfenicol, and thiamphenicol), ansamycin antibiotics (e.g., rifamycin and rifampin), carbacephems (e.g., loracarbef), carbapenems (e.g., biapenem and imipenem), cephalosporins (e.g., cefadroxil, cefamandole, cefatrizine, cefazolin, cefpimizole, cefpiramide, and cefpirome), cephapirin, cefbuperazone, cefmetazole, and cefminox), folic acid analogs (e.g., trimethoprim), glycopeptides (e.g., vancomycin), lincosamides (e.g., clindamycin, and lincomycin), macrodides (e.g., azithromycin, carbomycin, clarithromycin, dirithromycin, erythromycin, and erythromycin acistrate), monobactams (e.g., aztreonam, carubonam, and tigemonam), nitrofurans (e.g., furaltadone, and furazolidone chloride), oxacephems (e.g., flomoxef, and moxalactam), oxazolidinones (e.g., linezolid), penicillins (e.g., amdinocillin, amdinocillin pivoxil, amoxicillin, bacampicillin, benzylpenicillinic acid, benzylpenicillin sodium, epicillin, fenbenicillin, floxacillin, penamaceillin, penethamate hydroiodide, penicillin O benethamine, penicillin 0, penicillin V, penicillin V benzathine, penicillin V hydrabamine, penimepcycline, and phencicillin potassium), quinolones and analogs thereof (e.g., cinoxacin, ciprofloxacin, clinafloxacin, flumequine, grepafloxacin, levofloxacin, and moxifloxacin), streptogramins (e.g., quinupristin and dalfopristin), sulfonamides (e.g., acetyl sulfamethoxypyrazine, benzylsulfamide, nopyrsulfamide, phthalysulfacetamide, sulfachlysoidine, and sulfacytine), sulfones (e.g., diethylsulfone, glucosulfone sodium, and solasulfone), and tetracyclines (e.g., apicycline, clortetracycline, clomocycline, and demeclocycline). Additional examples include cycloserine, mupirocin, tuberin amphomycin, bacitracin, capreomycin, colistin, enduracidin, enniomycin, and 2,4 diaminopyrimidines (e.g., brodimoprim), isoniazid, rifampin, pyrazinamide, ethambutol, streptomycin, erythromycin, azithromycin, clarithromycin, doxycycline, minocycline, clindamycin, ofloxacin, and chloramphenicol.

[00463] In some embodiments, the combination treatment comprises lithium and an antifungal agent selected from, e.g., 5-fluorocytosine, Abafungin, Acrisorcin, Amorolfine, Albacnazole, Albendazole, Amorolfine, Anidulafungin, Arasertaconazole, Azithromycin, Becliconazole, Benzodithiazole, Bifonazole, Butenafine, Butoconazole, Calbistrin,
In some embodiments, the combination treatment comprises lithium treatment in combination with one or more treatments selected from, e.g., cell therapy (such as a stem cell), a formulation for gene therapy (such as, e.g., a virus, virus-like particle, virosome), an antibody or antigen-binding fragment thereof, an herb, a vitamin (e.g., a form of vitamin E, a vitamin A derivative, such as, e.g., all-trans retinoic acid (ATRA), a B vitamin, such as, e.g., inositol, panthenol, or biotin, or a vitamin D3 analog), a mineral, essential oils, an antioxidant or free radical scavenger, amino acids or amino acid derivatives, a shampoo ingredient (e.g., ammonium chloride, ammonium lauryl sulfate, glycol, sodium laureth sulfate, sodium lauryl sulfate, ketoconazole, zinc pyrithione, selenium sulfide, coal tar, a salicylate derivative, dimethicone, or plant extracts or oils), a conditioning agent, a soap product, a moisturizer, a sunscreen, a waterproofing agent, a powder, talc, or silica, an oil-control agent, alpha-hydroxy acids, beta-hydroxy acids (e.g., salicylic acid), poly-hydroxy acids, benzoyl peroxide, antiperspirant ingredients, such as astringent salts (e.g., zinc salts, such as zinc pyrithione, inorganic or organic salts of aluminum, zirconium, zinc, and mixtures thereof, aluminum chloride, aluminum chlorohydrate, aluminum chlorohydrex, aluminum chlorohydrex PEG, aluminum chlorohydrex PG, aluminum dichlorohydrate, aluminum dichlorohydrex PEG, aluminum dichlorohydrex PG, aluminum sesquichlorohydrate, aluminum sesquichlorohydrex PEG, aluminum sesquichlorohydrex PG, aluminum sulfate, aluminum zirconium octachlorohydrate, aluminum zirconium octachlorohydr ex GLY (abbreviation for glycine), aluminum zirconium pentachlorohydrate, aluminum zirconium pentachlorohydrex GLY, aluminum zirconium tetra chlorohydrate, aluminum zirconium trichlorohydrate, aluminum zirconium tetrachlorohydrate GLY, and aluminum zirconium trichlorohydrate GLY, potassium aluminum sulphate, (also known as alum
(KA1(S0 4 )2 12H2O), aluminum undecylenoyl collagen amino acid, sodium aluminum lactate+ aluminum sulphate (Na2HAl(OOCCHOHCH(OH)6) + A 2(S0 4 )3), sodium aluminum chlorohydroxylactate, aluminum bromohydrate (Al2Br(OH)4nH2O), aluminum chloride (AlCl3nH2O), complexes of zinc salt and of sodium salt, complexes of lanthanum and cerium, and the aluminum salt of lipoamino acids (R—CO—NH—CHR’—CO—OA1—(OH)2 with R = C6-i and R’-amino acid), retinoids (e.g., retinoic acid, retinol, retinal, or retinyl esters), sunscreens (e.g., derivatives of para-aminobenzoic acid (PABA), cinnamate and salicylate, avobenzophenone (Parsol 1789®), octyl methoxycinnamate (Parsol™ MCX) and 2-hydroxy-4-methoxy benzophenone (also known as oxybenzone and available as Benzophenone™, and preservatives), an anti-acne medication, an anti-age cream, a sebum production inhibitor and/or pore size reducing agent (e.g., carboxyalkylates of branched alcohols and/or alkoxylates thereof, e.g., tridecyl carboxyalkylates, cerulenin or a cerulenin analog, including pharmaceutically acceptable salts or solvates thereof, another fatty acid synthase inhibitor, such as triclosan or analogs thereof, a polyphenol extracted from green tea (EGCG), available from Sigma Corporation (St. Louis, Missouri), or a-methylene- γ-butyrolactone), a massage agent, an exfoliant, an anti-itch agent, a pro-inflammatory agent, an immunostimulant (e.g., interferon, cytokines, agonists or antagonists of various ligands, receptors and signal transduction molecules of the immune system, immunostimulatory nucleic acids, an adjuvant that stimulates the immune response and/or which causes a depot effect). In certain embodiments, adjuvants and/or other stimulators of local cytokines are used in conjunction with the intermittent lithium treatment or pulse lithium treatment.

Without being bound by any theory, one rationale for administering adjuvants and/or other stimulators of local cytokines in conjunction with the lithium treatment is that the production of local cytokines may induce changes in an infected wound and recruit new stem cells to the wound.

[00465] In yet another embodiment, pharmaceutical compositions of the invention are used in combination with one or more adjuvants. The adjuvant(s) can be administered separately or present in a pharmaceutical composition in admixture with pharmaceutical compositions of the invention of the invention. A systemic adjuvant is an adjuvant that can be delivered parenterally. Systemic adjuvants include adjuvants that creates a depot effect, adjuvants that stimulate the immune system and adjuvants that do both. An adjuvant that creates a depot effect as used herein is an adjuvant that causes the antigen to be slowly released in the body, thus prolonging the exposure of immune cells to the antigen. This class of adjuvants includes but is not limited to alum (e.g., aluminum hydroxide, aluminum
phosphate); or emulsion-based formulations including mineral oil, non-mineral oil, water-in-oil or oil-in-water-in-oil emulsion, oil-in-water emulsions such as Seppic ISA series of Montanide adjuvants (e.g., Montanide ISA 720, AirLiquide, Paris, France); MF-59 (a squalene-in-water emulsion stabilized with Span 85 and Tween 80; Chiron Corporation, Emeryville, Calif; and PROVAX (an oil-in-water emulsion containing a stabilizing detergent and a micelle-forming agent; IDEC, Pharmaceuticals Corporation, San Diego, Calif).

Other adjuvants stimulate the immune system, for instance, cause an immune cell to produce and secrete cytokines or IgG. This class of adjuvants includes but is not limited to immunostimulatory nucleic acids, such as CpG oligonucleotides; saponins purified from the bark of the Q. saponaria tree, such as QS21 (Antigenics, MA); derivatives of lipopolysaccharides (LPS) such as monophosphoryl lipid A (MPL; Ribi ImmunoChem Research, Inc., Hamilton, Mont.), muramyl dipeptide (MDP; Ribi) and threonyl-muramyl dipeptide (t-MDP; Ribi).

Other systemic adjuvants are adjuvants that create a depot effect and stimulate the immune system. These compounds are those compounds which have both of the above-identified functions of systemic adjuvants. This class of adjuvants includes but is not limited to ISCOMs (Immunostimulating complexes which contain mixed saponins, lipids and form virus-sized particles with pores that can hold antigen; CSL, Melbourne, Australia); AS01B (GlaxoSmithKline adjuvant system) which is a liposome based formulation containing MPL and QS21; AS02A (GlaxoSmithKline adjuvant system) which is an oil-in-water-based formulation containing MPL and QS21, and AS15 (GlaxoSmithKline adjuvant system) which is a formulation containing QS21, CpG oligonucleotides and MPL.

The mucosal adjuvants useful according to the invention are adjuvants that are capable of inducing a mucosal immune response in a subject when administered to a mucosal surface in conjunction with a pharmaceutical composition of the invention. Mucosal adjuvants include but are not limited to CpG nucleic acids (e.g. International Publication No. WO 99/61056) and Bacterial toxins: e.g., Cholera toxin (CT).

In other embodiments, the combination treatment comprises lithium in combination with a cell cycle regulator, a hormonal agonist, a hormonal antagonist (e.g., flutamide, bicalutamide, tamoxifen, raloxifene, leuprolide acetate (LUPRON), LH-RH antagonists), an inhibitor of hormone biosynthesis and processing, a steroid (e.g., dexamethasone, retinoids, deltoids, betamethasone, Cortisol, cortisolone, prednisone, dehydrotestosterone, glucocorticoids, hydrocortisone, mineralocorticoids, estrogen, testosterone, progestins), antiestrogens (e.g., mifepristone, onapristone), an antiandrogen
(e.g., cyproterone acetate), an antiestrogen, an antihistamine (e.g., mepyramine, diphenhydramine, and antazolone), an anti-inflammatory (e.g., corticosteroids (such as, e.g., Dermatop®), NTHEs, and COX-2 inhibitors, adrenocorticoids, beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, prednisone, hydrocortisone), an anesthetic (e.g., vocal anesthesia, lidocaine, bupivacaine, etidocaine, etc., with or without epinephrine or sodium bicarbonate) a retinoid (e.g., 13-cis-retinoic acid, adapalene, all-trans-retinoic acid, and etretinate), isotretinoin, PMMA, Restylane, poly-L-lactic acid, collagen, hyaluronic acid, which may be present in microspheres, or other skin fillers, a cosmetic (e.g., intended to increase soft tissue volume), an immunosuppressant (e.g., cyclosporine, tacrolimus, rapamycin, everolimus, and pimecrolimus), an anti-cancer agent (such as, e.g., fluorouracil (5-FU or f5U) or other pyrimidine analogs, methotrexate, cyclophosphamide, vincristine), a mood stabilizer (e.g., valproic acid or carbamazepine), an antimitabolite, non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, diclofenac, and COX-2 inhibitors), pain relievers, leukotriene antagonists (e.g., montelukast, methyl xanithenes, zafirlukast, and zileuton), beta2-agonists (e.g., albuterol, biterol, fenoterol, isoetharie, metaproterenol, pirbuterol, salbutamol, terbutalin formoterol, salmeterol, and salbutamol terbutaline), anticholinergic agents (e.g., ipratropium bromide and oxtropium bromide), sulphasalazine, penicillamine, dapsone, antihistamines, anti-fungal agents, antimalarial agents (e.g., hydroxychloroquine), anti-viral agents (e.g., nucleoside analogs (e.g., zidovudine, acyclovir, gangcyclovir, vidarabine, idoxuridine, trifluridine, and ribavirin), foscarnet, amantadine, rimantadine, saquinavir, indinavir, ritonavir, and AZT), antibiotics (e.g., a polymyxin, dactinomycin (formerly actinomycin), bleomycin, erythromycin, penicillin, mithramycin, and anthramycin (AMC)), and anti-microbials (e.g., benzyl benzoate, benzalkonium chloride, benzoic acid, benzyl alcohol, butylparaben, ethylparaben, methylparaben, propylparaben, camphorated metacresol, camphorated phenol, hexylresorcinol, methylbenzethonium chloride, cetrimide, chlorhexidine, chlorobutanol, chlorocresol, cresol, glycerin, imidurea, phenol, phenoxyethanol, phenylethylalcohol, phenylmercuric acetate, phenylmercuric borate, phenylmercuric nitrate, potassium sorbate, sodium benzoate, sodium propionate, sorbic acid, and thiomersal (thimerosal)).

[00470] In some embodiments, the combination treatment comprises lithium treatment in combination with one or more narcotic analgesics, selected from the group of, e.g., alfentanil, benzylmorphine, codeine, codeine methyl bromide; codeine phosphate, codeine sulfate, desomorphine, dihydrocodeine, dihydrocodeinone enol acetate, dihydromorphine, ethylmorphine, hydrocodone, hydromorphone, methadone hydrochloride, morphine,
morphine hydrochloride, morphine sulfate, nicomorphine, normethadone, normorphine, opium, oxycodone, oxymorphone, phenoperidine, and propiram. In some embodiments, the combination treatment comprises intermittent lithium treatment or a pulse lithium treatment in combination with one or more non-narcotic analgesics, selected from the group of, e.g., aceclofenac, acetaminophen, acetanilide, acetylsalicylsalicylic acid; aspirin, carbamazepine, dihydroxyaluminum acetylsalicylate, fenoprofen, fluproquazone, ibufenac, indomethacin, ketorolac, magnesium acetylsalicylate, morpholine salicylate, naproxen, phenacetin, phenyl salicylate, salacetamide, salicyclicamide, sodium salicylate, and tolfenamic acid. Other pain treatments that may be used in combination with the lithium treatments described herein include nerve blocks or non-traditional pain medications, such as, e.g., Lyrica (pregabalin) or Neurontin (gabapentin).

[00471] In some embodiments, a combination treatment comprises a lithium treatment in combination with an agent that also modulates wound healing, including any treatment known in the art to modulate wound healing, including any treatment described in International Patent Application No. PCT/US20 10/048439, which is incorporated by reference in its entirety. In one embodiment, the lithium treatment is administered in combination with a treatment that enhances one or more of the steps of wound healing discussed above, including any treatment described herein or known in the art to enhance wound healing. By enhancement of a step of wound healing or enhancement of wound healing is meant the hastening of healing, improvement of healing, or reduction of scarring, etc.

[00472] In some embodiments, the lithium treatment is administered in combination with a wound dressing or skin replacement, such as, for example, gauze, calcium-alginates, impregnated gauzes, films, foams, hydrogels, hydrocolloids, adsorptive powders and pastes, silicone, mechanical vacuum, dermal matrix replacements, dermal living replacements, or skin living replacements, a collagen dressing, cadaveric skin, or other matrix useful to promote healing of the wound such as described herein or known in the art. See, e.g., Table 10.3 in Lorenz & Longaker, which is incorporated by reference herein in its entirety.

[00473] In some embodiments, the lithium treatment is administered in combination with a pain reliever, antibiotic and anti-bacterial use or other anti-infectives (such as, e.g., tea tree oil), debridement, drainage of wound fluid, mechanical removal of bacteria, removal of devitalized tissue (such as, e.g., by surgery or maggot therapy), irrigation (e.g., by pulsed lavage), vacuum-assisted closure (otherwise referred to as negative pressure wound therapy), warming, oxygenation (e.g., using hyperbaric oxygen therapy), antioxidant therapy,
revascularization therapy, moist wound healing, removing mechanical stress, use of elastase inhibitors, or adding cells or other materials to secrete or enhance levels of healing factors.

[00474] In some embodiments, the lithium treatment is administered in combination with the upregulation of endogenous growth factors or exogenous application of growth factors, which may accelerate normal healing and improve healing efficacy. Such growth factors include, but are not limited to, vascular endothelial growth factor (VEGF), insulin-like growth factor 1-2 (IGF), PDGF, transforming growth factor-β (TGF-β), epidermal growth factor (EGF), EGF-receptor, members of the FGF family, and others described herein and listed in, e.g., Table 10.2 in Lorenz & Longaker, which is incorporated by reference herein in its entirety. Such growth factors can be applied exogenously or may be applied by spreading onto the wound a gel of the patient’s own platelets, implanting cultured keratinocytes into the wound, or treating the wound with artificial skin substitutes that have fibroblasts and keratinocytes in a matrix of collagen.

[00475] In some embodiments, the lithium treatment is administered in combination with a treatment that reduces the time it takes for an infected wound to heal or that reduces the extent of the wound. Such treatments are known in the art and include, for example, periodic rotation of the patient or wounded tissue or use of an air mattress, use of a lower pressure cast or relieving excessive suture tension, cleansing of the wound, debridement of tissue, particularly necrotic tissue, improvement of circulation and oxygen delivery to the tissue by, e.g., hyperbaric oxygen therapy or other oxygen administration, whirlpool therapy, ultrasound therapy, electrical stimulation, magnetic therapy have been utilized to aid the body in healing wounds coverage of wound with vascularized tissue, revascularization of the wounded tissue, treatment of circulatory obstruction or other treatment that improves circulation, treatment of ischemia, edema, or hypoxia, or improvement of the hematocrit (e.g., to at least 15%). Other treatments to enhance wound healing that may be used in combination with the lithium treatments described herein include treatment of tissue necrosis, treatment or prevention of infection (e.g., with antibiotics such as povidone-iodine, chlorhexidine gluconate, hexachlorophene, or silver sulfadiazine and others described herein (particularly for burn wound care), irrigation (e.g., with saline), and/or debridement), improvement of nutrition (e.g., increasing intake of vitamins, e.g., vitamin A, C, B1, B2, B5, or B6, or trace metals, such as, e.g., zinc and copper, amino acids such as arginine, glutamine, or Bromelain, Curcumin, etc.), herbal supplements (e.g., Aloe Vera, Centella), diabetes treatment (for example, to improve vascular conditions, or by administering glucose), skin graft, treatment with hormones (such as estrogen) or treatment with growth factors (e.g.,
epidermal growth factor, Insulin-like Growth Factor, human growth hormone, fibroblast
growth factor, vascular endothelial growth factor, interleukin-6, and interleukin-10).

[00476] In another embodiment, the lithium treatment is administered in combination
with a treatment that slows the natural adult wound healing process. In certain embodiments,
such combination treatments are used in the presence of a sterile wound dressing that
obviates the need to heal the wound quickly (for example, in natural wound healing, the
wound heals quickly in order to avoid infection). In one embodiment, the lithium treatment
is administered in combination with a treatment that causes the postnatal wound healing
process to resemble the fetal wound healing process. In some embodiments, this is
accomplished by placing the wounded skin into a womb-like environment, for example, using
a dressing and/or heat.

[00477] In one embodiment, the lithium treatment is administered in combination with an
agent that reduces or inhibits the inflammatory phase of wound healing, using, e.g., an anti-
inflammatory agent such as a NSAID or a topical glucocorticoids, an anti-androgen, or an
antagonist of TNFa, TGFp, NFkB, IL-1, IL-6, IL-8, IL-10, IL-18, or an antagonist of one or
more other proinflammatory cytokines. In an alternative embodiment, the lithium treatment
is administered in combination with an agent that slows the wound healing process by
extending the inflammatory phase, e.g., an androgen (see, e.g., Gilliver et al, 2007, Clin.
Dermatol. 25:56-62). In one embodiment, the treatment is administered in combination with
an agent that suppresses the proliferative phase of wound healing, or the maturation and
remodeling phase of wound healing. For example, in one embodiment, the treatment is
administered in combination with an agent that slows or interferes with fibrin deposition,
clotting caused by fibrin, or fibrin-induced immunity. In one embodiment, the treatment is
administered in combination with a treatment that inhibits the activity of fibrinogen. In one
embodiment, the treatment is administered in combination with an agent that decreases the
activity of myofibroblasts. In particular embodiments, the treatment is administered in
combination with a treatment that reduces collagen synthesis, deposition, or accumulation,
for example, collagenases. In particular embodiments, the treatment is administered in
combination with a treatment that maintains the wound in an open state for a longer than
normal period of time. In another embodiment, a treatment is administered in combination
with rapamycin or corticosteroids.

[00478] For example, in one embodiment, a biocompatible, synthetic skin substitute is
placed on the wound, especially if the wound is deep, covers large area, and is bulk ablated.
This process can help minimize or prevent the rapid wound contraction that occurs after loss
of a large area of tissue, frequently culminating in scar tissue formation and loss of skin function. In one embodiment, the biocompatible synthetic skin substitute is impregnated with depots of a slow releasing lithium formulation described herein. This method of treatment may enable treating a large area in one session at the treatment clinic. In some embodiments, other molecules are also co-eluted at the site through the skin substitute, such as, e.g., anesthetics and antibiotics, to prevent further pain and minimization of infection, or any other compound described herein. The skin substitute, in the presence or absence of a lithium compound and/or other compounds described herein, may also be pre-cooled and applied to the wound to provide a feeling of comfort to the patient. This mode of lithium or other compound application may prevent the lithium or other compound from being cleared away from the wound site as the wound heals.

[00479] In some embodiments, a lithium treatment is administered in combination with a treatment that also reduces the appearance or extent of scarring, including any treatment known in the art to modulate wound healing, including any treatment described in International Patent Application No. PCT/US20 10/048439. In some embodiments, a lithium treatment is administered in combination with a treatment that improves the appearance and/or function of scarred skin, including any such treatment described herein or known in the art. For example, in one embodiment, a lithium treatment is administered in combination with a treatment for scar revision or a treatment that improves the texture or appearance of skin, such as skin graft, serial expansion of surrounding skin, laser treatment, or dermabrasion as described in Section 5.5. In some embodiments, a pulse lithium treatment is administered in combination with re-excision with subsequent healing by primary intention, treatment with steroids (e.g., corticosteroid injection), silicone scar treatments (e.g., dimethicone silicone gel or silicone sheeting), use of porcine fillers or other cosmetic fillers (e.g., inserted under atrophic scars), ribosomal 6 kinase (RSK) antagonists, antagonists of pro-inflammatory cytokines, such as TGFP2 or TNF, osteopontin antagonists, the use of pressure garments, needling, dermabrasion, collagen injections, low-dose radiotherapy, or vitamins (e.g., vitamin E or vitamin C or its esters).

[00480] In some embodiments, a lithium treatment is administered in combination with a treatment that also reduces surgical scarring, e.g., by placement of elective incisions parallel to the natural lines of skin tension (Langer's lines) or by applying sutures in a "zigzag" pattern. In some embodiments, the lithium treatment is administered in combination with a treatment of wounds that also minimizes scarring, by, for example, administering physical therapy to a subject (e.g., range-of-motion exercises), reducing separation of wound edges,
minimizing collagen synthesis, deposition, or accumulation or otherwise causing the process of healing by secondary intention to better resemble healing by primary intention. Other interventions that reduce scarring and which may be used in combination with the methods described herein include meticulous hemostasis of wound healing (including control of bleeding by coagulation, desiccation, or ligation techniques), which decreases amount of hematoma to be cleared and thus decreases the inflammatory phase of wound healing, exercising care during dermal closure (e.g., avoiding forceps crush-injury of the epidermis and dermis), avoidance of necrotic tissue at the wound edge, which reduces inflammation, cleansing of the wound, and applying skin grafts where needed.

5.5.1 COMBINATION TREATMENTS COMPRISING INTEGUMENTAL PERTURBATION

[00481] The lithium treatments described herein may be administered in combination with methods of integumental perturbation. In a particular embodiment, a lithium treatment described herein is used together with a method for integumental perturbation in order to improve the skin, for example, to promote wound healing or scar revision.

[00482] Wounding is itself a form of integumental perturbation. Consequently, in one embodiment, a lithium treatment described herein is administered in combination with a method of scar revision (which involves wounding), for example, serial expansion, surgical excision of the wound and surrounding normal tissue (e.g., in which a jagged surgical incision is created so that the lines of tension of the skin are parallel to the incisions), dermabrasion to remove epidermis and papillary dermis, needling, subcision, or laser, such as pulsed dye laser or nonablative fractional laser.

[00483] In certain embodiments, enhancement of wound healing or scar revision is accomplished by lithium treatment alone, for example, in acutely wounded skin or skin affected by a chronic non-healing wound, i.e., skin already subjected to integumental perturbation. In other embodiments, enhancement of wound healing or scar revision is accomplished by a combination of integumental perturbation and a lithium treatment. Integumental perturbation can be used to induce, for example, a burn, excision, dermabrasion, full-thickness excision, or other form of abrasion or wound.

[00484] Mechanical means of integumental perturbation include, for example, use of sandpaper, a felt wheel, ultrasound, supersonically accelerated mixture of saline and oxygen, tape-stripping, spiky patch, or peels. Chemical means of integumental perturbation can be achieved, for example, using phenol, trichloroacetic acid, or ascorbic acid. Electromagnetic
means of integumental perturbation include, for example, use of a laser (e.g., using lasers, such as those that deliver ablative, non-ablative, fractional, non-fractional, superficial or deep treatment, and/or are C0₂-based, or Erbium-YAG-based, neodymium:yttrium aluminum garnet (Nd:YAG) laser, etc.). Integumental perturbation can also be achieved through, for example, the use of visible, infrared, ultraviolet, radio, or X-ray irradiation. In one embodiment, integumental perturbation is by light energy, such as described in Leavitt et al., 2009, Clin. Drug. Invest. 29:283-292. Integumental perturbation can also be achieved through surgery, for example, a biopsy, a skin transplant, skin graft, follicular unit extraction, hair transplant, cosmetic surgery, open-heart surgery, etc.

[00485] In some embodiments, integumental perturbation is by laser treatment. Exemplary laser treatments for integumental perturbation include Fraxel, laser abrasion, Erbium-YAG laser, Ultrapulse C0₂ fractional laser, Ultrapulse C0₂ ablative laser, Smooth Peel Full-ablation Erbium laser (Candela).

[00486] In one embodiment, integumental perturbation by laser treatment is by a fractional laser. One example of a fractional laser treatment is treatment with an Erbium-YAG laser at around 1540 nm or around 1550 nm (for example, using a Fraxel® laser (Solta Medical)). Another example is a C0₂ laser at 10,600 nm. In some embodiments, the laser treatment is ablative and fractional. For example, fractional tissue ablation can be achieved using a C0₂ laser at 10,600 nm or an Erbium-YAG laser at 2940 nm (e.g., the Lux 2940 laser, Pixel laser, or ProFractional laser). In another embodiment, the mode of laser treatment is non-ablative. Lasers that are non-ablative include the pulsed dye laser (vascular)(at, e.g., 585-595 nm), the 1064 Nd:YAG laser, or the Erbium-YAG laser at 1540 nm or 1550 nm (e.g., the Fraxel® laser). In some embodiments, the mode of laser treatment is fractional and non-ablative, which can be accomplished, e.g., using the Erbium-YAG laser with an emission at or around 1540 nm or 1550 nm.

[00487] In some embodiments, integumental perturbation is by dermabrasion (also referred to herein as "DA"). Dermabrasion may be carried out using any technique known in the art or as described herein, for example, using the novel dermabrasion tip described herein. In certain embodiments, dermabrasion may be carried out using standard DA with aluminum oxide crystals using the Asepctico Econo-Dermabrader, Advance Microderm DX system, or M2-T system; standard DA with Bell Hand Engine with diamond fraize; etc.

[00488] For example, in some embodiments, DA is carried out using an abrasive wheel. In some embodiments, DA with an abrasive wheel is used in order to achieve pinpoint bleeding. In other embodiments, DA may be carried out using an abrasive wheel to achieve
larger globules of bleeding and frayed collagen. In other embodiments, non-powered devices such as abrasive cloths can also be used to achieve the DA, with the optional achievement of the same endpoint(s).

[00489] In some embodiments, DA is accomplished using a device typically used for microdermabrasion.

[00490] In some embodiments, DA is accomplished by removal of surface skin by particle bombardment (also referred to herein as "particle mediated dermabrasion" ("PMDA")), for example, with alumina-, ice- or silica-based particles. In some such embodiments, micron-sized particles are propelled toward the surface of the skin via short strokes of a handpiece, such as a particle gun, as known in the art. The velocity of particles is controlled through positive or negative pressure. The depth of skin removed by particle bombardment DA (e.g., PMDA) is a function of the volume of particles impacting the skin, the suction or positive pressure, the speed of movement of the handpiece, and the number of passes per area of the skin.

[00491] In some embodiments, integumental perturbation by one or more of the aforementioned methods achieves removal of part or all of the epidermis. In some embodiments, integumental perturbation by one or more of the aforementioned methods achieves removal of part of the epidermis. In some embodiments, integumental perturbation removes the entire epidermis. In some embodiments, integumental perturbation disrupts the papillary dermis. In some embodiments, integumental perturbation removes the papillary dermis. In some embodiments, integumental perturbation removes the reticular dermis. The depth of integumental perturbation depends on the thickness of the skin at a particular treatment area. For example, the skin of the eyelid is significantly thinner than that of the scalp. The occurrence of pinpoint bleeding indicates that the epidermis and portions of the dermis have been removed. Deeper penetration can result in much more bleeding, and the perturbation can go as deeps as the hypodermis.

[00492] In some embodiments, integumental perturbation by one or more of the aforementioned methods is to a skin depth of 60 µm. In some embodiments, integumental perturbation is to a skin depth of 60-100 µm. In some embodiments, integumental perturbation is to a skin depth of 100 µm. In some embodiments, integumental perturbation is to a skin depth of 150 µm. In some embodiments, integumental perturbation is to a skin depth of 100-500 µm. In some embodiments, integumental perturbation is to a skin depth of less than 500 µm. In some embodiments, integumental perturbation is to a skin depth of 500-1000 µm. In some embodiments, integumental perturbation is to a skin depth of 1 mm or
more. In some embodiments, integumental perturbation is to a skin depth of 1 mm to 3 mm. In some embodiments, integumental perturbation is to a skin depth of 1 mm to 5 mm.

5.5.2 COMBINATION TREATMENTS TO INCREASE HAIR FOLLICLES

[00493] The approaches described herein permit wound healing and scar revision under sterile and controlled conditions that recreates and harnesses the fetal skin's plastic and regenerative capacity. For example, the present invention is based, in part, on the appreciation that hair follicles play a role in wound healing. Inducing the formation of new hair follicles in wounds, or enhancing the entry of hair follicles into wounds (for example, by transplanting hair follicles into wounds) may harness their regenerative capacity and provide a transformational approach to scar revision and the management of wounds.

[00494] For example, the invention is based in part on the discovery that there is a correlation between the extent of wound contraction and the deposition of adnexal structures, such as new hair follicles, in wounded areas. In experiments to assess the effect of 8% topical lithium on hair follicle neogenesis following full thickness excision wounding of mouse skin, it was found that there was a direct relationship between increased number of hair follicles and decreased wound size. Thus, without being bound by any theory for how the invention works, lithium treatments may promote wound healing and scar revision by, at least in part, promoting the entry of hair follicles into the wound as it heals. This may occur by inducing the generation of new hair follicles and/or promoting migration of hair follicles into the wound site.

[00495] In addition to the foregoing reasons, it may be advantageous to promote hair growth in wounded skin (which may be wounded as part of the process of scar revision) in order to promote proper hair patterning in the skin (e.g., scalp, arm, leg, chest, etc.) as the wound heals.

[00496] Thus, in certain embodiments, a lithium treatment described herein may be in combination with a method for enhancing hair growth or preventing or delaying hair loss. For example, the lithium treatments, e.g., a pulse or intermittent lithium treatment, described herein can be administered concurrently or alternating sequentially with one or more of the following treatments that prevent follicle senescence, for example, anti-oxidants such as glutathione, ascorbic acid, tocopherol, uric acid, or polyphenol antioxidants); inhibitors of reactive oxygen species (ROS) generation, such as superoxide dismutase inhibitors; stimulators of ROS breakdown, such as selenium; mTOR inhibitors, such as rapamycin; or
sirtuins or activators thereof, such as resveratrol, or other SIRT1, SIRT3 activators, or nicotinamide inhibitors.

[00497] The lithium treatments, e.g., an intermittent lithium treatments or a pulse lithium treatment, provided herein can also be administered concurrently or alternating sequentially with one or more of the following treatments that promote hair growth: minoxidil, kopexil (for example, the product Keranique™), finasteride, bimatoprost (Latisse), CaCl₂, or adenosine, or techniques of integumental perturbation such as, e.g., by mechanical means, chemical means, electromagnetic means (e.g., using a laser such as one that delivers ablative, non-ablative, non-fractional, superficial, or deep treatment, and/or are C0₂-based, or Erbium-YAG-based, or neodymium:yttrium aluminum garnet (Nd:YAG) laser, etc.), irradiation, radio frequency (RF) ablation, or surgical procedures (e.g., hair transplantation, strip harvesting, follicular unit extraction (FUE), scalp reduction, etc.).

[00498] Treatments that promote hair growth, or, alternatively, treatments that prevent hair growth, may also be used in combination with a lithium treatment, e.g., an intermittent lithium treatment or a pulse lithium treatment, described herein in order to promote the establishment of desired hair patterning in the healed wound or revised scar, thereby improving the appearance of the treated skin. For example, treatments that regulate gender-specific specialized human hair follicles, including those under the influence of sex-steroid regulation, or that regulate the differentiation of stem cells into gender-specific specialized human hair follicles, possibly resulting in follicles having features that are different from natural follicles in the target location of skin (e.g., normal sized follicles with terminal hair where previously miniaturized follicles with vellus hair were present) may be administered. For example, treatment of grafted skin with a combination of lithium and a modulator of specific hair patterning may reduce donor dominance and enhance the ability of the graft to acquire properties of the recipient site. Thus, a lithium treatment, e.g., an intermittent lithium treatment or a pulse lithium treatment, may be used concurrently or in sequential combination with either a treatment that enhances hair growth (described above) or a cytotoxic drug, a hair growth retardant, such as eflornithine HC1 (Vaniqa), 5-fluorouracil (5-FU) (e.g., Efudex 5% cream), or other epilation or depilation methods to prevent or reduce hair growth.

5.5.3 REGIMENS FOR COMBINATION TREATMENTS

[00499] In certain embodiments, lithium treatment in combination with the aforementioned methods and agents improves the effectiveness of these methods, making the treatment more effective, efficient, cost-effective, pain-free, and/or user friendly. For
example, fewer treatments may be required. In certain embodiments, a conventional antibiotic, wound healing, or scar revision treatment on its own is not cosmetically satisfactory, does not adequately restore function of the skin, or the benefits are too short-lived. When one of these treatments is combined with a pulse lithium treatment, the treated skin area may be more cosmetically satisfactory, the effects of the treatment longer lasting, or skin function is restored.

[00500] For any of the combination treatments described above, in specific embodiments, the lithium treatment can be administered prior to, concurrently with, or subsequent to the administration of a second (or third, or more) treatment.

[00501] In one embodiment, the lithium treatment is administered to a subject at reasonably the same time as the other treatment. This method provides that the two administrations are performed within a time frame of less than one minute to about five minutes, or up to about sixty minutes from each other, for example, at the same doctor's visit.

[00502] In another embodiment, the lithium treatment and other treatment are administered at exactly the same time.

[00503] In yet another embodiment, the lithium treatment and the other treatment are administered in a sequence and within a time interval such that lithium treatment and the other treatment can act together to provide an increased benefit than if they were administered alone. In another embodiment, the lithium treatment and other treatment are administered sufficiently close in time so as to provide the desired outcome. Each can be administered simultaneously or separately, in any appropriate form and by any suitable route. In one embodiment, the lithium treatment and the other treatment are administered by different routes of administration. In an alternate embodiment, each is administered by the same route of administration. The lithium treatment can be administered at the same or different sites of the subject's body. When administered simultaneously, the lithium treatment and the other treatment may or may not be administered in admixture or at the same site of administration by the same route of administration.

[00504] In various embodiments, the lithium treatment and the other treatment are administered less than 1 hour apart, at about 1 hour apart, 1 hour to 2 hours apart, 2 hours to 3 hours apart, 3 hours to 4 hours apart, 4 hours to 5 hours apart, 5 hours to 6 hours apart, 6 hours to 7 hours apart, 7 hours to 8 hours apart, 8 hours to 9 hours apart, 9 hours to 10 hours apart, 10 hours to 11 hours apart, 11 hours to 12 hours apart, no more than 24 hours apart or no more than 48 hours apart. In other embodiments, the lithium treatment and other treatment are administered 2 to 4 days apart, 4 to 6 days apart, 1 week apart, 1 to 2 weeks
apart, 2 to 4 weeks apart, one month apart, 1 to 2 months apart, 2 to 3 months apart, 3 to 4 months apart, 6 months apart, or one year or more apart. In some embodiments, the lithium treatment and the other treatment are administered in a time frame where both are still active. One skilled in the art would be able to determine such a time frame by determining the half life of each administered component.

[00505] In one embodiment, the lithium treatment and the other treatment are administered within the same patient visit. In one embodiment, the lithium treatment is administered prior to the administration of the other treatment. In an alternate embodiment, the lithium treatment is administered subsequent to the administration of the other treatment.

[00506] In certain embodiments, the lithium treatment and the other treatment are cyclically administered to a subject. Cycling treatment involves the administration of the lithium treatment for a period of time, followed by the administration of the other treatment for a period of time and repeating this sequential administration. The first treatment may be with the lithium treatment or with the other treatment, depending on the subject's prior treatment history and the intended outcome. Not only does such cycling treatment have the advantages described herein (attributable, at least in part, to the synchronization of the hair and/or Follicle Cycle), cycling treatment can also reduce the development of resistance to one or more of the treatments, avoid or reduce the side effects of one of the treatments, and/or improve the efficacy of the treatment. In such embodiments, alternating administration of the lithium treatment may be followed by the administration of another treatment (or vice versa) 1 year later, 6 months later, 3 months later, 1 month later, 3 weeks later, 2 weeks later, 1 week later, 4 to 6 days later, 2 to 4 days later, or 1 to 2 days later, wherein such a cycle may be repeated as many times as desired. In certain embodiments, the lithium treatment and the other treatment are alternately administered in a cycle of 3 weeks or less, once every two weeks, once every 10 days or once every week. Such time frames can be extended or reduced depending on whether a controlled release formulation of either the lithium compound or the other treatment formulation is used, and/or depending on the progress of the treatment course. See the examples in Section 6 to 9 for specific treatment variations.

[00507] For embodiments in which the lithium treatment accompanies skin or hair transplantation (e.g., follicular unit extraction), an area of skin that was pre-treated with lithium (and optionally another treatment) is used as a source for transplanted skin or follicles. Treatment with lithium at the wound(s) from which transplanted tissue was obtained and/or the site of implantation is initiated for one week, and then discontinued and optionally followed by another treatment.
5.6 PREVENTION AND TREATMENT OF MICROBIAL INFECTIONS

[00508] In accordance with the invention, a pharmaceutical composition which comprises a lithium compound described herein, is administered to treat a subject with a microbial infection, i.e., a bacterial infection, a fungal infection, a yeast infection, or a parasitic infection. In one embodiment, "treatment" or "treating" refers to an amelioration of a microbial infection, or at least one discernible symptom thereof. In another embodiment, "treatment" or "treating" refers to an amelioration of at least one measurable physical parameter associated with a microbial infection, not necessarily discernible by the subject. In yet another embodiment, "treatment" or "treating" refers to inhibiting the progression of a microbial infection, either physically, e.g., stabilization of a discernible symptom, physiologically, e.g., stabilization of a physical parameter, or both.

[00509] In certain embodiments, the pharmaceutical compositions of the present invention are administered to a subject as a preventative measure against such microbial infections. As used herein, "prevention" or "preventing" refers to a reduction of the risk of acquiring a given microbial infection. In one mode of the embodiment, the pharmaceutical compositions of the present invention are administered as a preventative measure to a subject having a genetic predisposition to a microbial infection.

[00510] For example, in certain embodiments, administration of a pharmaceutical composition of the invention leads to an inhibition or reduction of infectious agents by at least 99%, at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, at least 50%, at least 45%, at least 40%, at least 35%, at least 30%, at least 25%, at least 20%, or at least 10% relative to the growth in absence of said pharmaceutical composition.

5.7 PATIENT POPULATIONS AND INDICATIONS

[00511] The lithium treatments described herein can be administered to any subject who may benefit from such treatment. The subject is preferably a human subject, including male, female, intermediate/ambiguous (e.g., XO), and transsexual subjects. In certain embodiments, the subject is a Caucasian subject. In certain embodiments, the subject is an African subject or an African-American subject. In certain embodiments, the subject is a human infant. In certain embodiments, the subject is a human child. In certain embodiments, the subject is a human adolescent. In certain embodiments, the subject is undergoing puberty. In certain embodiments, the subject is a young adult. In certain embodiment, the
subject is a middle-aged adult. In certain embodiments, the subject is a premenopausal adult. In certain embodiments, the subject is undergoing menopause. In certain embodiments, the subject is postmenopausal. In certain embodiments, the subject is elderly. In certain embodiments, the subject is a newbon human, a human of 1 year old or less, 2 years old or less, 2 years old, 5 years old, 5 to 10 years old, 10 to 15 years old, e.g., 12 years old, 15 to 20 years old, 20 to 25 years old, 25 to 30 years old, 30 years old or older, 30 to 35 years old, 35 years old or older, 35 to 40 years old, 40 years old or older, 40 to 45 years old, 45 to 50 years old, 50 years old or older, 50 to 55 years old, 55 to 60 years old, 60 years old or older, 60 to 65 years old, e.g., 65 years old, 65 to 70 years old, 70 to 75 years old, 75 to 80 years old, 80 to 85 years old, 85 to 90 years old, 90 to 95 years old or 95 years old or older. In some embodiments, the subject is a male 20 to 50 years old. In some embodiments, the subject is a male or female 12 to 40 years old. In some embodiments, the subject is not a female subject. In some embodiments, the subject is not pregnant or expecting to become pregnant. In some embodiments, the subject is not a pregnant female in the first trimester of pregnancy. In some embodiments, the subject is not breastfeeding.

[00512] In some embodiments, the lithium treatments described herein can be administered to a patient, preferably a mammal, most preferably a human, who has been diagnosed with a microbial infection, i.e., an infection caused by bacteria, fungi, yeast, or a parasite. The lithium treatments described herein can be administered to a patient, preferably a mammal, most preferably a human, who is suffering from a disorder associated with, characterized by or caused by a microbial infection, i.e., an infection caused by bacteria, fungi, yeast, or a parasite. Such subjects may or may not have been previously treated for the microbial infection or may have failed treatment.

[00513] In a specific embodiment, the lithium treatments described herein are administered as a preventative measure to a patient. According to this embodiment, the patient can have a genetic predisposition to a microbial infection or a disorder associated with, characterized by or caused by a microbial infection, such as a family history of the microbial infection or the disorder, or a non-genetic predisposition to the microbial infection or a disorder associated with, characterized by or caused by a microbial infection.

[00514] In accordance with the invention, the lithium compositions of the present invention are administered to humans in need of inhibition of microbial cells, e.g., bacterial cells, fungal cells, parasitic cells. In certain embodiments, the growth of such cells is inhibited.
In certain embodiments, the lithium treatments described herein are administered to a subject that is at risk of having a microbial infection. In certain embodiments, the lithium treatments described herein are administered to a subject that has or is at risk of developing a cutaneous microbial infection, e.g., a cutaneous bacterial infection or a cutaneous fungal infection. In certain embodiments, the lithium treatments described herein are administered to a subject that has or is at risk of developing an infected wound, e.g., a bacterial-infected wound or a fungal-infected wound.

In an embodiment, the lithium treatments described herein are administered to a subject to prevent, reduce, or eradicate colonization of one or more microbes. As used herein, the term "colonization" refers to presence, growth, and multiplication of an organism without observable clinical symptoms or immune reaction. In an embodiment, the lithium treatments described herein are administered to a subject to prevent, reduce, or eradicate colonization in or on one or more of the following: nares, anterior nares, axillae, chronic wounds, decubitus ulcer surface, perineum, around gastrostomy sites, around tracheostomy sites, in sputum, in urine, or in the bowel. In an embodiment, the lithium treatments described herein are administered to a subject to prevent, reduce, or eradicate nasal colonization. In an embodiment, the lithium treatments described herein are administered to a subject to prevent, reduce, or eradicate colonization of one or more microbes in order to reduce the risk of developing a subsequent infection in that subject. In an embodiment, the lithium treatments described herein are administered to a subject to prevent, reduce, or eradicate colonization of one or more microbes, including but not limited to *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, drug-resistant *Enterococci*. In an embodiment, the lithium treatments described herein are administered to a subject to prevent, reduce, or eradicate colonization in order to reduce the risk of developing a subsequent infection by one or more microbes, including but not limited to: *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococci*, drug-resistant *Enterococci*.

In specific embodiments, colonization that can be prevented or reduced using the lithium treatments described herein is colonization with methicillin-resistant *Staphylococcus aureus* in the nares, axillae, chronic wounds or decubitus ulcer surface, perineum, around gastrostomy and tracheostomy sites, in the sputum or urine. In other embodiments, colonization that can be prevented or reduced using the lithium treatments described herein is colonization with *Enterococci* in the bowel and the female genital tract. In a specific
embodiment, colonization that can be prevented or reduced using the lithium treatments described herein is colonization with drug-resistant *Enterococci* in the bowel.

[00518] In an embodiment, the lithium treatments described herein are administered to subjects at high risk of methicillin-resistant *Staphylococcus aureus* infection, such as adult patients and health care workers, to prevent infection or reduce the risk of infection with methicillin-resistant *Staphylococcus aureus*. In another embodiment, the lithium treatments described herein are administered to adult patients and health care workers for the reduction or eradication of nasal colonization with methicillin-resistant *S. aureus* as part of a comprehensive infection control program to reduce the risk of spreading colonization or infection among patients and health care workers and those they come into contact with at high risk of methicillin-resistant *S. aureus* infection during institutional outbreaks of infections with this pathogen. In another embodiment, a combination treatment comprising lithium carbonate and mupirocin is administered to adult patients and health care workers for the reduction or eradication of nasal colonization with methicillin-resistant *S. aureus* in a as part of a comprehensive infection control program to reduce the risk of spreading colonization or infection among patients, health care workers, and those they come into contact with at high risk of methicillin-resistant *S. aureus* infection or colonization during community outbreaks of infections with this pathogen (for example, Community -Acquired MRSA).

[00519] In an embodiment, topical formulations of the lithium treatments described herein are administered as a skin sanitizer and/or hand sanitizer to prevent infection by bacteria including, but not limited to, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Propionibacterium acnes, Staphylococcus epidermidis, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloaca, Streptococcus pyogenes, corynebacterium species, enterococi, Proteus mirabilis, group D streptococci, other gram-positive aerobes, and Bacteroides fragilis.* In an embodiment, topical formulations of the lithium treatments described herein are administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi. In an embodiment, topical formulations of the lithium treatments described herein are administered as a skin sanitizer and/or hand sanitizer to prevent infection by fungi and bacteria. In an embodiment, the skin sanitizer formulation and/or hand sanitizer formulation is aqueous based or glycerol based. In another embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.
In a particular embodiment, the lithium treatments described herein are administered to a subject with a wound to prevent the development of a microbial infection or a microbial colonization in or around said wound. In a specific embodiment, the lithium treatments described herein are administered directly to a wound to prevent the development of a microbial infection or a microbial colonization in or around said wound.

In certain embodiments, the lithium treatments described herein are used to treat chronic microbial infections and colonizations or chronic disorders associated with, characterized by or caused by microbial infections and colonizations. In particular embodiments, the lithium treatments described herein are used to treat chronic cutaneous microbial infections and colonizations or disorders associated with, characterized by or caused by cutaneous microbial infections or colonizations. In specific embodiments, the lithium treatments described herein are used to treat chronic microbial wound infections and colonizations or chronic disorders associated with, characterized by or caused by microbial wound infections and colonizations. The subject with a chronic microbial infection may, e.g., have a suppressed immune system (e.g., post-operative patients, chemotherapy patients, and patients with immunodeficiency disease), have an impaired renal or liver function, be elderly, be a child, be an infant, have a neuropsychiatric disorder, take a psychotropic drug, have a history of seizures, or be on medication that would negatively interact with the anti-microbial therapies.

In certain embodiments, a lithium treatment described herein is administered to a patient who has been diagnosed with a disease caused by infection or colonization with a bacteria, e.g., the patient has been infected by *E. coli*, *Klebsiella* (e.g., *Klebsiella pneumoniae* and *Klebsiella oxytoca*), *Staphylococcus* (e.g., *Staphylococcus aureus*, *Staphylococcus epidermidis*), *Streptococcus* (e.g., *Streptococcus pneumoniae*), *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Pseudomonas* (e.g., *Pseudomonas aeruginosa*), *Clostridium* (e.g., *Clostridium (C.) tetani*, *C. botulinum*, *C. perfringens*), *Enterococcus*, *Enterobacter cloacae*, *Bacillus* (e.g., *Bacillus (B.) anthracis*, *B. cereus*, *B. circulans*, *B. subtilis*, *B. megaterium*), *Acinetobacter baumannii*, *M. tuberculosis*, *Chlamydia*, *N. gonorrhea*, *Shigella*, *Salmonella*, *Proteus*, *Gardnerella*, *Nocardia*, *Planococcus*, *Corynebacteria*, *Rhodococcus*, *Vibrio* (e.g., *Vibrio Cholera*, *Treponema pallidum*, *Pseudomonas*, *Bordetella pertussis*, *Brucella*, *Franciscella tularensis*, *Helicobacter pylori*, *Leptospira interrogans*, *Legionella pneumophila*, *Yersinia* (e.g. *Yersinia (Y.) pestis* *Y. enterocolitica*, *Y. pseudotuberculosis*, *Streptococcus* (types A and B), *Pneumococcus*, *Meningococcus*, *Haemophilus influenza* (type b), *Toxoplasma gondii*, *Comlylobacteriosis*, *Bordetella pertussis*, *Bacillus anthracis*).

[00523] In certain embodiments, lithium treatments comprising lithium carbonate are used to treat subjects diagnosed with or at risk for developing one or more diseases that are caused by one or more fungi selected from the group consisting of by Trichophyton rubrum, Trichophyton mentagrophytes, and Epidermophyton floccum, Candida (e.g., Candida (C) albicans, C glabrata, C krusei, C tropicalis), Cryptococcus (e.g., Cryptococcus neoformans), Malassezia (e.g., M. furfur, M. pachydermatis, M. globosa, M. restricta, M. slooffiae, M. sympodialis, M. nana, M. yamatoensis, M. dermatis, M. obtusa), Microsporum canis, Trichophyton tonsurans, Microsporum audouini, Microsporum gypseum, Trichophyton rubrum, Trichophyton tonsurans, Trichophyton mentagrophytes, Trichophyton interdigitalis, Trichophyton verrucosum, Trichophyton sulphureum, Trichophyton schoenleini, Trichophyton megnini, Trichophyton gallinae, Trichophyton crateriform, Trichomonas and Haemophilus vaginalis, Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum, and Sporothrix schenckii, Trypanosoma (e.g., Trypanosoma (T.) ambystoma, T. avium, T. bovis, T. brucei, T. carassii, T. cruzi, T. congolense, T. equinum, T. equiperdum, T. evansi, T. everetti, T. hosei, T. levisi, T. melophagium, T. paddai, T. parroti, T. percae, T. rangeli, T. rotatorium, T. rugosae, T. sergenti, T. simiae, T. siniperca, T. suis, T. theileri, T. teleosts, T. nagana), Aspergillus fumigatus, Aspergillus flavus, and Aspergillus clavatus.

[00524] In an embodiment, the lithium treatments described herein are used to treat bacterial infections and colonizations in humans and animals that are caused by methicillin-resistant staphylococcus aureus (MRSA). In an embodiment, the lithium treatments described herein are used to treat bacterial infections and colonizations in humans and animals that are caused by bacteria that are antibiotic resistant. In an embodiment, the lithium treatments described herein are used to treat bacterial infections and colonizations in humans and animals that are caused by multidrug-resistant (MDR) Acinetobacter baumannii.

[00525] In an embodiment, the lithium treatments described herein are used to treat bacterial infections and colonizations in humans and animals that are caused by P. aeruginosa. In a particular embodiment, the lithium treatments comprising lithium carbonate described herein are used to treat bacterial infections and colonizations in humans and animals that are caused by P. aeruginosa. In a specific embodiment, the lithium treatments
comprising lithium carbonate described herein are used to treat cutaneous bacterial infections and colonizations in humans and animals that are caused by *P. aeruginosa*.

[00526] In an embodiment, the lithium treatments described herein are used to treat bacterial infections and colonizations in humans and animals that are caused by *S. aureus*. In a particular embodiment, the lithium treatments comprising lithium carbonate described herein are used to treat bacterial infections and colonizations in humans and animals that are caused by *S. aureus*. In a specific embodiment, the lithium treatments comprising lithium carbonate described herein are used to treat cutaneous bacterial infections and colonizations in humans and animals that are caused by *S. aureus*.

[00527] In certain embodiments, the lithium treatments described herein are used to treat chronic bacterial infections and colonizations or chronic disorders associated with, characterized by or caused by bacterial infections and colonizations. In particular embodiments, the lithium treatments described herein are used to treat chronic cutaneous bacterial infections and colonizations or disorders associated with, characterized by or caused by cutaneous bacterial infections and colonizations. In specific embodiments, the lithium treatments described herein are used to treat chronic bacterial wound infections and colonizations or disorders associated with, characterized by or caused by bacterial wound infections and colonizations.

[00528] In certain embodiments, the lithium treatments described herein are used to treat subjects diagnosed with or at risk for developing diseases caused by or associated with bacterial infections and colonizations, e.g., Acinetobacter infections (*Acinetobacter baumannii*), Actinomycosis (*Actinomyces israelii, Actinomyces gerencseriae* and *Propionibacterium propionicus*) African sleeping sickness, African trypanosomiasis (*Trypanosoma brucei*), Amebiasis (*Entamoeba histolytica*), Anaplasmosis (*Anaplasma genus*), Anthrax (*Bacillus anthracis*), Arcanobacterium haemolyticum infection (*Arcanobacterium haemolyticum*), Ascariasis (*Ascaris lumbricoides*) Aspergillosis (*Aspergillus genus*), Astrovirus infection (Astroviridae family), Babesiosis (*Babesia genus*), *Bacillus cereus* infection (*Bacillus cereus*), Bacterial pneumonia (multiple bacteria), Bacterial vaginosis (BV) (multiple bacteria), Bacteroides infection (*Bacteroides genus*), Balantidiasis (*Balantidium coli*), Baylisascaris infection (*Baylisascaris genus*), Black piedra (*Piedraia hortae*), Blastocystis hominis infection (*Blastocystis hominis*), Blastomycosis (*Blastomyces dermatitidis*), Borrelia infection (*Borrelia genus*), Botulism and Infant botulism (*Clostridium botulinum*), Brazilian hemorrhagic fever *Sabia Brucellosis* (*Brucella genus*), *Burkholderia* infection (usually *Burkholderia cepacia* and other *Burkholderia* species), Buruli ulcer
(Mycobacterium ulcerans), Calicivirus infection (Norovirus and Sapovirus),
Campylobacteriosis (Campylobacter genus), Cat-scratch disease (Bartonella henselae),
Cellulitis (usually Group A Streptococcus) and Staphylococcus Chagas Disease,American
trypanosomiasis (Trypanosoma cruzi), Chancroid (Haemophilus ducreyi), Chlamydia
(Chlamydia trachomatis), Chlamydophila pneumoniae infection (Chlamydophila pneumoniae, Cholera (Vibrio cholerae), Chromoblastomycosis (usually Fonsecaea pedrosoi),
Clonorchiasis (Clonorchis sinensis), Clostridium difficile infection (Clostridium difficile),
Coccidioidomycosis (Coccioides immitis and Coccioides posadasii), Colorado tick fever
(CTF), Cryptococcosis (Cryptococcus neoformans) Cryptosporidiosis (Cryptosporidium
genus), Cutaneous larva migrans (CLM) (usually Ancylostoma braziliense), Cyclosporiasis
(Cyclospora cayetanensis), Cysticercosis (Taenia solium), Dientamoebiasis (Dientamoeba
fragilis), Diphtheria (Corynebacterium diphtheriae), Diphyllobothriasis (Diphyllobothrium),
Dracunculiasis (Dracunculus medinensis), Echinococcosis (Echinococcus genus),
Ehrlichiosis (Ehrlichia genus), Enterobiasis (Pinworm infection) (Enterobius vermicularis),
Enterococcus infection (Enterococcus genus), Epidemic typhus (Rickettsia prowazekii),
Fasciolopsis buskii, Fasciolasis (Fasciola hepatica and Fasciola gigantica),
Fatal familial insomnia (Filarioidea superfamilly), Food poisoning (by Clostridium
perfringens Clostridium perfringens), Free-living amebic infection, Fusobacterium infection
(Fusobacterium genus), Gas gangrene, Clostridial myonecrosis (usually Clostridium
perfringens); other Clostridium species, Geotrichosis (Geotrichum candidum, Gerstmann-
Straussler-Scheinker syndrome (GSS) (GSS prion Giardiasis Giardia intestinalis), Glanders
(Burkholderia mallei), Gnathostomiasis (Gnathostoma spinigerum and Gnathostoma
hispidum), Gonorrhea (Neisseria gonorrhoeae), Granuloma inguinale (Donovanosis)
(Klebsiella granulomatis), Group A streptococcal infection (Streptococcus pyogenes), Group
B streptococcal infection (Streptococcus agalactiae), Helicobacter pylori infection
(Helicobacter pylori), Hemolytic-uremic syndrome (HUS) (Escherichia coli), Hemorrhagic
fever with renal syndrome (HFRS) *Bunyaviridae family), Histoplasmosis (Histoplasma
capsulatum), Hookworm infection (Ancylostoma duodenale and Necator americanus), Human
ewingii ehrlichiosis (Ehrlichia ewingii), Human granulocytic
anaplasmosis (HGA) (Anaplasma phagocytophilum), Human monocytic ehrlichiosis
(Ehrlichia chaffeensis), Hymenolepiasis (Hymenolepis nana), Isosporiasis (Isospora belli),
Keratitis, Kingella kingae infection (Kingella kingae), Legionellosis (Legionnaires' disease
(Legionella pneumophila), Legionellosis (Pontiac fever) (Legionella pneumophila),
Leishmaniasis (Leishmania genus), Leprosy (Mycobacterium leprae and Mycobacterium
lepromatosis), Leptospirosis (Leptospira genus), Listeriosis (Listeria monocytogenes), Lyme disease (Lyme borreliosis) (usually *Borrelia burgdorferi* and other *Borrelia* species), Lymphatic filariasis), Elephantiasis (*Wuchereria bancrofti* and *Brugia malayi*), Malaria (*Plasmodium* genus), Melioidosis (Whitmore’s disease) (*Burkholderia pseudomallei*), Lyme disease (Lyme borreliosis) (usually *Borrelia burgdorferi* and other *Borrelia* species), Mycetoma (numerous species of bacteria), Melioidosis (Whitmore’s disease) (*Burkholderia pseudomallei*), Malaria (*Plasmodium* genus), Melioidosis (Whitmore’s disease) (*Burkholderia pseudomallei*), Meningitis multiple Meningococcal disease (*Neisseria meningitidis*), Metagonimiasis (usually *Metagonimus yokogawai*), Microsporidiosis (Microsporidia phylum, Murine typhus (Endemic typhus) (*Rickettsia typhi*), Mycoplasm pneumonia (*Mycoplasma pneumoniae*), Mycetoma (numerous species of bacteria), Neonatal conjunctivitis (Ophthalmia neonatorum) (most commonly *Chlamydia trachomatis* and *Neisseria gonorrhoeae*), Nocardiosis (usually *Nocardia asteroides* and other *Nocardia* species), Onchocerciasis (River blindness) (*Onchocerca volvulus*), Paracoccidioidomycosis (South American blastomycosis) (*Paracoccidioides brasiliensis*), Paragonimiasis (usually *Paragonimus westermani* and other *Paragonimus* species), Pasturellosis (*Pasteurella* genus), Pediculosis capitis (Head lice) (*Pediculus humanus capitis*), Pediculosis corporis (Body lice) (*Pediculus humanus corporis*), Pediculosis pubis (Pubic lice, Crab lice) (*Phthirus pubis*), Pelvic inflammatory disease (PID) multiple Pertussis (Whooping cough) (*Bordetella pertussis*), Plague (*Yersinia pestis*), Pneumococcal infection (*Streptococcus pneumoniae*), Pneumocystis pneumonia (PCP) (*Pneumocystis jirovecii*), Pneumonia (multiple), Prevotella infection (*Prevotella* genus), Primary amoebic meningoencephalitis (PAM) (usually *Naegleria fowleri*), Psittacosis (*Chlamydophila psittaci*), Q fever (*Coxiella burnetii*), Rat-bite fever (*Streptobacillus moniliformis* and *Spirillum minus*), Rhinosporidiosis (*Rhinosporidium seeberi*), Rickettsial infection (*Rickettsia* genus), Rickettsialpox (*Rickettsia akari*), Rocky mountain spotted fever (RMSF) (*Rickettsia rickettsii*), Salmonellosis (*Salmonella* genus), Scabies (*Sarcoptes scabiei*), Schistosomiasis (*Schistosoma* genus), Sepsis (multiple), Shigellosis (Bacillary dysentery) (*Shigella* genus), Smallpox (Variola) (Variola major or Variola minor), Sporotrichosis (*Sporothrix schenckii*), Staphylococcal food poisoning (*Staphylococcus* genus), Staphylococcal infection (*Staphylococcus* genus), Strongyloidiasis (*Strongyloides stercoralis*), Syphilis (*Treponema pallidum*), Taeniasis (*Taenia* genus), Tetanus (Lockjaw) (*Clostridium tetani*), Toxocariasis (Ocular Larva Migrants (OLM)), Trichinellosis Tuberculosis (usually *Mycobacterium tuberculosis*), Tularemia (*Francisella tularensis*), Ureaplasma urealyticum infection (*Ureaplasma urealyticum*), Yersinia pseudotuberculosis infection (*Yersinia pseudotuberculosis*), Yersiniosis (*Yersinia enterocolitica*).

[00529] In certain embodiments, lithium treatments comprising lithium carbonate are used to treat subjects diagnosed with or at risk for developing one or more of the diseases or
bacterial infections and colonizations selected from the group set forth in preceding paragraph.

[00530] In certain embodiments, a lithium treatment described herein is administered to a patient who has been diagnosed with a disease caused by infection with a fungus, e.g., the patient has been infected by *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*, *Candida* (e.g., *Candida* (C) albicans, *C. glabrata*, *C. krusei*, *C. tropicalis*), *Cryptococcus* (e.g., *Cryptococcus neoformans*), *Malassezia* (e.g., *M. furfur*, *M. pachydermatis*, *M. globosa*, *M. restricta*, *M. slooffiae*, *M. sympodialis*, *M. nana*, *M. yamatoensis*, *M. dermatis*, *M. obtusa*), *Microsporum canis*, *Trichophyton tonsurans*, *Microsporum audouini*, *Microsporum gypseum*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton mentagrophytes*, *Trichophyton interdigitalis*, *Trichophyton verrucosum*, *Trichophyton sulphureum*, *Trichophyton schoenleini*, *Trichophyton megnini*, *Trichophyton gallinae*, *Trichophyton crateriform*, *Trichomonas and Haemophilus vaginalis*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, and *Sporothrix schenckii*, *Trypanosoma* (e.g., *Trypanosoma* (T.) ambystoma, *T. avium*, *T. boissoni*, *T. brucei*, *T. carassii*, *T. cruzi*, *T. congolense*, *T. equinum*, *T. equiperdum*, *T. evansi*, *T. everetti*, *T. hosei*, *T. levisi*, *T. melophagium*, *T. paddai*, *T. parroti*, *T. percae*, *T. rangeli*, *T. rotatorium*, *T. rugosae*, *T. sergenti*, *T. simiae*, *T. siniperca*, *T. suis*, *T. theileri*, *T. teleosts*, *T. nagana*), *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus clavatus*.

[00531] In certain embodiments, lithium treatments comprising lithium carbonate are used to treat subjects diagnosed with or at risk for developing one or more diseases that are caused by one or more fungi selected from the group consisting of *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*, *Candida* (e.g., *Candida* (C) albicans, *C. glabrata*, *C. krusei*, *C. tropicalis*), *Cryptococcus* (e.g., *Cryptococcus neoformans*), *Malassezia* (e.g., *M. furfur*, *M. pachydermatis*, *M. globosa*, *M. restricta*, *M. slooffiae*, *M. sympodialis*, *M. nana*, *M. yamatoensis*, *M. dermatis*, *M. obtusa*), *Microsporum canis*, *Trichophyton tonsurans*, *Microsporum audouini*, *Microsporum gypseum*, *Trichophyton rubrum*, *Trichophyton tonsurans*, *Trichophyton mentagrophytes*, *Trichophyton interdigitalis*, *Trichophyton verrucosum*, *Trichophyton sulphureum*, *Trichophyton schoenleini*, *Trichophyton megnini*, *Trichophyton gallinae*, *Trichophyton crateriform*, *Trichomonas and Haemophilus vaginalis*, *Blastomyces dermatitidis*, *Coccidioides immitis*, *Histoplasma capsulatum*, and *Sporothrix schenckii*, *Trypanosoma* (e.g., *Trypanosoma* (T.) ambystoma, *T. avium*, *T. boissoni*, *T. brucei*, *T. carassii*, *T. cruzi*, *T. congolense*, *T. equinum*, *T. equiperdum*, *T. evansi*, *T. everetti*, *T. hosei*, *T. levisi*, *T. melophagium*, *T. paddai*, *T. parroti*, *T. percae*, *T. rangeli*, *T. rotatorium*, *T. rugosae*, *T. sergenti*, *T. simiae*, *T. siniperca*, *T. suis*, *T. theileri*, *T. teleosts*, *T. nagana*), *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus clavatus*. 

[00532] In certain embodiments, the lithium treatments described herein are used to treat subjects diagnosed with or at risk for developing diseases caused by or associated with fungal infections, e.g., athletes foot (Tinea pedis), beard ringworm (Tinea Barbae), facial ringworm (Tinea Faciale), jock itch (Tinea Cruris), pityriasis versicolor (Tinea Versicolor), hand ringworm (Tinea Manuum), nail ringworm (Tinea Unguium), scalp ringworm (Tinea Capitis), ringworm (Tinea corporis), conditions caused by candidiasis including oral thrush, intertrigo, vulvovaginitis, diaper rashes, paronychia and chronic mucocutaneous candidiasis.

[00533] In certain embodiments, lithium treatments comprising lithium carbonate are used to treat subjects diagnosed with or at risk for developing one or more of the diseases or fungal infections selected from the group consisting of athletes foot (Tinea pedis), beard ringworm (Tinea Barbae), facial ringworm (Tinea Faciale), jock itch (Tinea Cruris), pityriasis versicolor (Tinea Versicolor), hand ringworm (Tinea Manuum), nail ringworm (Tinea Unguium), scalp ringworm (Tinea Capitis), ringworm (Tinea corporis), conditions caused by candidiasis including oral thrush, intertrigo, vulvovaginitis, diaper rashes, paronychia and chronic mucocutaneous candidiasis.

[00534] In certain embodiments, a lithium treatment described herein is administered to a patient who has been diagnosed with a disease caused by infection with a yeast, e.g., the patient has been infected by Aciculoconidium, Botryosascus, Brettanomyces, Bullera, Bulleromyces, Candida, Citeromyces, Clavispora, Cryptococcus, Cystofilobasidium, Debaromyces, Debaryomyces, Dekkera, Dipodascus, Endomyces, Endomycopsis, Erythrobasidium, Fellomyces, Filobasidium, Guilliermondella, Hanseniaspora, Hansenula, Hasegawaea, Hyphopichia, Issatchenka, Kloeckera, Kluveromyces, Konagataella, Leucosporidium, Lipomyces, Lodderomyces, Malassezia, Mastigomyces, Metschnikowia, Mrakia, Nadsonia, Octosporomyces, Oosporidium, Pachysolen, Petasospora, Phaffia, Pichia, Pseudozyma, Rhodosporidium, Rhodotorula, Saccharomyces, Saccharomyces, Saccharomyces, Schizoblastosporion, Schizosaccharomyces, Schwanniomyces, Selenotila, Sirobasidium, Sporidiobolus, Sporabolomyces, Stephanoascus, Sterigmatomyces, Syringospora, Torulaspora, Torulopsis, Tremelloid, Trichosporon, Trigonopsis, Udeniomyces, Walthomyces, Wickerhamia, Williopsis, Wingea, Yarrowia, Zygoabospora, Zygoabospora, Zyglotomycos, and/or Zygosaccharomyces.
In certain embodiments, a lithium treatment described herein is administered to a patient who has been diagnosed with a disease caused by infection with a parasite, e.g., the patient has been infected by Babesia, Cryptosporidium, Entamoeba histolytica, Leishmania, Giardia lamblia, Plasmodium, Toxoplasma, Trichomonas, Trypanosoma, Ascaris, Cestoda, Ancylostoma, Brugia, Fasciola, Trichinella, Schistosoma, Taenia, Cimicidae, Pediculus, and/or Sarcoptes.

In some embodiments, the lithium compositions described herein are not used to treat infections caused by viruses. In a particular embodiment, the lithium compositions described herein are not used to treat infections caused by DNA viruses. In a specific embodiment, the lithium compositions described herein are not used to treat infections caused by Herpes Simplex Virus (HSV).

In an embodiment, the lithium compositions described herein are used to treat seborrheic dermatitis. In another embodiment, the lithium compositions described herein are used to prevent microbial infections caused by or associated with seborrheic dermatitis. In a particular embodiment, lithium compositions comprising lithium carbonate are used to treat Seborrheic dermatitis. In another particular embodiment, lithium compositions comprising lithium carbonate are used to prevent microbial infections caused by or associated with Seborrheic dermatitis.

In some embodiments, a lithium treatment described herein is administered to a patient with a microbial infection before symptoms of the infection manifest or before symptoms of the infection become severe (e.g., before the patient requires hospitalization). In some embodiments, a lithium treatment described herein is administered to a patient with a microbial infection after symptoms of the infection manifest or after symptoms of the infection become severe (e.g., after the patient requires hospitalization).

In a specific embodiment, the subject to receive or receiving a lithium treatment described herein is receiving or has received other anti-microbial therapies. In another embodiment the subject to receive a lithium treatment described herein is receiving other anti-microbial therapies and lithium treatments of the invention are administered to the subject before any adverse effects or intolerance of these other anti-microbial therapies occurs. In an alternative embodiment, the subject to receive or receiving a lithium treatment described herein has not received or is not receiving other anti-microbial therapies.

In certain embodiments, a pharmaceutical composition of the invention is administered to a subject refractory to one or more therapies. In one embodiment, that a microbial infection is refractory to a therapy means that at least some significant portion of
the microorganisms causing the infection are not killed or their cell division is not arrested. The determination of whether the microorganisms are refractory can be made either in vivo or in vitro by any method known in the art for assaying the effectiveness of a therapy on microorganisms, using the art-accepted meanings of "refractory" in such a context. In various embodiments, a microbial infection is refractory where the amount of microorganism has not been significantly reduced, or has increased. In other embodiments, that a microbial infection is refractory means that at least some significant portion of microorganisms are not killed or their cell division arrested.

[00541] In some embodiments, a subject to be administered lithium compound or composition thereof described herein is an animal. In certain embodiments, the animal is a bird. In certain embodiments, the animal is a canine. In certain embodiments, the animal is a feline. In certain embodiments, the animal is a horse. In certain embodiments, the animal is a cow. In certain embodiments, the animal is a mammal, e.g., a horse, swine, mouse, or primate. In specific embodiments, a subject to be administered a lithium compound or composition thereof described herein is a human.

[00542] In some embodiments, a candidate subject for a lithium treatment described herein is any subject at risk for, has, or has had a wound or scar.

[00543] In one embodiment, a lithium treatment is delivered to an area of skin of a subject in which enhanced wound healing or scar revision is desired, for example, the scalp, face (e.g., the eyebrow, eyelashes, upper lip, lower lip, chin, cheeks, beard area, or mustache area) or neck, or another part of the body, such as, e.g., the chest, breasts, sternum, abdomen, arms, armpits (site of axillary hair), legs, hands, feet, or genitals. In some embodiments, a wounded or scarred part of the skin is treated. In some embodiments, the wounded or scarred part of the skin is a flexion surface or involves the extremities, breasts, sternum, face, or neck.

[00544] Wounds treatable by the methods described herein include, but are not limited to, any form of wound known in the art or to be discovered. Non-limiting examples of wounds treatable by the methods described herein include acute wounds (surgical and non-surgical, including blast wounds and other battlefield wounds), chronic or non-healing wounds, pressure sores (also referred to as decubitus ulcers or bed sores), pressure necrosis, lower extremity ulcers, radiation injury (such as, e.g., caused by radiation overdose), an erythema, skin abrasion, or a non-healing wound caused by wounding (e.g., a surgical incision) of irradiated skin. In some embodiments, the methods described herein are used to enhance healing of wounds caused by blisters, cutaneous trauma (such as, e.g., acute cutaneous trauma), and surgery, such as described in Mulvaney & Harrington, 1994, Chapter 7.
"Cutaneous trauma and its treatment," in *Textbook of Military Medicine: Military Dermatology*, Office of the Surgeon General, Department of the Army, Virtual Naval Hospital Project, which is incorporated by reference herein in its entirety. In some embodiments, the methods described herein are used to enhance (e.g., hasten, improve, minimize scarring, etc.) healing of wounds by primary intention. In some embodiments, the methods described herein are used to enhance healing of wounds by secondary intention. In some embodiments, the methods described herein are used to enhance healing of wounds by tertiary intention.

[00545] In one embodiment, the wound to be treated by the methods described herein has wound dehiscence, which is the premature "bursting" open of a wound along surgical suture. In some embodiments, the patient is at risk for wound dehiscence, based on one or more of the following risk factors: age, diabetes, obesity, poor knotting or grabbing of stitches, and trauma to the wound after surgery, or inadequate ability to form scars.

[00546] In some embodiments, the methods described herein are used to treat a radiation scar, acne scar, curettage scar, spread scar, split-thickness scar, flap necrosis, scarring following infection, leg ulcer, burn scar, sternotomy scar, or as treatment to minimize scarring following curettage, following surgical excision, following follicular unit transplantation, or following Cesarean section.

[00547] In some embodiments, methods described herein are used to enhance healing of transplanted skin at recipient sites (e.g., skin grafts or hair transplantation, such as long-term frontal hair scalp or eyebrow plugs), so that, for example, the skin blends in with the skin at the recipient site with regard to thickness, pigmentation, hair patterning, etc. In one exemplary embodiment, a scar that results from skin grafting where the graft edges join the host skin, common in battlefield wounds, is treated by the methods described herein. In general any "flap" surgery or "free flap" graft will result in these scars. In another embodiment, the methods described herein are used to enhance healing of a split thickness skin graft. In one embodiment, the split-thickness donor skin tissue for grafting of wound sites is taken from the scalp, as described in Weyandt, *et al.*, 2009, Dermatol. Surg. 35:1873-1879, which is incorporated herein by reference in its entirety. Without being bound by any theory, lithium treatment may benefit this process by facilitating the "recipient dominance" phase (that temporally follows "donor dominance"). It is postulated that pulse or intermittent lithium treatment can make skin grafts (even pinch grafts) take on attributes of the recipient site by stimulating "local" tissue stem cells to form site-appropriate follicles. Such an intervention can help not only autologous grafts, but also allogeneic grafts, fetal cell grafts.
(like placenta stem cell "bandaids"), and also stem cell grafts (ex vivo expanded mesenchymal stem cells).

[00548] Scars treatable by the methods described herein include, but are not limited to, any form of scar known in the art or to be discovered. Non-limiting examples of scars that can be revised or otherwise treated by the methods described herein include scars that form by secondary intention, atrophic scars, hypertrophic scars, keloid scars, hypopigmented scars, hyperpigmented scars, depressed scars (including ice-pick scars), and spread scars. Scars form following a variety of causes including, e.g., cosmetic procedures and skin transplants are not really clinical categories of scars. Also treatable by the methods described herein are scars caused by a disease or disorder such as scarring (cicatricial) alopecia, scars caused by excessive wound healing, scars caused by joint contracture, or scars caused by burns or wounds. The methods described herein may also be used to treat wounded skin, or skin that may become wounded, in order to prevent, minimize, or reduce scar formation. In one embodiment, the scar is caused by surgery, such as a open heart surgery, joint surgery, face lift, skin graft, or hair transplant, etc.

[00549] In a particular embodiment, the subject for a lithium treatment described herein is intended is a patient who has scarring (cicatricial) alopecia, a condition of permanent hair loss in which the hair follicle is destroyed by inflammation and replaced with scar tissue. In some embodiments, the scarring alopecia is moderate to severe. There is primary cicatricial (scarring) alopecia and secondary cicatricial alopecia. In primary, the follicle is the direct target. See Harries, M.J., Sinclair, R.D., Macdonald-Hull, S., Whiting, D.A., Griffiths, C.E., and Paus, R. 2008. Br. J. Dermatol. 159:1-22. In secondary, the follicle is destroyed by events outside the follicle such as infection or trauma. The current aim of treatment is to reduce symptoms and to slow or stop PCA progression, namely the scarring process. See also Ross, 2007. Primary cicatricial alopecia: clinical features and management. Dermatol. Nurs. 19:137-43.

[00550] In some embodiments, the subject has wounding or scarring caused by, exacerbated by, or associated with medication, such as corticosteroid use, chemotherapy (e.g., anti-cancer therapy or cytotoxic drugs or other antiproliferative agents), thallium compounds, vitamins (e.g., vitamin A), retinoids, anti-viral therapy, or psychological therapy. In some embodiments, the subject has wounding or scarring caused by, exacerbated by, or associated with radiation (including therapeutic radiation treatment), trauma (chronic or acute, mild or severe), physical trauma, endocrine dysfunction, surgery (including, for example, face lift, hair transplant, cosmetic surgery, and surgery of flexion surfaces, the
extremities, breasts, sternum, and neck), sutures, x-ray atrophy, burning or other wound or injury, stress, aging, an inflammatory disease or condition (acute or chronic), an autoimmune disease or disorder, malnutrition (including, e.g., vitamin or trace metal deficiency, scurvy), anemia, diabetes, obesity, a circulatory disorder, such as, e.g., arterial or venous insufficiency, occlusive vascular disease, microvascular occlusive disease, vasoconstriction, hypovolemia, venous valvular disease, impaired oxygen delivery or tissue perfusion, caused by, e.g., ischemia, hypoxia, stroke, embolism or other circulatory obstruction, edema, sepsis, an infection (such as, e.g., a fungal, viral, or bacterial infection, including chronic deep bacterial, a biofilm, or fungal infections; of the wound itself or elsewhere, and which may cause weakening of the tissue), dehiscence, a disease associated with poor wound healing (e.g., Ehlers-Danlos), cellulites, dermatitis, psoriasis, acne, eczema, pregnancy, allergy, a severe illness (e.g., scarlet fever), myxedema, hypopituitarism, early syphilis, discoid lupus erythematosus, cutaneous lupus erythematosus, lichen planus, deep factitial ulcer, granuloma (e.g., sarcoidosis, syphilitic gummas, TB), inflamed tinea capitis (kerion, favus), a slow-growing tumor of the scalp or other skin tumor, or any other condition, disease, or disorder associated with or that causes damage to the skin known in the art or described herein.

5.8 EPIDERMAL THICKENING

[00551] In certain embodiments, the present invention provides a method for increasing the thickness of the epidermis of a subject. In certain embodiments, the method for increasing the thickness of the epidermis of a subject comprises perturbation of the skin followed by treatment with a lithium formulation. Illustrative methods for perturbation of the skin are set forth in Section 5.5 above, illustrative lithium formulations are set forth in Sections 5.1 and 5.2, and illustrative methods for administering the lithium formulation are set forth in Sections 5.2-5.5.

[00552] In certain specific embodiments, the perturbation of the skin is performed using dermabrasion or microdermabrasion by mechanical, chemical, or electromagnetic means. In certain specific embodiments, the lithium formulation comprises lithium carbonate, lithium gluconate, or lithium chloride. In certain more specific embodiments, the lithium formulation comprises the lithium salt at a concentration (w/w) of at about 1%, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at about 20%.

[00553] In a specific embodiment, a method for increasing the thickness of the epidermis of a subject comprises microdermabrasion followed by treatment with 8% (w/w) lithium
gluconate twice daily for about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 days.

[00554] In certain embodiments, the method of increasing the thickness of the epidermis is applied to a subject for cosmetic purposes. In certain embodiments, the subject is at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, or at least 90 years of age. In certain embodiments, the subject is a cigarette smoker at the time of application of the method of the invention. In certain embodiments, the subject, has been a cigarette smoker for at least 1 year, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at least 20 years.

[00555] In certain embodiments, the method of increasing the thickness of the epidermis of a subject is applied to the facial skin of the subject. In certain embodiments, the method of increasing the thickness of the epidermis is applied to an injured area of a subject.

[00556] In even further embodiments, a method disclosed herein for increasing the thickness of the epidermis is used to reduced wrinkles of the skin of a subject. More specifically, the method disclosed herein for increasing the thickness of epidermis is applied to an area of the body with wrinkled skin, such as facial skin (e.g., forehead, cheeks), skin of the neck etc. In certain embodiments, the number and/or depth of wrinkles is reduced by application of the method of the present invention by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at least 90% or by 100%. Evaluation of the skin before and after treatment can be performed as follows (Emsen, 2008, The Journal Of Craniofacial Surgery 19(3):812-816): (1) patient self evaluation, (2) dermatologist assessment (baseline and after dermasanding procedure), (3) digital standardized photography, and (4) colorimetry. Self-evaluation can consist of rating a list of attributes regarding the subject's perception on the look and feel of the subject's skin on a 1 to 10 scale (1 = undesirable, 10 = desirable). The dermatologist's evaluations can include efficacy variables. Efficacy variables can be scored on a 0 to 9 scale (0 = none, 9 = severe) and the following: (1) fine wrinkling, (2) skin dullness, (3) mottled hyperpigmentation, or solar lentigines, (4) blotchiness, (5) appearance of milia, (6) appearance of acne, and (7) appearance of large pores. The dermatologist can also perform a global assessment using a 0 to 9 scale (0 = very good, 9 = very poor) for the following variables: (1) overall skin texture, (2) overall skin tone, and (3) overall skin appearance.

[00557] In certain embodiments, the methods disclosed herein for increasing the thickness of the epidermis is used prophylactically to outdoor workers, such as farmers, fishermen, construction workers, lifeguards, outdoor enthusiasts, sunbathers, or people who regularly attend tanning salons or use tanning beds. In specific embodiments, methods disclosed
herein for increasing the thickness of the epidermis is used prophylactically to fair women under age 50. In specific embodiments, the methods disclosed herein for increasing the thickness of the epidermis is used prophylactically to people with skin phototypes I and II.

[00558] Without being bound by theory aged and / or sun exposed skin has a thin epidermis. The methods disclosed herein for increasing the thickness of the epidermis can be applied to aged and / or sun exposed skin of a subject. In certain embodiments, the thickness of the aged and / or sun exposed skin is at most 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at most 90% of the thickness of the epidermis of skin in the same body area without the effect of age and / or sun exposure. In certain embodiments, the methods disclosed herein for increasing the thickness of the epidermis can be applied to skin areas subject to UV induced skin aging. In other embodiments, the methods disclosed herein for increasing the thickness of the epidermis can be applied to skin areas subject to Non-UV related skin aging.

[00559] In certain embodiments, the methods disclosed herein for increasing the thickness of the epidermis can be applied to reduce any undesired effects of microdermabrasion.

[00560] In further embodiments, a method disclosed herein for increasing the thickness of the epidermis is used to increase the thickness of the epidermis in a subject with steroid induced atrophy. In certain embodiments, the thickness of the atrophied skin is at most 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at most 90% of the thickness of the epidermis of skin in the same body area without steroid induced atrophy. In more specific embodiments, the method disclosed herein for increasing the thickness of the epidermis is applied to a subject with skin thinning due to topical corticosteroid treatment. In a specific embodiment, the corticosteroid is Cortisol.

5.9 **COSMETIC COMPOSITIONS**

[00561] The following cosmetic compositions may be used alone or in combination with the antimicrobial treatments, wound healing, scar revision, epidermal thickening, and acne treatments described herein.

[00562] In particular embodiments, the cosmetic compositions provided herein are emulsions, *e.g.*, emulsions of the water-in-oil (W/O) or oil-in-water (O/W) type. As used herein, the term "emulsion" refers to a mixture of two or more immiscible liquids. Emulsions are part of a more general class of two-phase systems of matter called colloids. Although the terms colloid and emulsion are sometimes used interchangeably, emulsion tends to imply that
both the dispersed and the continuous phase are liquid. In an emulsion, one liquid (the dispersed phase) is dispersed in the other (the continuous phase).

[00563] In some embodiments, the cosmetic compositions described herein are emulsions that comprise two phases: an aqueous phase (described in more detail in Section 5.1) and a oily phase (described in more detail in Section 5.1). The emulsified cosmetics described herein include all cosmetics in which an oil phase comprising oil and fat content and an aqueous phase comprising aqueous components are stably dispersed, regardless of water-in-oil emulsification type or oil-in-water emulsification type. The cosmetic compositions described herein can also be multiple emulsions such as oil-in-water-in-oil emulsions and water-in-oil-in-water emulsions. The emulsions described herein can be prepared according to any method known in the art.

[00564] The cosmetic compositions provided herein further comprise surface active substances ("surfactants") which can increase the kinetic stability of emulsions greatly so that, once formed, the emulsion does not change significantly over years of storage.

[00565] Preparations of the cosmetic emulsion compositions described herein can be milk preparations, lotions, creams, e.g., emollient creams, ointments, gels, e.g., hyrdogels, powders, masks, packs, sprays, aerosols or sticks. The cosmetic formulations described herein can also be applied to textiles or nonwovens and thereby be provided to the skin via this medium.

5.9.1 AQUEOUS PHASE

[00566] The aqueous phase of the cosmetic compositions described herein comprises an appropriate aqueous vehicle, including, but not limited to, water, distilled water, saline, physiological saline or buffered saline (e.g., phosphate buffered saline (PBS)), sodium chloride for injection, Ringers for injection, isotonic dextrose for injection, sterile water for injection, dextrose lactated Ringers for injection, sodium bicarbonate, or albumin for injection.

[00567] In an embodiment, the aqueous phase contains water. An amount of water in the cosmetic is preferably adjusted depending on the form of the cosmetic in the range of from 1 to 90.0 mass %, based on a total mass of the cosmetic. In an embodiment, the aqueous phase optionally contains one or more water-miscible or at least partially water-miscible compounds, for instance polios; C.sub.2 to C.sub.8 lower monoalcohols, such as ethanol and isopropanol; and C.sub.3 to C.sub.4 ketones that are liquid at room temperature. As used herein, the term "polyol" refers any organic molecule comprising at least two free hydroxyl
groups. Examples of polios that may be mentioned include glycols, for instance butylene glycol, propylene glycol, dipropylene glycol, and isoprene glycol, glycerol and polyethylene glycols, for instance PEG-8, sorbitol and sugars, for instance glucose. The aqueous phase may also comprise any common water-soluble or water-dispersible additive.

[00568] Aqueous phase components for use in the cosmetic compositions described herein, include, for example, alcohols such as glycerin, water-soluble polymers such as hyaluronic acid and chondroitin sulfate, salts such as sodium lactate, sodium citrate, sodium glutamate, sodium 2-pyrrolidone carboxylate, sodium chloride, and magnesium chloride.

[00569] In some embodiments, the aqueous phase is from 10% to 99% by weight, preferably from 20% to 95% by weight, better still from 30% to 90% by weight and even better still from 40% to 85% by weight relative to the total weight of the composition.

[00570] The water-miscible compound(s), such as lower polios and alcohols, may be present in an amount ranging from, e.g., 0 to 30%, especially from 0.1% to 30% and better still in an amount ranging from 1% to 20%, relative to the total weight of the composition. These components are preferably present in an amount of 0.1 to 25 wt %.

[00571] The aqueous phase of the cosmetic compositions described herein may contain a water-soluble polymer, water-swellable polymer or a mixture thereof. Examples of these water-soluble or water-swellable polymer include gum Arabic, tragacanth gum, arabinogalactan, locust bean gum (carob gum), guar gum, karaya gum, carrageenan, pectin, agar-agar, quince seed (i.e., marmelo), starch from rice, corn, potato or wheat, algae colloid, and trant gum; bacteria-derived polymers such as xanthan gum, dextran, succinoglucon, and pullulan; animal-derived polymers such as collagen, casein, albumin, and gelatin; starch-derived polymers such as carboxymethyl starch and methylhydroxypropyl starch; cellulose polymers such as methyl cellulose, ethyl cellulose, methylhydroxypropyl cellulose, carboxymethyl cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, nitrocellulose, sodium cellulose sulfate, sodium carboxymethyl cellulose, crystalline cellulose, and cellulose powder; alginic acid-derived polymers such as alginate, sodium alginate and propylene glycol alginate; vinyl polymers such as polyvinyl methylether, polyvinylpyrrolidone, and carboxyvinyl polymer; polyoxyethylene polymers such as polyethylene glycol; polyoxyethylene/polyoxypropylene copolymers; acrylic polymers such as sodium polyacrylate, polyethyl acrylate, and polyacrylamide; polyethyleneimine; cationic polymers; and inorganic thickening agents such as, bentonite, aluminum magnesium silicate, montmorillonite, videlite, nontronite, saponite, hectorite, and silicic anhydride. Film forming polymers such as polyvinyl alcohol and polyvinylpyrrolidone are also included.
[00572] An amount of the water-soluble or water-swellable polymer (D) in the cosmetic ranges preferably from 0.01 to 25 mass %, based on a total mass of the cosmetic.

[00573] The aqueous phase of the cosmetic emulsions described herein preferably contains a monovalent cation (e.g., Li⁺, Na⁺, K⁺, Rb⁺, NH₄⁺) known in the art. Of these, lithium is preferred. As used herein, a monovalent lithium salt (e.g., lithium hydroxide, lithium gluconate, lithium chloride, lithium stearate, lithium orotate, etc.) refers to a salt form of lithium in which there is one lithium cation for each anion of the salt. A challenge in preparing cosmetic emulsions that contain a monovalent cation, such as lithium, is that some sources, of such cations e.g., lithium hydroxide and lithium carbonate, are corrosive. The cosmetic formulations described herein overcome this challenge, as shown in the examples. Any form of lithium may be used in the lithium treatment. For example, any commonly used lithium salts can be used including lithium hydroxide (LiOH), lithium carbonate (Li₂CO₃), lithium citrate (Li₃C₆H₅O₇), lithium sulfate (Li₂SO₄), lithium aspartate, lithium orotate, lithium succinate, and lithium gluconate. A lithium formulation well-suited for use in the compositions and methods disclosed herein is lithium hydroxide, for example, a topical ointment of 0.01 to 0.9% lithium hydroxide. Another lithium formulation well-suited for use in the compositions and methods disclosed herein is lithium carbonate, for example, a topical ointment of 0.01 to 0.9% lithium carbonate. Any compound or composition that can release a lithium ion (also referred to herein as lithium cation, Li⁺, or ionized lithium) is suitable for use in the cosmetic compositions and methods. Such compounds include but are not limited to a prodrug, salt or solvate (e.g., a hydrate) of lithium (sometimes referred to herein as "lithium compounds"). Optionally, the lithium compounds can be formulated with a vehicle, carrier, diluent, or excipient, or a mixture thereof.

[00574] In some embodiments, a cosmetic composition comprising lithium or a monovalent lithium salt, e.g., lithium hydroxide or lithium carbonate, at a concentration in the range of 0.01 to 2.0 mg Li+/gram is used. In some embodiments, a cosmetic composition comprising lithium or a monovalent lithium salt, e.g., lithium hydroxide or lithium carbonate, at a concentration in the range of 0.01 mg to 0.05 mg, 0.05 to 0.1 mg, 0.1 mg to 0.5 mg, 0.5 to 1.0 mg, 1.0 mg to 1.5 mg, 1.5 mg to 2.0 mg Li+/gram is used. In another embodiment, the concentration of lithium in the cosmetic compositions described herein is 1.37 mg Li+/gram or less. In another embodiment, the concentration of lithium in the cosmetic compositions described herein is less than 0.1 mg Li+/gram. In another embodiment, the concentration of lithium in the cosmetic compositions described herein is 0.34 mg Li+/gram. In an
embodiment, a cosmetic composition of the invention comprises 0.34 mg Li+/gram of lithium hydroxide.

The concentration of lithium in a particular topical cosmetic composition to deliver the intended dose of lithium will depend on the release properties of the lithium ion, the hydrophobicity of the lithium salt form, the partition coefficient of the lithium salt form, etc.

Lithium formulations comprising the foregoing lithium and monovalent lithium salt, e.g., lithium hydroxide or lithium carbonate, concentrations may be achieved using, for example, a formulation comprising, w/w, lithium ions at a concentration of 0.10% lithium, 0.15% lithium, 0.20% lithium, 0.25% lithium, 0.30% lithium, 0.35% lithium, 0.40% lithium, 0.45% lithium, 0.50% lithium, 0.55% lithium, 0.60% lithium, 0.65% lithium, 0.70% lithium, 0.75% lithium, 0.80% lithium, 0.85% lithium, 0.90% lithium, 0.95% lithium, 1.0% lithium, 1.5% lithium, 2.5% lithium, 3.0% lithium, 3.5% lithium, or 4.0% lithium. In some embodiments, the form of lithium for topical administration comprises, w/w, 0.1% to 0.5% lithium ions, 0.2% to 0.5% lithium ions, 0.5% to 1% lithium ions, or more. In some embodiments, the form of lithium for topical administration comprises, w/w, 0.206% lithium ions. In some embodiments, the form of lithium for topical administration comprises, w/w, 0.4% lithium ions.

The amount of a salt form of lithium to generate a topical lithium formulation with one of the aforementioned concentrations of lithium ion is readily deducible by one of ordinary skill in the art, and depends upon several factors including, e.g., the valency of the salt form, the stability of the salt form, the ability of the salt form to release the lithium ion, the hydrophobicity or hydrophilicity, etc.

In some embodiments, a topical cosmetic composition described herein comprises 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.15%, 0.15%-0.2%, or 0.2%-0.25%, 0.25%-0.3%, 0.3%-0.35%, 0.35%-0.4%, 0.4%-0.45%, 0.45%-0.5%, 0.5%-0.55%, 0.55%-0.6%, 0.6%-0.65%, 0.65%-0.7%, 0.7%-0.75%, 0.75%-0.8%, 0.8%-0.85%, or 0.85%-0.9%, or more lithium carbonate (w/w). In some embodiments, a topical cosmetic composition described herein comprises 0.73%> or less of lithium carbonate (w/w).

In some embodiments, a topical cosmetic composition described herein comprises 0.01%-0.05%, 0.05%-0.1%, 0.1%-0.15%, 0.15%-0.2%, or 0.2%-0.25%, 0.25%-0.3%, 0.3%-0.35%, 0.35%-0.4%, 0.4%-0.45%, 0.45%-0.5%, 0.5%-0.55%, 0.55%-0.6%, 0.6%-0.65%, 0.65%-0.7%, 0.7%-0.75%, 0.75%-0.8%, 0.8%-0.85%, or 0.85%-0.9%, or more lithium hydroxide (w/w). In some embodiments, a topical cosmetic composition described herein
comprises 0.83% or less of lithium carbonate (w/w). In some embodiments, a topical cosmetic composition described herein comprises 0.206% lithium hydroxide (w/w). In some embodiments, a topical cosmetic composition described herein comprises 0.4% lithium hydroxide (w/w).

[00580] In some embodiments, the cosmetic compositions treatment described herein comprise mixtures of one or more lithium salts. For example, a mixture of a fast-dissolving lithium salt can be mixed with a slow dissolving lithium salt proportionately to achieve the release profile. In certain embodiments, the lithium salts do not comprise lithium chloride. In some embodiments, the lithium salt can be the salt form of anionic amino acids or poly(amino) acids. Examples of these are glutamic acid, aspartic acid, polyglutamic acid, polyaspartic acid. By reciting lithium salts of the acids set forth above, applicants do not mean only the lithium salts prepared directly from the specifically recited acids. In contrast, applicants mean to encompass the lithium salts of the acids made by any method known to one of ordinary skill in the art, including but not limited to acid-base chemistry and cation-exchange chemistry. In another embodiment, a large anion or multianionic polymer such as polyacrylic acid can be complexed with lithium, then complexed with a cationic compound, such as finasteride, to achieve a slow release formulation of both lithium ion and finasteride. Similarly, a lithium complex with a polyanion can be complexed further with the amines of minoxidil, at pHs greater than 5. Lithium compounds for use in the methods provided herein may contain an acidic or basic moiety.

5.9.2 OILY PHASE

[00581] The oily phase of the cosmetic compositions described herein may comprise at least one oil. The at least one oil, for example, may be chosen from polar oils and apolar oils including hydrocarbon-based liquid oils and oily liquids at room temperature.

[00582] For a oily phase structured with an apolar polymer of the hydrocarbon-based type, this fatty phase may contain more than 30%, for example more than 40% by weight, or from 50% to 100%, by weight, of at least one liquid apolar, such as hydrocarbon-based oil, relative to the total weight of the oily phase.

[00583] For example, the at least one polar oil useful in the invention may be chosen from hydrocarbon-based plant oils with a high content of triglycerides comprising fatty acid esters of glycerol in which the fatty acids may have varied chain lengths from C.sub.4 to C.sub.24, these chains possibly being chosen from linear and branched, and saturated and unsaturated chains; these oils are chosen from, for example, hydrogenated cottonseed oil, jojoba oil,
wheat germ oil, corn oil, sunflower oil, karite butter, castor oil, sweet almond oil, macadamia oil, apricot oil, soybean oil, cotton oil, alfalfa oil, poppy oil, pumpkin oil, sesame oil, marrow oil, rapeseed oil, avocado oil, hazelnut oil, grape seed oil, blackcurrant seed oil, evening primrose oil, millet oil, barley oil, quinoa oil, olive oil, rye oil, safflower oil, candle nut oil, passion flower oil and musk rose oil; or alternatively caprylic/capric acid triglycerides such as those sold by Stearineries Dubois or those sold under the names Miglyol 810, 812 and 818 by Dynamit Nobel; synthetic oils or esters of formula R.sub.5COOR.sub.6 in which R.sub.5 is chosen from linear and branched fatty acid residues containing from 1 to 40 carbon atoms and R.sub.6 is chosen from, for example, a hydrocarbon-based chain containing from 1 to 40 carbon atoms, on condition that R.sub.5+R.sub.6 gtoreq.10, such as, for example, purcellin oil (cetostearyl octanoate), isononyl isononanoate, C.sub.12-C.sub.15 alkyl benzoates, isopropyl myristate, 2-ethylhexyl palmitate, isostearyl isostearate and alkyl or polyalkyl octanoates, decanoates or ricinoleates; hydroxylated esters such as isostearyl lactate and diisostearyl malate; and pentaerythritol esters; synthetic ethers containing from 10 to 40 carbon atoms; C.sub.8 to C.sub.26 fatty alcohols such as oleyl alcohol; and C.sub.8 to C.sub.26 fatty acids such as oleic acid, linolenic acid or linoleic acid.

The at least one apolar oil according to the invention may include a hydrocarbon chosen from linear and branched, volatile and non-volatile hydrocarbons of synthetic and mineral origin, such as volatile liquid paraffins (such as isoparaffins and isododecane) or non-volatile liquid paraffins and derivatives thereof, liquid petrolatum, liquid lanolin, polydecenes, hydrogenated polyisobutene such as Parleam.RTM., and squalane; silicone oils, polydimethylsiloxanes and phenylsilicones that would otherwise not function herein as a swelling agent; and mixtures thereof. The structured oils, for example those structured with polyamides such as those of formula (I) or the polyurethanes or polyureas or polyurea-urethanes, may be, in one embodiment, apolar oils, such as an oil or a mixture of hydrocarbon oils chosen from those of mineral and synthetic origin, chosen from hydrocarbons such as alkanes such as Parleam.RTM. oil, isoparaffins including isododecane, and squalane, and mixtures thereof. These oils may, in one embodiment, be combined with at least one phenylsilicone oil.

The oily phase, in one embodiment, contains at least one non-volatile oil chosen from, for example, hydrocarbon-based oils of mineral, plant and synthetic origin, synthetic esters or ethers, silicone oils and mixtures thereof.

In practice, the total oily phase may be present, for example, in an amount ranging from about 0.1% to about 99% by weight relative to the total weight of the composition;
further examples include ranges of from about 5.0% to about 95.5%, from about 10% to about 80%, from about 20% to about 75%, and from about 1.0% to about 60% by weight relative to the total weight of the composition.

[00587] The cosmetic compositions of the present invention may comprise surfactants (i.e. a surface active agent) generally in a concentration varying from about 0.01% (w/v) to about 40% (w/v), and more preferably from about 0.05% (w/v) to about 15% (w/v) and most preferably from about 0.1% (w/v) to about 10% (w/v) selected from the group consisting of anionics, cationics, nonionics and amphotericics or mixtures thereof. The surfactants used herein may act in a variety of ways including without limitation as a cleansing agent, detergent, emulsifiers, e.g., polyglyceryl-10 decaoleate and polyglyceryl-6-octostearate, wetting agent, foam booster, foam depressant, conditioner or germicide. A wide variety of surfactants may be used in the formulation of the products herein disclosed. They include the surfactants disclosed in U.S. Pat. No. 5,151,209 to McCall et al; U.S. Pat. No. 5,151,210 to Steuri et al; U.S. Pat. No. 5,120,532 to Wells et al; and U.S. Pat. No. 5,635,469 to Fowler et al. all of which are incorporated herein by reference in their entirety.

[00588] Anionic surfactants that may be used in the formulation of the emulsions of the present invention include without limitation branched and unbranched alkyl and acyl hydrocarbon compounds, sodium dodecyl sulfate (SDS); sodium lauryl sulfate (SLS); sodium lauryl ether sulfate (SLES); sarcosinate; fatty alcohol sulfates, including sodium, potassium, ammonium or triethanolamine salts of C.sub.10 to C.sub.18 saturated or unsaturated forms thereof; ethoxylated fatty alcohol sulfates, including alkyl ether sulfates; alkyl glyceryl ether sulfonate, alpha sulpho fatty acids and esters; fatty acid esters of isethionic acid, including Igepon A; acyl (fatty) N-methyltaurides, including Igepon T; dialkylsulfo succinate esters, including C.sub.8, C.sub.10 and C.sub.12 forms thereof; Miranot BT also referred to as lauroamphocarboxyglycinate and sodium tridecath sulfate; N-acylated amino acids, such as sodium N-lauroyl sarcosinate or gluconate; sodium coconut monoglyceride sulfonate; and fatty acid soaps, including sodium, potassium, DEA or TEA soaps.

[00589] Among the cationic surfactants that are useful are monoalkyl trimethyl quaternary salts; dialkyl dimethyl quaternary salts; ethoxylated or propoxylated alkyl quaternary ammonium salts, also referred to in the art as ethoquats and propoquats; cetyl benzylmethylalkyl ammonium chloride; quaternized imidazolines, which are generally prepared by reacting a fat or fatty acid with diethylenetriamine followed by quaternization, and non-fat derived cationic polymers such as the cellulosic polymer, Polymer JR (Union Carbide).
Further useful cationic surfactants include lauryl trimethyl ammonium chloride; cetyl pyridinium chloride; and alkyltrimethylammonium bromide. Cationic surfactants are preferably used in the formulation of hair care products and more preferably in the formulation of rinses and conditioners.

Useful nonionic surfactants include polyethoxylated compounds and polypropoxylated products. Polyethoxylated and polypropoxylated compounds may be prepared by reacting fatty alcohols with ethylene oxide or glycol or by reacting fatty alcohols with propylene oxide or glycol. These materials have the general formula R(X).sub.n OR’ wherein R is H or C.sub.10 to C.sub.30 alkyl group, X is —OCH.sub.2.CH.sub.2 —(i.e. when derived from ethylene oxide or glycol) or --OCH.sub.2.CHCH.sub.3 —(i.e. when derived from propylene oxide or glycol), n is an integer from about 1 to 100, and R’ is H or a C.sub.10 to C.sub.30 alkyl group. Polyethoxylated and polypropoxylated products may also be prepared by reacting fatty acids with ethylene oxide or glycol or propylene oxide or glycol respectively. These materials have the general formula RCO(X).sub.n.OH wherein R is H or a C.sub.10 to C.sub.30 alkyl group, X is --OCH.sub.2.CH.sub.2 —(i.e. when derived from ethylene oxide or glycol) or --OCH.sub.2.CHCH.sub.3 —(i.e. when derived from propylene oxide or glycol) and n is an integer from about 1 to 100. Still other nonionic surfactants are the condensation products of a mixture of fatty acids and fatty alcohols reacting with ethylene glycol or oxide or propylene glycol or oxide. These materials have the general formula RCO(X)nR’ wherein R and R’ are H or C.sub.10 to C.sub.30 alkyl groups, X is -OCH.sub.2.CH.sub.2 —(i.e. when derived from ethylene oxide or glycol) or —OCH.sub.2.CHCH.sub.3 —(i.e. when derived from propylene oxide or glycol), and n is an integer from about 1 to 100.

Examples of ethoxylated and propoxylated non-ionic surfactants include ethoxylated anhydrohexitol fatty esters, for example Tween 20; mono- and diethanolamides; Steareth-20, also known as Volpo20; polyethylene glycol fatty esters (PEGs), such as PEG-8-stearate, PEG-8 distearate; block co-polymers, which are essentially combinations of hydrophylic polyethoxy chains and lipophilic polypropoxy chains and generically known as Poloaxamers.

Still other useful non-ionic surfactants include fatty esters of polyglycols or polyhydric alcohols, such as mono and diglyceride esters; mono- and di-ethylene glycol esters; diethylene glycol esters; sorbitol esters also referred to as Spans; sucrose esters; glucose esters; sorbitan monooleate, also referred to as Span80; glyceryl monostearate; and sorbitan monolaurate, Span20 or Arlacel 20.
Yet other useful nonionic surfactants include polyethylene oxide condensates of alkyl phenols and polyhydroxy fatty acid amide surfactants which may be prepared as for example disclosed in U.S. Pat. No. 2,965,576 to E. R. Wilson.

Examples of amphoteric surfactants which can be used in the compositions of the present invention include the betaines, which can be prepared by reacting an alkylidimethyl tertiary amine, for example lauryl dimethylamine with chloroacetic acid. Betaines and betaine derivatives include higher alkyl betaine derivatives including coco dimethyl carboxymethyl betaine; sulfopropyl betaine; alkyl amido betaines; and cocoamido propyl betaine. Sulfosultaines which may be used include for example, cocamidopropyl hydroxy sulfate. Still other amphoteric surfactants include imidazoline derivatives and include the products sold under the trade name "Miranol" described in U.S. Pat. No. 2,528,378 which is incorporated herein by reference in its entirety. Still other amphoteric surfactants include phosphates for example, cocamidopropyl PG-dimonium chloride phosphate and alkylidimethyl amine oxides.

ADDITIVES

The cosmetic compositions described herein may also contain one or more moisturizers such as aloe vera, shea butter, allantoin, sodium hyaluronate, and protein hydrolysates. The cosmetic compositions described herein may also contain one or more soothing agents such as aloe vera. The cosmetic compositions described herein may also contain one or more regenerating agents such as aloe vera and collagen.

The cosmetic compositions described herein may also contain one or more adjuvants, including for example those that are common in cosmetics or dermatology. Adjuvants that may be mentioned in particular include gelling agents, active agents, preserving agents, antioxidants, fragrances, solvents, salts, fillers, sunscreens (=UV-screening agents), dyestuffs, basic agents (triethanolamine, diethanolamine or sodium hydroxide) or acidic agents (citric acid), and also lipid vesicles or any other type of vector (nanocapsules, microcapsules, etc.), hydrophilic surfactants, and mixtures thereof. These adjuvants are used in the usual proportions in the cosmetics field, for example from 0.01% to 30% of the total weight of the composition, and, depending on their nature, they are introduced into the aqueous phase of the composition or into the oily phase, or alternatively into vesicles or any other type of vector. These adjuvants and the concentrations thereof must be such that they do not modify the desired property for the emulsion of the invention.

Depending on the desired viscosity of the cosmetic compositions described herein, it is possible to incorporate therein one or more hydrophilic or lipophilic gelling agents.
Examples of hydrophilic gelling agents that may be mentioned include modified or unmodified carboxyvinyl polymers, such as the products sold under the names Carbopol (INCI name: carbomer) and Pemulen (INCI name: Acrylates/C 10-30 alkyl acrylate crosspolymer) by the company Noveon; polyacrylamides; optionally crosslinked and/or neutralized 2-acrylamido-2-methylpropane sulphonic acid polymers and copolymers, for instance the poly(2-acrylamido-2-methylpropanesulphonic acid) sold by the company Hoechst under the name "Hostacerin AMPS" (INCI name: ammonium polyacryldimethyltauramide); crosslinked anionic copolymers of acrylamide and of AMPS, which are in the form of a W/O emulsion, such as those sold under the name Sepigel 305 (CTFA name: Polyacrylamide/C13-14 Isoparaffin/Laureth-7) and under the name Simulgel 600 (CTFA name: Acrylamide/Sodium acryloyldimethyltaurate copolymer/Isohexadecane/Polyisorbate 80) by the company SEPPIC; polysaccharide biopolymers, for instance guar gum, alginates and modified or unmodified celluloses; and mixtures thereof. When they are present, these gelling agents must be introduced in an amount such that they do not modify the properties of the composition according to the invention. Lipophilic gelling agents that may especially be mentioned include modified clays such as modified magnesium silicate (Bentone Gel VS38 from Rheox), or hectorite modified with distearyldimethylammonium chloride (CTFA name: Distearidimonium hectorite) sold under the name Bentone 38 CE by the company Rheox.

Fillers that may be present in the cosmetic compositions described herein, include the pigments such as titanium oxide, zinc oxide or iron oxide and organic pigments; kaolin; silica; talc; boron nitride; organic spherical powders, fibres; and mixtures thereof. Organic spherical powders that may be present in the cosmetic compositions described herein include polyamide powders and especially Nylon.RTM., powders such as Nylon-1 or Polyamide 12, sold under the name Orgasol by the company Atochem; polyethylene powders; Teflon.RTM.; microspheres based on acrylic copolymers, such as those made of ethylene glycol dimethacrylate/lauryl methacrylate copolymer, sold by the company Dow Corning under the name Polytrel; expanded powders such as hollow microspheres and especially the microspheres sold under the name Expancel by the company Kemanord Plast or under the name Micropearl F 80 ED by the company Matsumoto; silicone resin microbeads such as those sold under the name Tospearl by the company Toshiba Silicone; polymethyl methacrylate microspheres, sold under the name Microsphere M-100 by the company Matsumoto or under the name Covabead LH85 by the company Wackherr; ethylene acrylate copolymer powders, such as those sold under the name Flobeads by the company Sumitomo.
Seika Chemicals; powders of natural organic materials such as starch powders, especially of maize starch, wheat starch or rice starch, which may or may not be crosslinked, such as the starch powders crosslinked with octenyl succinate anhydride, sold under the name Dry-Flo by the company National Starch. Examples of fibres that may be mentioned include polyamide fibres, especially such as Nylon 6 (or Polyamide 6) (INCI name: Nylon 6) fibres, Nylon 6,6 (or Polyamide 66) (INCI name: Nylon 66) fibres, or such as poly-p-phenyleneterephthalamide fibres; and mixtures thereof. These fillers may be present in amounts ranging from 0 to 20% by weight and preferably from 0.5% to 10% by weight relative to the total weight of the cosmetic compositions described herein.

[00600] Active agents that may be present in the cosmetic compositions described herein include but are not limited to flavonoids; anti-inflammatory agents; procyanidol oligomers; vitamins, for instance vitamin A (retinol), vitamin E (tocopherol), vitamin K, vitamin C (ascorbic acid), vitamin B5 (panthenol), vitamin B3 or PP (niacinamide), derivatives of these vitamins (especially esters) and mixtures thereof; polios, for instance glycerol, glycols, for instance polyethylene glycols, and sugar derivatives enzymes (for example lactoperoxidase, lipase, protease, phospholipase and cellulases); keratolytic agents and/or desquamating agents, such as salicylic acid and its derivatives, .alpha.-hydroxy acids, for instance lactic acid and glycolic acid and derivatives thereof, and ascorbic acid and its derivatives; urea; caffeine; depigmenting agents such as kojic acid, hydroquinone and caffeic acid; salicylic acid and its derivatives; retinoids such as carotenoids and vitamin A derivatives; hydrocortisone; melatonin; algal extracts, fungal extracts, plant extracts, yeast extracts or bacterial extracts; steroids; antibacterial active agents, for instance 2,4,4'-trichloro-2'-hydroxy-diphenyl ether (or triclosan), 3,4,4'-trichloro-carbanilide (or triclocarban) and the acids indicated above, and especially salicylic acid and its derivatives; tensioning agents such as synthetic polymers, plant proteins, polysaccharides of plant origin optionally in the form of microgels, starches, wax dispersions, mixed silicates and colloidal particles of mineral fillers; ceramides; anti-inflammatory agents; calmatives; mattifying agents; agents for preventing hair loss and/or for promoting regrowth of the hair; anti-wrinkle agents; essential oils; and mixtures thereof; and any active agent that is suitable for the composition.

[00601] Steroids that may be present in the cosmetic compositions described herein include dehydroepiandrosterone (or DHEA), and also (1) its precursors and biological derivatives, in particular the salts and esters of DHEA, such as DHEA sulphate and salicylate, 7-hydroxy DHEA, 7-keto DHEA, 7-hydroxy and 7-keto DHEA esters, especially 3-.beta.-acetoxy-7-oxo DHEA, and (2) its precursors and chemical derivatives, in particular
sapogenins such as diosgenin or hecogenin, and/or derivatives thereof such as hecogenin acetate, and/or natural extracts containing them and especially extracts of Dioscorea plants, such as wild yam.

[00602] UV-screening agents may be present in the cosmetic compositions described herein, such as organic or mineral (or physical UV sunblocks). They may be present in an active-material amount ranging from 0.01% to 20% by weight of active material, preferably from 0.1% to 15% by weight and better still 0.2% to 10% by weight relative to the total weight of the composition.

[00603] Examples of UV-A-active and/or UV-B-active organic screening agents that may be added to the cosmetic compositions described herein, include but are not limited to derivatives containing a sulphonic function, such as sulphone-containing or sulphonate-containing derivatives of benzylidenecamphor, of benzophenone or of phenylbenzimidazole, more particularly benzylidenecamphor derivatives, for instance benzene-1,4-bis(3-methylidenecamphor-10-sulphonic acid) (INCI name: Terephthalylidenedicamphor-sulphonic acid) manufactured under the name "Mexoryl SX" by the company Chimex, 3-benzylidenecamphor-4'-sulphonic acid (INCI name: Benzylidenecamphorsulphonic acid), manufactured under the name "Mexoryl SL" by the company Chimex, 2-[4-(camphormethylidene)phenyl]benz-imidazole-5-sulphonic acid and phenylbenzimidazole-sulphonic acid (INCI name: Phenylbenzimidazolesulphonic acid), sold under the name Eusolex 232 by the company Merck; para-aminobenzoic acid derivatives; salicylic derivatives such as ethylhexyl salicylate sold under the trade name Neo Heliopan OS by Haarmann & Reimer; dibenzoylmethane derivatives such as butylmethoxydibenzoylmethane sold especially under the trade name Parsol 1789 by Hoffmann La Roche; cinnamic derivatives such as ethylhexyl methoxycinnamate sold especially under the trade name Parsol MCX by Hoffmann La Roche; .beta.,.beta.,'-diphenylacrylate derivatives such as octocrylene (2-ethylhexyl alpha.,cyano-.beta.,.beta.,-diphenylacrylate) sold under the trade name Uvinul N539 by the company BASF; benzophenone derivatives such as Benzophenone-1 sold under the trade name Uvinul 400 by BASF, Benzophenone-2 sold under the trade name Uvinul D50 by BASF, Benzophenone-3 or Oxybenzone, sold under the trade name Uvinul M40 by BASF, Benzophenone-4 sold under the trade name Uvinul MS40 by BASF; benzylidene-camphor derivatives such as 4-methylbenzylidenecamphor sold under the trade name Eusolex 6300 by Merck; phenylbenzimidazole derivatives such as Benzimidazilate sold under the trade name Neo Heliopan AP by Haarmann & Reimer; triazine derivatives such as Anisotriazine sold under the trade name Tinosorb S by Ciba Geigy and ethylhexyltriazone
sold especially under the trade name Uvinul T 150 by BASF; phenylbenzotriazole derivatives such as Drometrizole Trisiloxane sold under the trade name Silatrizole by Rhodia Chimie and methylenebis-benzotriazolyl-tetramethylbutylphenol, sold in solid form under the trade name Mixxim BB/100 by Fairmount Chemical, or in micronized form as an aqueous dispersion under the trade name Tinosorb M by Ciba Specialty Chemicals; anthranilic derivatives such as methyl anthranilate sold under the trade name Neo Heliopan MA by Haarmann & Reimer; imidazoline derivatives; benzalmalonate derivatives; and mixtures thereof.

Physical sunblocks that may be added to the cosmetic compositions described herein, include but are not limited to pigments and nanopigments of coated or uncoated metal oxides, especially titanium oxide, iron oxide, zirconium oxide, zinc oxide or cerium oxide, and mixtures thereof, these oxides possibly being in the form of optionally coated microparticles or nanoparticles (nanopigments).

5.9.4 COSMETIC INDICATIONS

The cosmetic compositions described here can be administered to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance of the body. The cosmetic compositions described herein can be used as, e.g., skincare cosmetics, hair cosmetics, antiperspirants, makeup cosmetics, or UV-ray protective cosmetics. Examples include, but are not limited to, basic cosmetics such as milky lotion, cream, lotion, calamine lotion, sunscreen agent, suntan agent, after shave lotion, pre-shave lotion, pack, cleansing, face wash, and cosmetic for acne protection; makeup cosmetic such as face foundation, powder, foundation, eye shadow, eyeliner, eyebrow, rouge, lipstick and nail color; hairdressing products, such as shampoo, rinse, conditioner, hair color, hair tonic, setting agent, body powder, deodorant, hair remover, soap, body shampoo, bath powder, hand soap, and perfume.

The cosmetic compositions of the present invention can be used to treat or reverse skin changes associated with aging such as wrinkles, blotches and atrophy or elastotic changes associated with intrinsic aging of the skin as well as changes caused by external factors for example sunlight radiation; X-ray radiation; air pollution; wind; cold; dampness; dryness; heat; smoke and cigarette smoking; external infectious agents such as fungi and bacteria; and combinations thereof.

The cosmetic compositions described herein can be used for any cosmetic indication including, but not limited to, reducing wrinkles, reducing unwanted hair, promoting hair growth, reducing sun spots, tightening loose skin, improving skin tone and
color, reducing blotchiness, eliminating damaged blood vessels, reducing age spots, reducing acne scars, reducing varicose and spider veins, reducing cellulite, reducing acne, reducing actinic keratosis, reducing brown spots, reducing crows feet and laugh lines, reducing dark circles around the eyes, reducing the appearance of facial veins, reducing the appearance of freckles, reducing the appearance of leg veins, reducing melasma, reducing port wine stains, reducing rosacea, reducing strechmarks, reducing sebaceous hyperplasia, reducing venous lake, wart removal, wound healing, skin plumping, skin rejuvenation, or epidermal thickening.

The cosmetic compositions described herein can be applied after rejuvenation treatments such as laser treatments, microdermabrasion, and chemical peels.

In certain embodiments, the cosmetic compositions described herein can be used to increase the thickness of the epidermis of a subject. In certain embodiments, the method for increasing the thickness of the epidermis of a subject comprises perturbation of the skin followed by treatment with a cosmetic composition described herein.

In certain embodiments, the method of increasing the thickness of the epidermis is applied to a subject for cosmetic purposes. In certain embodiments, the subject is at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, or at least 90 years of age. In certain embodiments, the subject is a cigarette smoker at the time of application of the method of the invention. In certain embodiments, the subject, has been a cigarette smoker for at least 1 year, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or at least 20 days/years.

In even further embodiments, a method disclosed herein for increasing the thickness of the epidermis is used to reduce wrinkles of the skin of a subject. More specifically, the method disclosed herein for increasing the thickness of epidermis is applied to an area of the body with wrinkled skin, such as facial skin (e.g., forehead, cheeks), skin of the neck etc. In certain embodiments, the number and/or depth of wrinkles is reduced by application of the method of the present invention by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at least 90%, or by 100%. Evaluation of the skin before and after treatment can be performed as follows (Emsen, 2008, The Journal Of Craniomaxillofacial Surgery 19(3):812-816): (1) patient selfevaluation, (2) dermatologist assessment (baseline and after dermasanding procedure), (3) digital standardized photography, and (4) colorimetry. Self-evaluation can consist of rating a list of attributes regarding the subject's perception on the look and feel of the subject's skin on a 1 to 10 scale (1 = undesirable, 10 = desirable). The dermatologist's evaluations can include efficacy variables. Efficacy variables can be scored on a 0 to 9 scale (0 = none, 9 = severe) and the following: (1) fine wrinkling, (2) skin dullness,
(3) mottled hyperpigmentation, or solar lentigines, (4) blotchiness, (5) appearance of milia, (6) appearance of acne, and (7) appearance of large pores. The dermatologist can also perform a global assessment using a 0 to 9 scale (0 = very good, 9 = very poor) for the following variables: (1) overall skin texture, (2) overall skin tone, and (3) overall skin appearance.

[00612] In certain embodiments, the methods disclosed herein for increasing the thickness of the epidermis is used prophylactically to outdoor workers, such as farmers, fishermen, construction workers, lifeguards, outdoor enthusiasts, sunbathers, or people who regularly attend tanning salons or use tanning beds. In specific embodiments, the methods disclosed herein for increasing the thickness of the epidermis is used prophylactically to fair women under age 50. In specific embodiments, the methods disclosed herein for increasing the thickness of the epidermis is used prophylactically to people with skin phototypes I and II.

[00613] Without being bound by theory aged and/or sun exposed skin has a thin epidermis. The methods disclosed herein for increasing the thickness of the epidermis can be applied to aged and/or sun exposed skin of a subject. In certain embodiments, the thickness of the aged and/or sun exposed skin is at most 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at most 90%, of the thickness of the epidermis of skin in the same body area without the effect of age and/or sun exposure. In certain embodiments, the methods disclosed herein for increasing the thickness of the epidermis can be applied to skin areas subject to UV induced skin aging. In other embodiments, the methods disclosed herein for increasing the thickness of the epidermis can be applied to skin areas subject to Non-UV related skin aging.

[00614] In certain embodiments, the methods disclosed herein for increasing the thickness of the epidermis can be applied to reduce any undesired effects of microdermabrasion.

[00615] In further embodiments, a method disclosed herein for increasing the thickness of the epidermis is used to increase the thickness of the epidermis in a subject with steroid induced atrophy. In certain embodiments, the thickness of the atrophied skin is at most 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or at most 90% of the thickness of the epidermis of skin in the same body area without steroid induced atrophy. In more specific embodiments, the method disclosed herein for increasing the thickness of the epidermis is applied to a subject with skin thinning due to topical corticosteroid treatment. In a specific embodiment, the corticosteroid is Cortisol.

[00616] In certain embodiments, epidermal thickness is increased by application of the method of the present invention by at least 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%,...
50%, 55%, 60%, 65%, 70%, 75, 80%, 85%, 90%, 95%, 100%, 125%, 150%, 175%, or by at least 200%.

[00617] Epidermal thickness of a subject can be determined by any method known to the skilled artisan. In a specific embodiment, epidermal thickness of a subject can be determined by a high-frequency ultrasound (see, e.g., Hermann et al., Skin Pharmacol 1:128-136) or by multiphoton laser tomography (see, e.g., Koehler et al. 2010, Skin Research and Technology 16: 259-264) or by optical coherence tomography (see, e.g., Morgensen 2008, Dermatology 217:14-20).

5.9.5 ILLUSTRATIVE EMBODIMENTS

[00618] In certain embodiments, a cosmetic composition provided herein comprises the following components at the listed concentrations: Lithium Hydroxide monohydrate at 0.1% to 0.5%, 0.1% to 0.3%, 0.15% to 0.25%, at about 0.2%, or at 0.206%; Citric Acid at 6% to 10%, 7% to 9%; or at 8%; CMC at 1% to 3%, 1.5% to 2.5%, or at 2%; Methyl Paraben at 0.05% to 0.15%, or at 0.1%; Propyl Paraben at 0.01 to 0.1%, 0.02 to 0.08%; 0.03% to 0.06%, or 0.05%; distilled Water to 100%; 10% NaOH to adjust pH; Allantoin at 0.05% to 0.3%, at 0.1% to 0.2%, or at 0.16%; Alginate at 0.05% to 0.2%, 0.08% to 0.14%, or at 0.12%; and Glycerin at 5% to 15%, 7% to 12%, or at 10%.

[00619] In certain embodiments, a cosmetic composition provided herein comprises the following components at the listed concentrations: Lithium Hydroxide monohydrate at 0.1% to 0.5%, 0.1% to 0.3%, 0.15% to 0.25%, at about 0.2%, or at 0.206%; Citric Acid at 6% to 10%, 7% to 9%; or at 8%; Sodium Hyaluronate at 1% to 3%, 1.5% to 2.5%, or at 2%; Methyl Paraben at 0.05% to 0.15%, or at 0.1%; Propyl Paraben at 0.01 to 0.1%, 0.02 to 0.08%; 0.03% to 0.06%, or 0.05%; distilled Water to 100%; 10% NaOH to adjust pH; Allantoin at 0.05% to 0.3%, at 0.1% to 0.2%, or at 0.16%; Alginate at 0.05% to 0.2%, 0.08% to 0.14%, or at 0.12%; and Glycerin at 5% to 15%, 7% to 12%, or at 10%.

[00620] In certain embodiments, a cosmetic composition provided herein comprises the following components at the listed concentrations: Lithium Hydroxide monohydrate at 0.1% to 0.5%, 0.1% to 0.3%, 0.15% to 0.25%, at about 0.2%, or at 0.206%; Citric Acid at 6% to 10%, 7% to 9%; or at 8%; Collagen at 1% to 3%, 1.5% to 2.5%, or at 2%; Methyl Paraben at 0.05% to 0.15%, or at 0.1%; Propyl Paraben at 0.01 to 0.1%, 0.02 to 0.08%; 0.03% to 0.06%, or 0.05%; distilled Water to 100%; 10% NaOH to adjust pH; Aloe Vera Gel at 0.05% to 0.3%, at 0.1% to 0.2%, or at 0.16%; Alginate at 0.05% to 0.2%, 0.08% to 0.14%, or at 0.12%; and Glycerin at 5% to 15%, 7% to 12%, or at 10%.
In certain embodiments, a cosmetic composition provided herein is an emollient cream which is composed of a Phase I and a Phase II, wherein Phase I comprises Lithium Hydroxide at 0.1% to 1%, 0.2% to 0.8%, 0.3% to 0.6%, 0.35% to 0.5% or at 0.4%; Citric Acid at 10% to 20%, 12% to 18%, 14% to 16%, 15% to 17%, or at 16%; Hyaluronic Acid at 1% to 3%, 1.5% to 2.5%, or at 2%; Glycerin at 5% to 15%, 7% to 12%, or at 10%; Allantoin at 0.1% to 1%, 0.2% to 0.8%, 0.3% to 0.6%, 0.35% to 0.5%, at 0.4%, or at 0.32%; Sodium Chloride at 0.1% to 1%, 0.3% to 0.7%, or at 0.5%; Methyl Paraben at 0.1% to 0.3%, 0.15% to 0.25%, or at 0.2%, Propyl Paraben at 0.05 to 0.15%, 0.075 to 0.125% or at 0.1% or at 0.096%>; 10%, NaOH to adjust the pH; and Water to 100%; and wherein Phase II comprises Soybean Oil at 10% to 30%, 15% to 25%, 18% to 22% or at 20%; Hydrogenated Cottonseed Oil at 5% to 15%, 7.5% to 12.5%, at 9% to 11%, or at 10%; Polyglyceryl-10 decaoleate at 4% to 12%, 6% to 10%, 7% to 9% or at 8%; Polyglyceryl-6-octastearate at 1% to 7%, 2% to 6%, 3% to 5%, or at 4%; Jojoba Seed Oil at 2% to 8%, 3% to 7%, 4% to 6%, or at 5%; Shea Butter at 0.5% to 8%, 1% to 6%, 2% to 4%, 2.5% to 3.5% or at 3%; Olive Oil at 5% to 15%, 7% to 12%, or at 10%. In certain embodiments, the emollient cream further comprises Lithium Hydroxide at 0.1% to 0.5%, 0.1% to 0.3%, 0.15% to 0.25%, at about 0.2%; Citric Acid at 6% to 10%, 7% to 9%; or at 8%; Hyaluronic Acid at 0.25% to 2.5%, 0.5% to 2%, 0.75% to 1.5%, or at 1%; Glycerin at 1% to 9%, 2% to 8%, 3% to 8%, 4% to 6%, or at 5%; Allantoin 0.05% to 0.3%, at 0.1% to 0.2%, or at 0.16%; Sodium Chloride at 0.05% to 0.5%, 0.1% to 0.4%, 0.2% to 0.3%, or at 0.25%; Methyl Paraben at 0.05% to 0.15%, or at 0.1%; Propyl Paraben at 0.01 to 0.1%, 0.02 to 0.08%; 0.03% to 0.06%, or 0.05%; 10% NaOH to adjust the pH; Water to 100%; Soybean Oil at 5% to 15%, 7% to 12%, or at 10%; Hydrogenated Cottonseed Oil 5% to 15%, 7% to 12%, or at 10%; Polyglyceryl-10 decaoleate at 1% to 6%, 2% to 5%, 3% to 5%, or at 4%; Polyglyceryl-6-octastearate at 1% to 3%, 1.5% to 2.5%, or at 2%; Jojoba Seed Oil 1% to 5%, 1.5% to 4%, 2% to 3%, or at 2.5%; Shea Butter at 0.5% to 4%, 1% to 3%, 1.25% to 2%, or at 1.5%; and Olive Oil at 5% to 15%, 7% to 12%, or at 10%.

In certain embodiments, a cosmetic composition provided herein comprises the following components at the listed concentrations: Lithium Carbonate at 0.1% to 0.5%, 0.1% to 0.3%, 0.15% to 0.25%, at about 0.2%, or at 0.206%; Citric Acid at 6% to 10%, 7% to 9%; or at 8%; CMC at 1% to 3%, 1.5% to 2.5%, or at 2%; Methyl Paraben at 0.05% to 0.15%, or at 0.1%; Propyl Paraben at 0.01 to 0.1%, 0.02 to 0.08%; 0.03% to 0.06%, or 0.05%; distilled Water to 100%; 10% NaOH to adjust pH; Allantoin at 0.05% to 0.3%, at 0.1% to 0.2%, or at...
0.16%; Alginate at 0.05% to 0.2%, 0.08% to 0.14%, or at 0.12%; and Glycerin at 5% to 15%, 7% to 12%, or at 10%.

[00623] In certain embodiments, a cosmetic composition provided herein comprises the following components at the listed concentrations: Lithium Carbonate at 0.1% to 0.5%, 0.1% to 0.3%, 0.15% to 0.25%, at about 0.2%, or at 0.206%; Citric Acid at 6% to 10%, 7% to 9%; or at 8%; Collagen at 1% to 3%, 1.5% to 2.5%, or at 2%; Methyl Paraben at 0.05% to 0.15%, or at 0.1%; Propyl Paraben at 0.01 to 0.1%, 0.02 to 0.08%; 0.03% to 0.06%, or 0.05%; distilled Water to 100%; 10% NaOH to adjust pH; Aloe Vera Gel at 0.05% to 0.3%, at 0.1% to 0.2%, or at 0.16%; Alginate at 0.05% to 0.2%, 0.08% to 0.14%, or at 0.12%; and Glycerin at 5% to 15%, 7% to 12%, or at 10%.

[00624] In certain embodiments, a cosmetic composition provided herein is an emollient cream which is composed of a Phase I and a Phase II, wherein Phase I comprises Lithium Carbonate at 0.1% to 1%, 0.2% to 0.8%, 0.3% to 0.6%, 0.35% to 0.5% or at 0.4%; Citric Acid at 10% to 20%, 12% to 18%, 14% to 16%, 15% to 17%, or at 16%; Hyaluronic Acid at 1% to 3%, 1.5% to 2.5%, or at 2%; Glycerin at 5% to 15%, 7% to 12%, or at 10%; Allantoin at 0.1% to 1%, 0.2% to 0.8%, 0.3% to 0.6%, 0.35% to 0.5%, at 0.4%, or at 0.32%; Sodium Chloride at 0.1% to 1%, 0.3% to 0.7%, or at 0.5%; Methyl Paraben at 0.1% to 0.3%, 0.15% to 0.25%, or at 0.2%, Propyl Paraben at 0.05 to 0.15%, 0.075 to 0.125% or at 0.1% or at 0.096%>; 10%, NaOH to adjust the pH; and Water to 100%; and wherein Phase II comprises Soybean Oil at 10% to 30%, 15% to 25%, 18% to 22% or at 20%; Hydrogenated Cottonseed Oil at 5% to 15%, 7.5% to 12.5%, at 9% to 11%, or at 10%; Polyglyceryl-10 decaoleate at 4% to 12%, 6% to 10%, 7% to 9% or at 8%; Polyglyceryl-6-octastearate at 1% to 7%, 2% to 6%, 3% to 5%, or at 4%; Jojoba Seed Oil at 2% to 8%, 3% to 7%, 4% to 6%, or at 5%; Shea Butter at 0.5% to 8%, 1% to 6%, 2% to 4%, 2.5% to 3.5% or at 3%; Olive Oil at 5% to 15%, 7% to 12%, or at 10%. In certain embodiments, the emollient cream further comprises Lithium Carbonate at 0.1% to 0.5%, 0.1% to 0.3%, 0.15% to 0.25%, at about 0.2%; Citric Acid at 6% to 10%, 7% to 9%; or at 8%; Hyaluronic Acid at 0.25% to 2.5%, 0.5% to 2%, 0.75% to 1.5%, or at 1%, Glycerin at 1% to 9%, 2% to 8%, 3% to 8%, 4% to 6%, or at 5%; Allantoin 0.05% to 0.3%, at 0.1% to 0.2%, or at 0.16%; Sodium Chloride at 0.05% to 0.5%, 0.1% to 0.4%, 0.2% to 0.3%, or at 0.25%; Methyl Paraben at 0.05% to 0.15%, or at 0.1%; Propyl Paraben at 0.01 to 0.1%, 0.02 to 0.08%; 0.03% to 0.06%, or 0.05%; 10% NaOH to adjust the pH; Water to 100%; Soybean Oil at 5% to 15%, 7% to 12%, or at 10%; Hydrogenated Cottonseed Oil 5% to 15%, 7% to 12%, or at 10%; Polyglyceryl-10 decaoleate at 1% to 6%, 2% to 5%, 3% to 5%, or at 4%; Polyglyceryl-6-octastearate at 1% to 3%, 1.5%
to 2.5%, or at 2%; Jojoba Seed Oil 1% to 5%, 1.5% to 4%, 2% to 3%, or at 2.5%; Shea Butter
at 0.5% to 4%, 1% to 3%, 1.25% to 2%, or at 1.5%; and Olive Oil at 5% to 15%, 7% to 12%,
or at 10%.

5.10 METHODS FOR EVALUATING TREATMENT

[00625] The effectiveness of the methods and compositions described herein can be
demonstrated using the following methods.

5.10.1 ANTI-MICROBIAL ASSAYS

[00626] The lithium treatments described supra can be tested for anti-microbial activity.
Any of the standard anti-microbial assays well-known in the art can be used to assess the
anti-microbial activity of a lithium compound. The anti-microbial effect on different species
of microorganisms can be tested. The tests recommended by the National Committee for
Clinical Laboratories (NCCLS) (See National Committee for Clinical Laboratories Standards.
1995, Proposed Standard M27T. Villanova, Pa., all of which is incorporated herein by
reference in its entirety) and other methods known to those skilled in the art (Pfaller et al.,
1993, Infectious Dis. Clin. N. Am. 7: 435-444) can be used to assess the anti-microbial effect
of a lithium compound. The anti-microbial activities of lithium compounds can be tested
using macrodilution methods and/or microdilution methods using protocols well-known to
those skilled in the art (see, e.g., Clancy et al., 1997 Journal of Clinical Microbiology,
35(11): 2878-82; Ryder et al., 1998, Anti-microbial Agents and Chemotherapy, 42(5): 1057-
61; U.S. 5,521,153; U.S. 5,883,120, U.S. 5,521,169, all of which are incorporated by
reference in their entirety). Briefly, a microbial strain is cultured in an appropriate liquid
media, and grown at an appropriate temperature, depending on the particular microbial strain
used for a determined amount of time, which is also depends on the particular microbial
strain used. An inoculum is then prepared photometrically and the turbidity of the
suspension is matched to that of a standard, e.g., a McFarland standard. The effect of the
lithium compound on the turbidity of the inoculum is determined visually or
spectrophotometrically. The minimal inhibitory concentration of the lithium compound
(MIC) is determined, which is defined as the lowest concentration of the lithium compound
which prevents visible growth of an inoculum as measured by determining the culture
turbidity.

[00627] The anti-microbial activity of a lithium compound can also be determined
utilizing colorimetric based assays well-known to one of skill in the art. One exemplary
colorimetric assay that can be used to assess the anti-microbial activity of a lithium compound is described by Pfaller et al. (1994, Journal of Clinical Microbiology, 32(8): 1993-6, which is incorporated herein by reference in its entirety; also see Tiballi et al., 1995, Journal of Clinical Microbiology, 33(4): 915-7). This assay employs a colorimetric endpoint using an oxidation-reduction indicator (Alamar Biosciences, Inc., Sacramento CA).

[00628] The anti-microbial activity of a lithium compound can also be determined utilizing photometric assays well-known to one of skill in the art (see, e.g., Clancy et al, 1997 Journal of Clinical Microbiology, 35(11): 2878-82; Jahn et al, 1995, Journal of Clinical Microbiology, 33(3): 661-667, each of which is incorporated herein by reference in its entirety). This photometric assay is based on quantifying mitochondrial respiration by viable fungi through the reduction of 3-(4,5-dimethyl-2thiazolyl)-2,5,-diphenyl-2H-tetrazolium bromide (MTT) to formazan. MIC's determined by this assay are defined as the highest concentration of the test compound associated with the first precipitous drop in optical density. In some embodiments, the lithium compounds are assayed for anti-microbial activity using macrodilution, microdilution and MTT assays in parallel.

[00629] The anti-microbial properties of the lithium compounds may also be determined from a microbial lysis assay, as well as by other methods, including, inter alia, growth inhibition assays, fluorescence-based microbial viability assays, flow cytometry analyses, and other standard assays known to those skilled in the art.

[00630] In some embodiments, the assays for growth inhibition of a microbial strain by a lithium compound are used to derive an "Ed. 50," value for the compound, which is defined as the concentration of the compound required to kill 50% of the microbial cell. Alternatively, growth inhibition by a lithium compound may also be characterized in terms of the minimum inhibitory concentration (MIC), which is the concentration of compound required to achieve inhibition of microbial cell growth. Such values are well known to those in the art as representative of the effectiveness of a particular anti-microbial agent against a particular organism or group of organisms. For instance, cytolysis of a microbial population by an anti-microbial compound can also be characterized, as described above by the minimum inhibitory concentration, which is the concentration required to reduce the viable microbial population by 99.9%. The value of MIC. 50 can also be used, defined as the concentration of a compound required to reduce the viable microbial population by 50% .

[00631] Another parameter useful in identifying and measuring the anti-microbial effectiveness of a lithium compound is the determination of the kinetics of the anti-microbial activity of the lithium compound. Such a determination can be made by determining anti-
microbial activity as a function of time. In a preferred embodiment, a lithium compound displays kinetics which result in efficient lysis of a microbial cell.

[00632] In a most preferred embodiment, a lithium compound displays selective toxicity to target microorganisms (in particular, bacteria and fungi) and minimal toxicity to animalia (preferably mammalian cells, and most preferably human cells). Determination of the toxic dose (or "LD$_{50}$") can be carried out using protocols well-known in the field of pharmacology. Ascertaining the effect of a lithium compound on animalia (preferably, mammalian cells and most preferably human cells) is preferably performed using tissue culture assays, well-known to those skilled in that art (see, for example, Gootz, T. D. (1990) Clin. Microbiol. Rev. 3:13-31, which is incorporated herein by reference in its entirety). For mammalian cells, such assay methods include, inter alia, trypan blue exclusion and MTT assays (Moore et al. (1994) Compound Research 7:265-269 which is incorporated herein by reference in its entirety). Where a specific cell type may release a specific metabolite upon changes in membrane permeability, that specific metabolite may be assayed, e.g., the release of hemoglobin upon the lysis of red blood cells (Srinivas et al. (1992) J. Biol. Chem. 267:7121-7127 which is incorporated herein by reference in its entirety). The lithium compounds are preferably tested against primary cells, e.g., using human skin fibroblasts (HSF) or fetal equine kidney (FEK) cell cultures, or other primary cell cultures routinely used by those skilled in the art. Permanent cell lines may also be used, e.g., Jurkat cells. In some embodiments, the lithium compounds are tested in cancer lines, including but not limited to Caco-2 (human colon carcinoma cell line) and Huh7 (human hepatoma cell line). In yet other embodiments, the lithium compounds of the invention may be tested in peripheral blood mononuclear cells (PBMC). In preferred embodiments, the lithium compounds are selected for use in animals, or animal cell/tissue culture based, at least in part, on having LD$_{so}$'s at least one order of magnitude greater than the MIC or ED$_{sub.50}$ as the case may be, and even more preferably at least two, three and even four orders of magnitude greater. That is, in preferred embodiments, where the lithium compounds are to be administered to an animal, a suitable therapeutic index is preferably greater than 10, and more preferably greater than 100, 1000 or even 10,000.

5.10.2 CELL VIABILITY AND CELL PROLIFERATION ASSAYS

[00633] Many assays well-known in the art can be used to assess the proliferation and viability of bacterial cells or mycotic agents following exposure to the lithium treatments provided herein. For example, cell proliferation can be assayed by measuring
Bromodeoxyuridine (BrdU) incorporation, (3H) thymidine incorporation, by direct cell count, or by detecting changes in transcription, translation or activity of known genes such as proto-oncogenes (e.g., fos, myc) or cell cycle markers (Rb, cdc2, cyclin A, D1, D2, D3, E, etc). The levels of such protein and mRNA and activity can be determined by any method well known in the art. For example, protein can be quantitated by known immunodiagnostic methods such as ELISA, Western blotting or immunoprecipitation using antibodies, including commercially available antibodies. mRNA can be quantitated using methods that are well known and routine in the art, for example, using northern analysis, RNase protection, or polymerase chain reaction in connection with reverse transcription.

[00634] Cell viability can be assessed by using trypan-blue staining or other cell death or viability markers known in the art. In a specific embodiment, the level of cellular ATP is measured to determined cell viability. In specific embodiments, cell viability is measured in three-day and seven-day periods using an assay standard in the art, such as the CellTiter-Glo Assay Kit (Promega) which measures levels of intracellular ATP. A reduction in cellular ATP is indicative of a cytotoxic effect. In another specific embodiment, cell viability can be measured in the neutral red uptake assay. In other embodiments, visual observation for morphological changes may include enlargement, granularity, cells with ragged edges, a filmy appearance, rounding, detachment from the surface of the well, or other changes. These changes are given a designation of T (100% toxic), PVH (partially toxic-very heavy-80%), PH (partially toxic-heavy-60%), P (partially toxic-40%), Ps (partially toxic-slight-20%), or 0 (no toxicity-0%), conforming to the degree of cytotoxicity seen. A 50% cell inhibitory (cytotoxic) concentration (IC$_{50}$) is determined by regression analysis of these data.

[00635] The toxicity and/or efficacy of a formulation in accordance with the invention can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD$_{50}$ (the dose lethal to 50% of the population) and the ED$_{50}$ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD$_{50}$/ED$_{50}$. A formulation identified in accordance with the invention that exhibits large therapeutic indices is preferred. While a formulation identified in accordance with the invention that exhibits toxic side effects may be used, care should be taken to design a delivery system that targets such agents to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

[00636] The data obtained from the cell culture assays and animal studies can be used in formulating a range of dosage of a formulation identified in accordance with the invention for
use in humans. The dosage of such agents lies preferably within a range of circulating concentrations that include the ED$_{50}$ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any agent used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range that includes the IC$_{50}$ (i.e., the concentration of the test formulation that achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high-performance liquid chromatography.

5.10.3 SPORE COUNT ASSAYS

[00637] Any assay well known in the art can be used to determine the spore count of microbial agents following exposure to the formulations provided herein. For example, the viable microbial spore count can be measured by colony counting, and then the total microbial spore count can be measured by direct microscopic counting. The ratio of viable to total microbial spore count yields the fraction of spores that remain viable within a given sample.

[00638] A procedure for colony counting to determine endospore concentration is, for example, comprised of the steps of (1) heat shocking a microbial sample to kill vegetative cells while microbial spores remain viable, (2) plating a known volume of the sample with a known dilution factor onto a growth medium, and (3) incubating the growth plates for 2 days. Finally, the resulting visible colonies can be counted and reported as colony forming units (CFU’s). A procedure for direct microscopic counting is, for example, comprised of the steps of (1) placing a microbial sample on a slide with an indentation of a known volume, and (2) counting the spores in each the several squares and multiplying the average count by an appropriate factor to yield the number of total cells per milliliter in the original suspension.

5.10.4 ANIMAL MODELS

5.10.4.1 ANIMAL MODELS FOR BACTERIAL INFECTIONS

[00639] Animal models for bacterial infections can be used to assess the efficacy of a lithium treatment described herein. Animal models for bacterial infections such as H. pylori-infection, genital mycoplasmosis, primary sclerosing cholangitis, cholera, chronic lung

**[00640]** The lithium treatment described herein can be tested for their ability to decrease the time course of bacterial infection, e.g., a cutaneous bacterial infection by at least 25%, at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99% relative to a negative control using methods well known in the art.

**[00641]** The efficacy of the lithium treatments described here in for the prevention, treatment and/or management of a fungal infection can be assessed in animal models for such infections. Animal models for fungal infections such as *Candida* infections, *zygomycosis*, *Candida mastitis*, progressive disseminated trichosporonosis with latent trichosporonemia, disseminated candidiasis, pulmonary paracoccidioidomycosis, pulmonary aspergillosis, *Pneumocystis carinii* pneumonia, cryptococcal meningitis, coccidiodal meningoencephalitis and cerebrospinal vasculitis, *Aspergillus niger* infection, *Fusarium keratitis*, paranasal sinus mycoses, *Aspergillus fumigatus* endocarditis, tibial dyschondroplasia, *Candida glabrata*
The lithium treatment described herein can be tested for their ability to decrease the time course of fungal infection, e.g., a cutaneous fungal infection by at least 25%, at least 50%, at least 60%, at least 75%, at least 85%, at least 95%, or at least 99% relative to a negative control using methods well known in the art.

The safety and efficacy of the lithium treatments described herein may also be measured in human subjects according to methods known in the art.

5.10.4.2 HUMAN SKIN XENOGRAFT MODELS

Human skin and hair have features that are relatively unique among terrestrial mammals. For example, in mouse models involving human diseases associated with scarring or wounding, the mice appear deficient in scarring and heal their wounds rapidly.

Similarly, there are differences with regard to hair patterning in animals compared to humans. First, the great majority of human skin appears hairless to the naked eye, while the vast majority of other terrestrial mammals are essentially covered with visible hair. Second, visible human hair appears and disappears in patterns that have spatial and temporal components. Third, the patterns of visible human hair are distinct in typical male and females (exhibit gender dimorphism). Accordingly, it is evident that relative to other mammals, humans have distinct hair patterning and humans have correspondingly distinct molecular, cellular and tissue mechanisms that regulate hair growth and that control human hair, patterning. Modulating human hair follicle neogenesis, and, consequently, wound healing and scar revision as a result of such modulation, requires considerations that are unique to humans and for which other animals are insufficient models.

It should be noted that certain non-human primates share features of hair patterning with humans, but not to the degree or extent. Old World Apes (gorillas and chimpanzees) have areas of skin that lack visible hair; on the face surrounding the eyes, nose and mouth; on ears; and the plantar surfaces of hands and feet. In addition, Rhesus Macaque has patterned alopecia in males and females. Gorillas have hair patterning with respect to color on dominant males: i.e., the "Silverback". While certain of these mechanisms share similarities to humans, the extent and degree of hair patterning in human remains relatively unique.

Preliminary evidence of hair follicle neogenesis has been demonstrated in human skin (obtained from the hair line during a face lift procedure) grafted onto the back of an immunodeficient SCID mouse. Such human skin xenograft models are useful for testing the safety and efficacy of lithium treatments described herein, as well as the combination
treatments described in Section 5.5 supra. Any method for producing human skin xenografts known in the art may be used.

[00648] Alternatively, a human skin xenograft (without skin appendages) can be considered as similar to a scar, and can be wounded and then treated pharmacologically to induce hair follicles and/or monitor revision of the scar. Xenografts can also be combined with inducible genetically modified cells to activate pathways known to form hair follicles.

[00649] In some embodiments, the safety and efficacy of a lithium treatment, optionally as part of a combination treatment described in Section 5.5 supra, is tested in a full thickness or a split thickness human skin xenograft (e.g., obtained surgically from scar revisions; from foreskin; or cadaveric), or may be tested in a three-dimensional organotypic human skin culture on SCID mice.

[00650] Success of a lithium treatment for wound healing or scar revision described herein can be measured by:

- improvement of pigmentation of the scarred or wounded area
- improved thickness of the scarred or wounded area
- improved surface contour of the scarred or wounded area
- improved texture of the scarred or wounded area
- improved overall cosmetic outcome
- hair follicle regeneration
- return of adnexal structures to the area
- increased proportion of hair follicles in anagen or decreased proportion of follicles in telogen
- increased numbers of follicular units with 3 or more hair follicles.

[00651] Any method known in the art may be used to evaluate the safety and efficacy of a lithium treatment protocol or pulse lithium protocol, or of the combination treatments described in Section 5.5. Preferably, a human skin xenograft model is used. For example, an intermittent lithium treatment or pulse lithium treatment may be administered with a full thickness excision, laser, inflammatory stimulus, or dermabrasion procedure for integumental perturbation. A synergistic effect of an intermittent lithium treatment or pulse lithium treatment on another treatment for enhancing wound healing or scar revision may be measured as an improvement over a control subject receiving only one of the two treatments (i.e., the intermittent lithium treatment or pulse lithium treatment alone or the second treatment alone).
5.10.4.3 OTHER ANIMAL MODELS

[00652] Another animal model for use in evaluating treatment that may more closely mimic the biology of human skin is a guinea pig model (see, Stenn & Paus, 2001, Physiol. Revs. 81:449-494). The methods for evaluating treatment in animals described elsewhere in this section may be applied to guinea pigs according to methods known in the art. See also, e.g., Kramer et al., 1990, Dermatol Monatsschr. 176:417-20; and Simon et al., 1987, Ann Plast Surg 19:519-23.


[00654] Other animal models that may be of use in evaluating the treatments described herein include other pig models (e.g., minipig), cat, or stumptailed macaque models.

5.10.4.4 TOXICOLOGY STUDIES

[00655] In certain embodiments, toxicological studies for the lithium formulations described herein are carried out in accordance with the methods outlined in the table below.

<table>
<thead>
<tr>
<th>Study Title</th>
<th>Species</th>
<th>Groups</th>
<th>Proposed Study Design and Measurable End-Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 day repeat dose study + 14 day recovery</td>
<td>Rodent Species: Rat Sprague Dawley</td>
<td>Four Dose Groups (0 (vehicle) and 3 treatment groups) with and without wound. 8 groups in total. N=10/group, 5 recovery at Control and High Dose, 6/sex/group for TK</td>
<td>Dosing: Twice daily with washing at 23h and irritation reading at 24h and application of next dose. Recovery time: 14 days  Application Area: Up to 1/10th the body surface area  Necropsy: Day 29  Blood Sampling for Li+ measurement AUC(0-24h), T_max, C_max at baseline (pre-treatment) Day 1, 7 and 28 at selected time points, Test article will be retained for concentration analysis at the end of the study.  Measurable End-Points:  1. Clinical observation, mortality/morbidity; daily</td>
</tr>
<tr>
<td>Study Title</td>
<td>Species</td>
<td>Groups</td>
<td>Proposed Study Design and Measurable End-Points</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>--------------------------</td>
<td>-----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>3 month Repeat Dose study + 1 month recovery</td>
<td>Rodent Species: Rat</td>
<td>1. Five Dose Groups (sham, 0 (vehicle) and 3 treatment groups)</td>
<td>2. Body weight and food consumption: pre-dose, weekly, before necropsy 3. Ophthalmoscopy 4. Cutaneous Irritation: daily, test site will be scored using Draize Scoring 5. Pre-Dose and Terminal Bleeding for standard clinical chemistry, hematology, coagulation, and urinalysis panels 6. TK: Day 1 and Day 28 7. Histology/Pathology: Full histopathology from high dose and control groups will be microscopically examined. Tissues and organs from other groups will be preserved and examined based on findings of the high dose group. Skin, including a distal site such as the skin on the head, will be examined to determine if there is any effect of absorbed Li+ after circulation to a distal skin site. 8. Organ Weights: Standard Panel 9. Necropsy: Full on all animals</td>
</tr>
<tr>
<td></td>
<td>Sprague Dawley</td>
<td>2. N=10/group, 5 recovery at Control, sham, and high dose, 6/sex/group for TK; N=95/sex</td>
<td>Measurables as above. Recovery time = 28 days Irritation, Mortality, Body weight, Food consumption, Clinical chemistry, Ophthalmoscopy, TK (Day 1/week 4, week 13), Necropsy observations, Organ weights, Histopathology at control and high; other tissues TBD.</td>
</tr>
<tr>
<td>Repeat Dose 1 Month Study + 14 day recovery period</td>
<td>MiniPig (Hanford or Goettingen)</td>
<td>1. Four Dose Groups, 0 (vehicle) and 3 treatment groups with and without skin wounding. 10 groups in total. 2. N=5/sex /group, 2 recovery at Control, sham and high dose 3. N=31/sex (total 62) 4. 2g /kg</td>
<td>Recovery time = 14 days Measurable end-points as above. Plus: ECG at baseline and Week 4 Irritation, Mortality, Body weight, Food consumption, Clinical chemistry, Ophthalmoscopy, TK (Day and /Week 4.), Necropsy observations, Organ weights, Histopathology in all animals.</td>
</tr>
<tr>
<td>Repeat Dose 3 Month Study + 28 day recovery period</td>
<td>MiniPig (Hanford or Goettingen)</td>
<td>1. Five Dose Groups (sham, 0 vehicle and 3 treatment groups)</td>
<td>Measurable end-points as above. Plus: ECG at baseline and Week 12 Irritation, Mortality, Body weight, Food consumption, Clinical chemistry, Ophthalmoscopy, TK (Day 1, Week 4, Week 13), Necropsy observations, Organ weights, Histopathology in all animals.</td>
</tr>
<tr>
<td>42 Day Antigenicity</td>
<td>Guinea Pigs</td>
<td>Groups: 0 (vehicle) and 2 concentrations of Lithium formulation</td>
<td>Standard Protocol, wherein animals are treated daily topically on intact skin.</td>
</tr>
<tr>
<td>90 day standard Photoallergy/tox</td>
<td>Rat</td>
<td>Groups: 0 (vehicle) and 2 concentrations of Lithium formulation</td>
<td>Standard Panel</td>
</tr>
<tr>
<td>7 day Eye Irritation</td>
<td>NZ White Rabbits</td>
<td>1. Three Dose Groups (saline, 0 vehicle and the treatment group)</td>
<td>Single treatment group dosed at clinical concentration Animals observed for 7 days using Draize scale</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. N=3/group, same sex</td>
<td></td>
</tr>
</tbody>
</table>
5.10.5 METHODS FOR EVALUATING TREATMENT OF WOUND HEALING OR SCAR REVISION IN HUMANS

[00656] The safety and efficacy of the lithium treatments described herein may also be measured in human subjects according to methods known in the art.

[00657] For example, success of a lithium treatment described herein can be measured by:

- improvement of pigmentation of the scarred or wounded area
- improved surface contour of the scarred or wounded area
- improved texture of the scarred or wounded area
- improved skin depth, *i.e.*, thickness (if the scar started out as depressed relative to the plane of the skin) or thinness (if the scar started out as elevated relative to the plane of the skin) of the scarred or wounded area
- improved overall cosmetic outcome (*e.g.*, using the Visual Analogue Scale (VAS))
- subjective patient measures of improved outcome
- presence of elastin
- proper collagen orientation
- Improvement in viscoelasticity
- return of adnexal structures
- return of normal pore pattern
- increased number of hair germs
- hair follicle neogenesis or regeneration
- increased proportion of hair follicles in anagen or decreased proportion of follicles in telogen
- increased numbers of follicular units with 3 or more hair follicles
- reduction in the size of the wound or appearance of the scar compared to a wound or scar not treated with lithium
- conversion of the dermal epidermal junction from a flat junction between the dermis and epidermis (typical of a scar) to rete pegs (epithelial extensions that project into the underlying connective tissue) with interdigitating dermis, as assessed by *in vivo* scanning laser microscopy
- normalization of blood vessels as assessed using laser Doppler analysis.
- normal values according to the Vancouver Scar Scale (VSS). The VSS has 4 separate domains: pigmentation (graded 0 = normal, to 2 = hyperpigmentation), vascularity (graded 0 = normal, to 3 = purple), pliability (graded 0 = normal, to 5 = contracture) and height (graded 0 = normal, 3 = > 5 mm):

PIGMENTATION
0. Normal;
1. Hypopigmented;
2. Hyperpigmentation;

VASCULARITY
0. Normal: resembles the color over the rest of the body area;
1. Pink;
2. Red;
3. Purple.

PLIABILITY
0. Normal
1. Supple: flexible with minimal resistance;
2. Yielding: giving way to pressure;
3. Firm: inflexible, not easily moved, resistant to manual pressure
4. Banding: rope-like tissue that blanches with extension of scar;
5. Contracture: permanent shortening of scar, producing deformity or distortion.

HEIGHT
0. Normal: flat;
1. < 2 mm;
2. 2-5 mm;
3. > 5 mm.

[00658] In one embodiment, effectiveness of a lithium treatment described herein, as it relates to wound healing and scar revision, can be assayed by counting the number of hairs or hair follicles in the radius of a wounded area of skin.

[00659] In some embodiments, the lithium treatment improves one of the foregoing measures by 5% or more, by 10% or more, by 15% or more, by 20% or more, by 25% or more, by 30% or more, by 40% or more, by 50% or more, by 75% or more, or by 100% or
more. Such an improvement may be measured after 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, or one year or longer after initiation of the lithium treatment.

[00660] A synergistic effect of a lithium treatment on another treatment described herein may be measured as an improvement over a control subject receiving only one of the two treatments (i.e., the lithium treatment alone or the second treatment alone).

[00661] The efficacy of the intermittent lithium treatments described herein may be tested using skin explants, for example, prepared from skin biopsies or other surgical procedures. See, e.g., Ballanger et al, supra.

[00662] Human skin equivalents can be grown and assembled in vitro, with the advantage that they can be grown to theoretically to any size/shape; can be comprised of different types of cells, including keratinocytes (hair follicle derived and non-hair follicle derived), dermal cells (hair follicle derived and non-hair follicle derived), other cell types (e.g., mesenchymal stem cells); can contain cells that are genetically modified to include, e.g., markers or "inducible" signaling molecules; provide an unlimited and uniform source of human cells; from normal skin based on histology and marker studies; are generally devoid of skin appendages; and can be wounded and show similar wound healing events as in vivo.

5.11 GMP MANUFACTURING PLAN OF LITHIUM DOSAGE FORMS

[00663] GMP manufacturing is defined to include all operations of receipt of materials, production, packaging, repackaging, labeling, quality control, release, storage and distribution of the product and all associated process controls. In one embodiment, the dosage forms will be manufactured, filled and labeled in a cGMP-certified manufacturing facility. In one embodiment, a lithium hydrogel formulation described herein is manufactured and packaged into 5 mL sterile unit doses.

[00664] In one embodiment, development batches at each of the lithium strengths are manufactured and filled in previously-selected container closures and shipped to a sterilization facility for sterilization of the product. Sterilization validation of the product contained in the intended container closure is carried out. The sterilized samples are then shipped to a GMP certified analytical facility responsible for analysis of the drug product. The development batches are tested to specifications and ICH stability are initiated at an appropriate analytical laboratory.

[00665] In one embodiment, during the manufacture of development batches, process control parameters such as temperature of mixing and time of mixing are developed with
appropriate sampling points identified to ensure homogeneity of mixing of components. Appropriate batch records are developed in preparation of the GMP batches.

[00666] In one embodiment, NF or USP-grade raw materials for the GMP batches are selected from qualified vendors. GMP-grade active ingredients are purchased from a qualified vendor and raw material specifications set. In one embodiment, since lithium carbonate is a well-established active ingredient approved for use in the United States, a certificate of analyses will be accepted and set as specifications. Other active ingredients and excipients may be purchased and a certificate of analysis accepted as specifications.

[00667] In one embodiment, specifications are finalized prior to GMP manufacturing of the final drug product(s). Exemplary drug product specifications include assay (Li+), dose uniformity based on Li+, pH and appearance. Tolerances on specifications are based on batch to batch variation data. In some embodiments, specifications for sterility (USP <71>) and bioburden (USP <1227>) for sterile wound products are utilized.

[00668] In some embodiments, phase-appropriate method validation of all analytical methods are carried out using methods known in the art or described herein. The analysis of the GMP drug product is carried out. In some embodiments, a standard 36 month ICH stability study is initiated and performed on three sterile lots of topical gel (plus placebo) in their original container-closures. The T=0 time point will be used as the release data for the product.

[00669] In some embodiments, manufactured product is provided as sterile, single-dose units packaged in screw-cap LDPE tubes of appropriate volume. Selection of tubes may be conducted with respect to compatibility of the packaging material in contact with the formulation and integrity upon sterilization by gamma irradiation.

[00670] Sterilization may be carried out in accordance with methods known in the art. In one embodiment, the sterilization is carried out in two or three iterations to determine the gamma ray dose necessary for sterilization; 20 and 40 kilo-gray gamma ray doses may be used to sterilize development batches contained in various tube configurations and materials. At the conclusion of this process, the polymer material compatible with gamma irradiation is selected and the dose required for sterilization determined. If a specific ingredient degrades at the lowest dose of irradiation requisite for sterilization, that ingredient may be eliminated from the composition or replaced with another that is functionally equivalent and that is stable to the gamma irradiation process. At the conclusion of this process, the formulation may be optimized into its final composition.
5.12 ARTICLES OF MANUFACTURE

[00671] An article of manufacture, as contemplated by the present invention, comprises the lithium compositions described herein formulated as wound dressings, which can be in the form of barriers, membranes, or films. Alternatively, the lithium compositions described herein are added to dressing backings, such as barriers, membranes, or films. A barrier, membrane, or film can be supplied in a variety of standard sizes, which can be further cut and sized to the area being treated. The backing can be a conventional dressing material, such as a bandage or gauze to which a lithium compositions described herein is added or coated on, prior to application to the patient. Alternatively, the lithium compositions described herein can be formulated as a barrier, membrane, or film made out of strings, microbeads, microspheres, or microfibrils, or the composition can be formulated as a barrier-forming mat. Alternatively, the lithium compositions described herein can be formulated as a spray that can be sprayed on to skin or a wound as a protective layer (see Section 5.11 for a detailed description of a spray delivery system). Alternatively, the lithium compositions described herein can be formulated as a skin sanitizer and/or hand sanitizer. In an embodiment, skin sanitizer and/or hand sanitizer formulation is aqueous based or glycerol based. In another embodiment, the skin sanitizer and/or hand sanitizer can be formulated as a cream or an ointment.

[00672] In some embodiments, the articles of manufacture described herein are used to treat an existing microbial infection or colonization. In other embodiments, the articles of manufacture described herein are used to treat or prevent an infection of the skin or to treat or prevent an infection in or around a wound.

5.12.1 Spray Delivery System

[00673] Lithium carbonate has been shown to have unexpected potential as a broad-spectrum antimicrobial compared to other lithium salts such as lithium gluconate and lithium succinate. Additionally, antimicrobial activity has been shown to be dose-concentration related. That is, formulations with higher concentrations of ionized lithium (formulated as lithium carbonate hydrogels, 0.34 mg Li+/Gge; 10.64 mg Li+/Gge) have more of a biocidal effect. However, pharmacokinetics of lithium carbonate hydrogels shows rapid clearance from the site of administration (75% of the drug absorbed is cleared from the wound site within 4 hours). Thus, lithium carbonate hydrogels with both bolus and sustained release of Li+ may offer a sustained antimicrobial effect in addition to potential scarless wound healing.
Additionally, infected wounds need to be debrided and cleansed with a biocompatible cleansing agent prior to application of the antimicrobial therapy. Furthermore, a delivery system that can deliver both a cleansing agent and a sustained release system can be useful in the wound treatment units for both battlefield wounds and burns.

A complete spray delivery system that delivers both a cleansing agent (to clean and debride the wound) and an in-situ "gelling" antimicrobial gel that also accomplishes scarless wound healing is envisioned. The two-chamber spraying system would contain a liquid in each compartment. The spraying mechanism of each of the chambers would be deployed separately, as opposed to simultaneously. In this concept, the spraying mechanism for chamber 1 would deploy the spray stream at high energy, creating a highly effective cleansing method that delivers a sterile liquid to physically debride the infected wound. The sterile liquid delivered as a high energy spray would be comprised of ionized lithium at a concentration that is microbiocidal, combined with a surfactant molecule like benzalkonium chloride or cetyl Pyridinium chloride. Both molecules are FDA-approved as preservatives and also as potent antimicrobials. It is surmised that a high energy spray of a sterile, cleansing liquid prepares the wound tissue, for delivery of the antimicrobial gel.

The spraying mechanism for step 2 involves the 2nd chamber. The solution contained in chamber 2 would contain micronized lithium carbonate. Due to the high bond strength of the Li-carbonate linkage (1st Group I alkali in the periodic table), the kinetics of dissolution of this molecule is slow in aqueous solutions. While formulating lithium carbonate into an aqueous formulation, the pH of the solution has to be kept low and the solution heated to allow dissociation of the lithium-carbonate linkage and carbon dioxide to be released. Once the lithium-carbonate linkage is cleaved and carbon dioxide has been released, lithium can exist in a thermodynamically stable, ionized state in solution even when the pH is raised to 7. If lithium carbonate is to exist in a micronized state in an aqueous solution, this can be accomplished by keeping the micronized lithium carbonate at neutral pH. This can be helped further by lowering the temperature of the suspension to refrigeration (2-8 °C). Thus, the spray containers would be stored at refrigerated temperatures. Both chamber 1 and chamber 2 can be sprayed cold, creating a soothing spray to cleanse and treat an infected wound.

High-energy spraying the contents of chamber 1 (containing a formulation of the surfactant and dissolved lithium) on the wound cleanses and debrides the wound, while eliminating/killing surface bacteria from the wound.
After spraying the contents of chamber 2 (which contains a formulation of micronized lithium carbonate) on the wound, the dissolution of micronized lithium carbonate is triggered by the higher temperature of the wound releasing a sustained stream of ionized lithium. Sustained release of ionized lithium into the wound maintains an antimicrobial environment, preventing re-establishment of microbial organisms at the site. The micronized lithium carbonate would be formulated in a thermo-reversible polymer system that is liquid when refrigerated and a gel at skin temperatures. This would allow the polymer solution (with micronized lithium) to be held at the site of administration.

This method provides a complete treatment of the wound at the clinic, by debridement/cleansing followed by administration of a sustained release lithium gel. This step would occur at the clinic, where the spray system would be operated by the medical personnel. The person being treated would be provided with a tube of lithium carbonate hydrogel, which the person will apply twice daily after 2 days as a maintenance regimen.

It is not necessary that chamber 1 contains lithium as the wash. An alcoholic solution (±drug) may be used to first "prepare" the wound by thorough cleansing, followed by spraying the micronized lithium formulation as the sustained release antimicrobial. The spraying mechanism may be at high energy or low energy, depending upon the application.

Provided below in Examples 5 and 6 (Section 6) are descriptions of chamber spray systems for cleansing and treatment of infected wounds.

5.13 **TREATMENT OF ACNE**

The present invention also provides methods and formulations for the treatment and prevention of acne. More specifically, the present invention provides methods and formulations to treat and prevent acniform eruptions, such as acne vulgaris, pimples, zits, whiteheads, blackheads, and red, inflamed patches of skin (such as cysts), acne aestivalis (multiple, uniform, red, papular lesions reported to occur after sun exposure), acne conglobata (a highly inflammatory disease presenting with comedones, nodules, abscesses, and draining sinus tracts), acne cosmetica (acne caused by or made worse by cosmetics), acne fulminans (a severe form of acne, which can occur after unsuccessful treatment for another form of acne, acne conglobata), acne mechanica (an acniform eruption that has been observed after repetitive physical trauma to the skin such as rubbing), acne medicamentosa (drug-induced acne), acne miliaris necrotica (acne consisting of follicular vesicopustules, sometimes occurring as solitary lesions that are usually very itchy), acne necrotica (acne in which the primary lesion is a pruritic or painful erythematous follicular-based papule that
develops central necrosis and crusting and heals with a varioliform scar), acne rosacea (a red rash predominantly on the face), infantile acne/neonatal acne (a rash seen on the cheeks, chin, and forehead of infants) and occupational acne (acne caused by exposure to various industrial compounds).

[00683] In certain embodiments, the invention provides a method for the treatment or prevention of acne comprising administering to a patient in need thereof a composition comprising lithium. In certain, more specific embodiments, the composition for the treatment or prevention of acne comprises, w/w, lithium ions at a concentration of 0.10% lithium, 0.15% lithium, 0.20% lithium, 0.25% lithium, 0.30% lithium, 0.35% lithium, 0.40% lithium, 0.45% lithium, 0.50% lithium, 0.55% lithium, 0.60% lithium, 0.65% lithium, 0.70% lithium, 0.75% lithium, 0.80% lithium, 0.85% lithium, 0.90% lithium, or 0.95% lithium. In some embodiments, the composition for the treatment or prevention of acne comprises, w/w, 0.1% to 0.5% lithium ions, 0.2% to 0.5% lithium ions, 0.5% to 1% lithium ions.

[00684] In some exemplary embodiments, the composition for the treatment or prevention of acne comprises a salt form of lithium (e.g., lithium carbonate or other salts described in Section 5.1 above) at a concentration, w/w, of 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 12%, 15%, 16%, 18% and 20%. In some embodiments, a salt form of lithium for the treatment or prevention of acne comprises, w/w, 1% to 2% lithium salt (e.g., lithium carbonate or other form described in Section 5.1 above), 2% to 5% lithium salt, 5% to 10% lithium salt, 10% to 15% lithium salt, 15% to 20% lithium salt, 20% to 25% lithium salt, or 25% to 50% lithium salt. In one embodiment, the form for the treatment or prevention of acne is 1% to 20% w/w lithium salt.

[00685] In a specific embodiment, a formulation for the treatment or prevention of acne comprises lithium carbonate at a concentration of 1.46% w/w. In another embodiment, a formulation for the treatment or prevention of acne comprises lithium carbonate at a concentration of 2.91% w/w. In yet another embodiment, a formulation for the treatment or prevention of acne comprises lithium carbonate at a concentration of 5.66% w/w.

[00686] In some embodiments, a formulation for the treatment or prevention of acne comprises 0.25%-0.75%, 0.75%-1.5%, or 1.5%-3%, 3%-6%, or 8%-16% lithium carbonate (w/w). In some embodiments, a formulation for the treatment or prevention of acne comprises 1%-4% lithium gluconate (w/w). In some embodiments, a formulation for the treatment or prevention of acne comprises 4%-8% lithium gluconate (w/w). In some embodiments, a formulation for the treatment or prevention of acne comprises 8%-16% or more lithium gluconate (w/w). In some embodiments, a formulation for the treatment or
prevention of acne comprises 0.2%-1%, or 1%-5%, or more lithium chloride (w/w). In some embodiments, a formulation for the treatment or prevention of acne comprises 0.5%-2%, or 2%-4%, or 4%-8%, or 8%-16, or more lithium succinate (w/w). In some embodiments, a formulation for the treatment or prevention of acne comprises 0.5%-6%, 6%-12%, or 12%-25%, or more lithium stearate (w/w). In some embodiments, a formulation for the treatment or prevention of acne comprises 1%-4%, 4%-8%, or 8%-16%, or more lithium orotate (w/w). In some embodiments, a formulation for the treatment or prevention of acne comprises 0.25%- 1.5%, 1.5%-3.0%, or 3%-6%, or more 8% lithium citrate (w/w).

[00687] More details on these and other lithium formulations for use in the treatment or prevention of acne described herein are described in Sections 5.1-5.4 supra.

[00688] The lithium compositions can be administered alone or in combination with other agents or methods to enhance the treatment or prevention of acne. The other agent or method can be administered before, concurrently with, or after the lithium composition is administered. In certain embodiments, a composition for the treatment or prevention of acne can comprise lithium in combination with antibiotics, e.g., sulfonamides, trimethoprim/sulfamethoxazole, dapsone, sulfapyridine, sulfasalazine, tetracycline (tetracycline natural products, tetracycline semisynthetic compounds, or chemically modified tetracyclines), minocycline, macrolides, erythromycin, ampicillin, ciprofloxacin, cephalosporin, clindamycin, doxycycline, erythromycin stearate, erythromycin base, doxycycline hyclate, oxytetracycline, lymecycline, trimethoprim, cotrimoxazole, oxytetracycline, quinolones, aminoglycosides or chloramphenicol. In certain embodiments, a composition for the treatment or prevention of acne can comprise lithium in combination with retinoids, e.g., adapalene (Differin), tazarotene (Tazorac), tretinoin (Retin-A), tretinoin microsphere (Retin-A micro), isotretinoin (Accutane), arotinoid, MDI-301, retinaldehyde, retinol, bexarotene, motretinide or retinoyl-b-glucuronide. In certain embodiments, a composition for the treatment or prevention of acne can comprise lithium in combination with oral contraceptives or hormonal therapy agents, e.g., norethidrone, norethidrone acetate, ethynodiol diacetate, norgestimate, cyproterone acetate/ethinyl estradiol, spironolactone, flutamide, levonorgestrel, drospirenone or chloromadinone. In certain embodiments, a composition for the treatment or prevention of acne can comprise lithium in combination with agents and methods selected from, e.g., benzoyl peroxide, azelaic acid (Azelex), sulfacetamide (Klaron), sulfacetamide-sulfur (Sulfacet-R), salicylic acid, alpha hydroxy acids, botanicals, resorcinol, aluminum chloride, zinc, nicotinamide, complementary and alternative medications, lasers, light sources, photodynamic therapy and microdermabrasion.
In certain embodiments, a composition for the treatment or prevention of acne can comprise lithium in combination with anti-inflammatory agents, e.g., corticosteroids (such as, e.g., Dermatop®), NTHEs, COX-2 inhibitors, adrenocorticoids, beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, prednisone, hydrocortisone, and non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, diclofenac, and COX-2 inhibitors).

[00689] In certain embodiments, the invention provides compositions useful for treatment or prevention of acne. Such compositions comprise in certain embodiments lithium and one or more of the following: sulfonamides, trimethoprim/sulfamethoxazole, dapsone, sulfapyridine, sulfasalazine, tetracycline (tetracycline natural products, tetracycline semisynthetic compounds, or chemically modified tetracyclines), minocycline, macrodilides, erythromycin, ampicilin, ciprofloxacin, cephalosporin, clindamycin, doxycycline, erythromycin stearate, erythromycin base, doxycycline hyclate, oxytetracyline, lymecycline, trimethorprim, cotrimoxazole, oxytetracycline, quinolones, aminoglycosides, chloramphenicol; adapalene (Differin), tazarotene (Tazorac), tretinoin (Retin-A), tretinoin microsphere (Retin-A micro), isotretinoin (Accutane), arotinoid, MDI-301, retinaldehyde, retinol, bexarotene, motretinide, retinoyl-b-glucuronide; norethidrone, norethidrone acetate, ethynodiol diacetate, norgestimate, cyproterone acetate/ethinyl estradiol, spironolactone, flutamide, levonorgestrel, drospirenone, chlormadinone; benzoyl peroxide, azelaic acid (Azelex), sulfacetamide (Klaron), sulfacetamide-sulfur (Sulfacet-R), salicylic acid, alpha hydroxy acids, botanicals, resorcinol, aluminum chloride, zinc, nicotinamide, complementary and alternative medications, corticosteroids (such as, e.g., Dermatop®), NTHEs, COX-2 inhibitors, adrenocorticoids, beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, prednisone, hydrocortisone, and non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, diclofenac, and COX-2 inhibitors).

[00690] In certain embodiments, the lithium formulations can be administered in combination with one or more of the following: sulfonamides, trimethoprim/sulfamethoxazole, dapsone, sulfapyridine, sulfasalazine, tetracycline (tetracycline natural products, tetracycline semisynthetic compounds, or chemically modified tetracyclines), minocycline, macrodilides, erythromycin, ampicilin, ciprofloxacin, cephalosporin, clindamycin, doxycycline, erythromycin stearate, erythromycin base, doxycycline hyclate, oxytetracyline, lymecycline, trimethorprim, cotrimoxazole, oxytetracycline, quinolones, aminoglycosides, chloramphenicol; adapalene (Differin),
tazarotene (Tazorac), tretinoin (Retin-A), tretinoin microsphere (Retin-A micro), isotretinoin (Accutane), arotinoid, MDI-301, retinaldehyde, retinol, bexarotene, motretinide, retinoyl-b-glucuronide; norethidrone, norethidrone acetate, ethynodiol diacetate, norgestimate, cyproterone acetate/ethinyl estradiol, spironolactone, flutamide, levonorgestrel, drospirenone, chlormadinone; benzoyl peroxide, azelaic acid (Azelex), sulfacetamide (Klaron), sulfacetamide-sulfur (Sulfacet-R), salicylic acid, alpha hydroxy acids, botanicals, resorcinol, aluminum chloride, zinc, nicotinamide, complementary and alternative medications, corticosteroids (such as, e.g., Dermatop®), NTHEs, COX-2 inhibitors, adrenocorticoids, beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, prednisone, hydrocortisone, and non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, diclofenac, and COX-2 inhibitors).

[00691] In certain embodiments, the invention provides a kit useful for treating or preventing acne, comprising a first container with a formulation comprising lithium and a second container with a second formulation. The second formulation can comprise sulfonamides, trimethoprim/sulfamethoxazole, dapsone, sulfapyridine, sulfasalazine, tetracycline (tetracycline natural products, tetracycline semisynthetic compounds, or chemically modified tetracyclines), minocycline, macrolides, erythromycin, ampicillin, ciprofloxacin, cephalosporin, clindamycin, doxycycline, erythromycin stearate, erythromycin base, doxycycline hyclate, oxytetracycline, lymecycline, trimethoprim, cotrimoxazole, oxytetracycline, quinolones, aminoglycosides, chloramphenicol; adapalene (Differin), tazarotene (Tazorac), tretinoin (Retin-A), tretinoin microsphere (Retin-A micro), isotretinoin (Acutane), arotinoid, MDI-301, retinaldehyde, retinol, bexarotene, motretinide, retinoyl-b-glucuronide; norethidrone, norethidrone acetate, ethynodiol diacetate, norgestimate, cyproterone acetate/ethinyl estradiol, spironolactone, flutamide, levonorgestrel, drospirenone, chlormadinone; benzoyl peroxide, azelaic acid (Azelex), sulfacetamide (Klaron), sulfacetamide-sulfur (Sulfacet-R), salicylic acid, alpha hydroxy acids, botanicals, resorcinol, aluminum chloride, zinc, nicotinamide, complementary and alternative medications, corticosteroids (such as, e.g., Dermatop®), NTHEs, COX-2 inhibitors, adrenocorticoids, beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, prednisone, hydrocortisone, and non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, diclofenac, and COX-2 inhibitors). Any pharmaceutically acceptable compound that releases the lithium ion can be used for making the formulation containing lithium for treating or preventing acne described herein; such compounds include, but are not limited to lithium gluconate, lithium succinate, and other organic salts/ acids, lithium.
carbonate, lithium chloride and other inorganic salts/acids, and lithium hydroxide, as described in Section 5.1, supra. In certain embodiments, the formulation in the second container of the kit for treating or preventing acne can comprise one or more of the following: sulfonamides, trimethoprim/sulfamethoxazole, dapsone, sulfapyridine, sulfasalazine, tetracycline (tetracycline natural products, tetracycline semisynthetic compounds, or chemically modified tetracyclines), minocycline, macrolides, erythromycin, ampicilin, ciprofloxacin, cephalosporin, clindamycin, doxycycline, erythromycin stearate, erythromycin base, doxycycline hyclate, oxytetracline, lymecycline, trimethorprim, cotrimoxazole, oxytetraycline, quinolones, aminoglycosides, chloramphenicol; adapalene (Differin), tazarotene (Tazorac), tretinoin (Retin-A), tretinoin microsphere (Retin-A micro), isotretinoin (Accutane), arotinoid, MDI-301, retinaldehyde, retinol, bexarotene, motretinide, retinoyl-b-glucuronide; norethidrone, norethidrone acetate, ethynodiol diacetate, norgestimate, cyproterone acetate/ethinyl estradiol, spironolactone, flutamide, levonorgestrel, drospirenone, chlormadinone; benzoyl peroxide, azelaic acid (Azelex), sulfacetamide (Klaran), sulfacetamide-sulfur (Sulfacet-R), salicylic acid, alpha hydroxy acids, botanicals, resorcinol, aluminum chloride, zinc, nicotinamide, complementary and alternative medications, corticosteroids (such as, e.g., Dermatop®), NTHeS, COX-2 inhibitors, adrenocorticoids, beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, prednisone, hydrocortisone, and non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, diclofenac, and COX-2 inhibitors).

[00692] The formulation for treating or preventing acne can be a hand or body wash, an acne dressing, which can be in the form of barriers, membranes, or films, a gel, a lotion, a cream, an ointment, a surgical scrub, a skin sanitizer, or a spray that can be sprayed on to skin as a protective layer.

5.14  TREATMENT OF ONYCHOMYCOSIS

[00693] The present invention provides methods and formulations for reducing the risk of infection by members of the genera Microsporum, Trichophyton and Epidermophyton. More specifically, the present invention provides methods and formulations for reducing the risk of infection by members of the genera Microsporum, Trichophyton and Epidermophyton in the nail bed. Even more specifically, the present invention provides methods and formulations for the treatment or prevention of onychomycosis. In ceratin embodiments, the present invention provides methods and formulations for reducing the risk of infection by T. rubrum, T. mentagrophytes and E. floccosum in the nail bed. In certain, more specific embodiments,
the methods and formulations reduce the risk of infection by T. rubrum in the nail bed by
10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%.

[00694] In certain embodiments, the invention provides a method for the treatment or
prevention of onychomycosis comprising administering to a patient in need thereof a
composition comprising lithium. In certain, more specific embodiments, the composition for
the treatment or prevention of onychomycosis comprises, w/w, lithium ions at a concentration
of 0.10% lithium, 0.15% lithium, 0.20% lithium, 0.25% lithium, 0.30% lithium, 0.35%
lithium, 0.40% lithium, 0.45% lithium, 0.50% lithium, 0.55% lithium, 0.60% lithium, 0.65%
lithium, 0.70% lithium, 0.75% lithium, 0.80% lithium, 0.85% lithium, 0.90% lithium, or
0.95% lithium. In some embodiments, the composition for the treatment or prevention of
onychomycosis comprises, w/w, 0.1% to 0.5%> lithium ions, 0.2%> to 0.5%> lithium ions, 0.5%>
to 1% lithium ions.

[00695] In some exemplary embodiments, the composition for the treatment or prevention
of onychomycosis comprises a salt form of lithium (e.g., lithium carbonate or other salts
described in Section 5.1 above) at a concentration, w/w, of 1%, 2%, 3%, 4%, 5%, 6%, 7%,
8%, 9%, 10%, 12%, 15%, 16%, 18% and 20%. In some embodiments, a salt form of lithium
for the treatment or prevention of onychomycosis comprises, w/w, 1% to 2% lithium salt
(e.g., lithium carbonate or other form described in Section 5.1 above), 2% to 5% lithium salt,
5% to 10% lithium salt, 10% to 15% lithium salt, 15% to 20% lithium salt, 20% to 25%
lithium salt, or 25% to 50% lithium salt. In one embodiment, the form for the treatment or
prevention of onychomycosis is 1% to 20% w/w lithium salt.

[00696] In some embodiments, a formulation for the treatment or prevention of
onychomycosis comprises 0.25%-0.75%, 0.75%-1.5%, or 1.5%-3%, 3%-6%, or 8%-16%
lithium carbonate (w/w). In a specific embodiment, a formulation for the treatment or
prevention of onychomycosis comprises lithium carbonate at a concentration of 11.5% w/w.
In some embodiments, a formulation for the treatment or prevention of onychomycosis
comprises 1%-4% lithium gluconate (w/w). In some embodiments, a formulation for the
treatment or prevention of onychomycosis comprises 4%-8% lithium gluconate (w/w). In
some embodiments, a formulation for the treatment or prevention of onychomycosis
comprises 8%-16% or more lithium gluconate (w/w). In some embodiments, a formulation
for the treatment or prevention of onychomycosis comprises 0.2%-1%, or 1%-5%, or more
lithium chloride (w/w). In some embodiments, a formulation for the treatment or prevention
of onychomycosis comprises 0.5%-2%, or 2%-4%, or 4%-8%, or 8%-16, or more lithium
succinate (w/w). In some embodiments, a formulation for the treatment or prevention of
onychomycosis comprises 0.5%-6%, 6%-12%, or 12%-25%, or more lithium stearate (w/w). In some embodiments, a formulation for the treatment or prevention of onychomycosis comprises 1%-4%, 4%-8%, or 8%-16%, or more lithium orotate (w/w). In some embodiments, a formulation for the treatment or prevention of onychomycosis comprises 0.25%-1.5%, 1.5%-3.0%, or 3%-6%, or more 8% lithium citrate (w/w).

[00697] More details on these and other lithium formulations for use in the treatment or prevention of onychomycosis described herein are described in Sections 5.1-5.5 supra.

[00698] The lithium formulations can be administered alone or in combination with other agents or methods to enhance the prevention or treatment of onychomycosis. The other agent or method can be administered before, concurrently with, or after the lithium composition is administered. In certain embodiments, a composition for the prevention or treatment of onychomycosis can comprise lithium and one or more of the following: terbinafme, itraconazole, fluconazole, amorolfme, cyclopirox, tioconazole and Penlac®. In certain embodiments, the lithium formulations can be administered in combination with one or more of the following agents or methods: terbinafme, itraconazole, fluconazole, amorolfme, cyclopirox, tioconazole, Penlac®, laser treatment to remove top layers of nail, iontophoresis, low frequency ultrasound micro-holes, abrasion or removal of the nail plate, acid etching of nail, micro-cutting (PathFormer®), and permeation enhancing agents such as sodium thioglycoUate, papain, allantoin and benzalkonium chloride.

[00699] In certain embodiments, the invention provides a kit useful for treating or preventing onychomycosis, comprising a first container with a formulation comprising lithium and a second container with a second formulation. The second formulation can comprise one or more of terbinafme, itraconazole, fluconazole, amorolfme, cyclopirox, tioconazole, Penlac®, or permeation enhancing agents such as sodium thioglycoUate, papain, allantoin and benzalkonium chloride. Any pharmaceutically acceptable compound that releases the lithium ion can be used for making the formulation containing lithium for treating onychomycosis described herein; such compounds include, but are not limited to lithium gluconate, lithium succinate, and other organic salts/acids, lithium carbonate, lithium chloride and other inorganic salts/acids, and lithium hydroxide, as described in Section 5.1, supra. In certain embodiments, the formulation in the second container of the kit for treating or preventing onychomycosis can comprise one or more of terbinafme, itraconazole, fluconazole, amorolfme, cyclopirox, tioconazole, Penlac®, or permeation enhancing agents such as sodium thioglycoUate, papain, allantoin and benzalkonium chloride.
5.15 DERMABRASION TIP FOR A DEVICE, KIT, AND METHOD FOR GENERATING HAIR GROWTH

[00700] The embodiments of the present invention will now be described more fully hereinafter with reference to the accompanying drawings, in which preferred embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the illustrated embodiments set forth herein. Rather, these illustrated embodiments are provided so that this disclosure will be thorough and complete and will convey the scope of the invention to those skilled in the art.

[00701] In the following description, like reference characters designate like or corresponding parts throughout the figures. Additionally, in the following description, it is understood that terms such as "top," "bottom," "upper," "lower," "first," "second" and the like, are words of convenience and are not to be construed as limiting terms.

[00702] In a preferred embodiment of the present invention, the dermabrasion tips described herein are used with the inventions disclosed in US Provisional Patent Application No. 61/513,906, which is hereby incorporated by reference in its entirety. In particular, the Background (Section 2), the disclosure related to Figures 71-81, and Section 5 of US Provisional Patent Application No. 60/985,612 are hereby incorporated by reference. Additionally, this application hereby incorporates by reference International Application No. PCT/US2008/01979, US Provisional Patent Application No. 60/985,612, and US Patent Application Publication No. US 2010/0298760 in their entirety.

[00703] The preferred embodiments of the present invention are intended for use in the area of neogenic hair follicle formation. However, as used in the present application neogenic hair follicle formation is not only a treatment for baldness but also is a means to restore burned or scarred skin to normal function. Additionally, the embodiments of the present invention may involve mechanical or physical treatments that cause dermabrasion which may be used as part of a treatment for different skin conditions.

[00704] Embodiments of the present invention include dermabrasion tips that convert the rotational output of a traditional dermabrader to reciprocating motion. Additionally, the tips of the embodiments of the present invention may include an angular offset to improve ergonomics. The tips of the embodiments of the present invention have several advantages over the standard dermabrasion tips including: improved ergonomics, less blood and splatter, suitable for use in areas of transitioning hair, better clinician control, and they may be a single use disposable type tip. In one embodiment, a dermabrasion tip of the present invention does
not pull out hair. In one embodiment, a dermabrasion tip of the present invention
dermabrases with no or reduced blood splatter compared to conventional dermabrasion tips.

[00705] In one example of the embodiments of the present invention, a Torque Plus
dermabrader, Model AEU-12C, manufactured by Aseptico, Inc. in Woodinville, WA is used
to provide rotational input to a tip according to an embodiment of the present invention. In
this example, which is shown in Figure 67, the tip according to an embodiment of the present
invention attaches directly to the dermabrader and provides for the following improvements
over standard dermabrasion, which is shown in Figure 61: improved ergonomics;
compatible with dermabrasion hand piece and reduced cleaning time; tip transmission
converts to reciprocating motion; reciprocating tip doesn't splatter blood; reciprocating tip
doesn't pull out hair; and reciprocating tip provides for better control. Each of these
improvements is discussed in greater detail below.

[00706] Improved Ergonomics: In a standard dermabrasion tip, the axial orientation
provides for poor ergonomics because the clinician's hand continually interferes with the
patient. Additionally, standard human factors engineering teaches that Figure 60 is a poor
way to hold a finesse instrument. In contrast, in the tip according to an embodiment of the
present invention as shown in Figure 67, there is an approximate angle of 45 degrees
between the hand piece and the end-effector. This angle provides for holding the instrument
more like a pen or an artist's paint brush. Additionally, the angle improves clinician comfort
as well as overall control. Improved control is especially important in follicular applications
where the clinician is carefully abrading the skin in the areas of transitioning hair. In other
embodiments of the present invention, the improved ergonomics is a result of the conversion
of rotational input to a form of reciprocating movement (e.g., linear and combination linear
and radial).

[00707] The embodiments of the present invention may also include a dermabrasion tip
that has the ability to tilt or pivot during use to further improve the ergonomics of the
dermabrader. For example, the dermabrasion tips according to any of the embodiments of the
present invention described herein may be able to pivot about an axis located substantially on
or near the front end of the housing of a dermabrader. Additionally, the pivoting
dermabrasion tips may include a biasing means that will return the dermabrasion tip to an at
rest position when there is no counteracting means that will return the dermabrasion tip to an at
rest position when there is no counteracting force on the dermabrasion tips or when the
counteracting force is less than a predetermined amount of force. The force necessary to
move a biased pivoting dermabrasion tip according to an embodiment of the present
invention may be adjustable. Further, the range of motion of the pivoting or tilting
dermabrasion tip may be adjustable. This may include the ability to adjust the pivoting/tilting
dermabrasion tip such that no pivoting and/or tilting is permitted (i.e., a pivoting/tilting
dermabrasion tip may be adjusted such that it is a stationary dermabrasion tip).

[00708] Compatible with Dermabrasion Hand Piece and Reduced Cleaning Time: The tip
according to an embodiment of the present invention can be designed to be compatible for
use as a direct replacement for a standard dermabrasion tip. Figure 61 includes a schematic
showing a tip according to an embodiment of the present invention cooperating with a
standard dermabrader.

[00709] In an embodiment of the present invention, the custom tip can be made as a single
use disposable accessory to the standard dermabrader. The single use embodiment eliminates
the need to clean and autoclave a standard tip, which is problematic and time consuming as
debris has to be wire brushed from the tip prior to autoclave. This manual wire brushing can
also leave the tip inconsistently clean prior to autoclave.

[00710] As an added convenience to the clinician, the disposable tip embodiment of the
present invention can be packaged in a kit with a tube (or other similar container) containing
a specific composition/formulation or compositions/formulations.

[00711] Tip Transmission Converts to Reciprocating Motion: The tip according to an
embodiment of the present invention includes a transmission that converts the rotational
output of the dermabrasion hand piece to reciprocating motion. The transmission is shown,
e.g., in Figure 69. This conversion has distinct advantages in follicular applications. For
example, reciprocating motion does not pull out existing hair, reduces blood splatter, and
improves clinician control. These advantages are further discussed below.

[00712] Reciprocating Tip Doesn't Pull out Hair: With a standard rotating dermabrasion
tip the 360 degree rotation "winds up" existing hair as shown in Figure 58. This causes
longer hair to be pulled out of the scalp. For example, if the hair is longer than the
circumference of the standard rotating dermabrasion tip, it will wind up. In contrast, the
reciprocating converting tip according to an embodiment of the present invention, for
example, the tip shown in Figure 66, never completes a 360 degree rotation. Therefore, there
is no propensity to wind up hair. Thus, embodiments of the present invention are better
suited for treatment in balding/hair transition areas.

[00713] Reciprocating Tip Doesn't Splatter Blood: A standard rotating dermabrasion tip
transfers the 360 degree rotational inertia to blood and debris. During the dermabrasion
procedure using a standard rotating dermabrasion tip, blood and debris will be ejected, which
results in both safety and visualization problems. A standard rotating dermabrasion tip
ejecting blood and debris is shown in Figure 57. In contrast to the standard rotating dermabrasion tip, the tip according to an embodiment of the present invention, for example, the tip shown in Figure 65, reciprocates less than +/- 180 degrees such that no rotational inertia is transferred to blood and debris. This greatly reduces the volume and distance of any ejected material and improves overall procedural visualization and safety.

Reciprocating Tip Provides for Better Control: In a standard rotating dermabrasion tip, the 360 degree rotation causes the instrument to "walk" across the surface of the skin, which is problematic because it reduces clinician control. To maintain control the clinician has to constantly draw the tip backwards (fighting the "walking" tendency). Additionally, the standard rotating dermabrasion tip causes skin to deform which makes maintaining consistency in the dermabrasion procedure difficult. An example of this is shown in Figure 59. In contrast, the tip according to an embodiment of the present invention, for example, the tip shown in Figure 66, never completes a 360 degree rotation. Therefore, there is in no propensity to "walk" in the direction of rotation and there is less skin deformation. As a result, the embodiments of the present invention are better suited for finesse treatments especially in balding/hair transition areas.

In order to address the above-discussed drawbacks of conventional hand held dermabrasion devices, described herein is a dermabrasion tip that converts the rotational output of conventional dermabraders to a reciprocating motion. Additionally, in an embodiment of the present invention, an angle offset is utilized in order to improve ergonomics for the user. Embodiments of the present invention have several advantages over conventional dermabrasion tips including the improvements outlined above such as improved ergonomics, less blood and splatter, better clinician control, better suitability for use in areas of transitioning hair, and use as a single use disposable unit.

Radial Reciprocating Converting Dermabrasion Tip

Depicted in Figure 61 is an embodiment of a dermabrasion tip 220 that can be used with conventional dermabraders and which converts the rotational motion of a standard dermabrader to a reciprocating motion. As depicted, this embodiment is designed to be compatible as a direct replacement for a standard dermabrasion tip and thus, can be used with a standard dermabrasion hand piece 222. An example of a standard dermabrader with which the present dermabrasion tip can be used is depicted in Figure 62, which is a Torque Plus+ dermabrader, Model AEU-12C, manufactured by Aseptico, Inc. in Woodinville, WA.

Typically, the dermabrader includes a control unit 223, a dermabrasion hand piece 222, and a
cord 224 that connects the hand piece 222 to the control unit 223. As depicted in Figure 61, the present dermabrasion tip 220 simply fits over the front end 225 of a conventional dermabrasion hand piece 222 thereby converting the hand piece’s rotational motion to a reciprocating motion.

Conversion of the rotational motion of a conventional dermabrasion tip to the reciprocating motion in the present dermabrasion tip is achieved by way of a transmission. Although there are many ways to construct a means that converts rotational motion to reciprocating motion, such as gear based transmission, transmissions that use flexible linkages, or a combination of both gears and flexible linkages, one embodiment of a transmission will be described with reference to Figure 63.

As depicted in Figure 63, in the present embodiment, the dermabrasion tip 220 houses a gear/linkage converting transmission 226. In this embodiment, a first pair of bevel miter gears 228 converts the rotational output of the hand piece 222 to rotational output that is essentially orthogonal to the direction of rotation of the drive unit of the hand piece 222. The gears 228 can be constructed of Nylon, Acetal, or other suitable durable low-friction plastic, can be approximately 6.35 mm in diameter, and will typically have approximately 1.9 teeth/mm. However, one skilled in the art would readily understand that the embodiments of the present invention are not limited by the stated or shown size or teeth arrangements of the elements of the reciprocating converting dermabrasion tip 220. The first set of bevel gears 228 is driven by a drive shaft 230 that can be made, for example, from stainless steel and is approximately 2.36 mm in diameter. Such a drive shaft 230 is suitable for insertion into the front end 225 of the hand piece 222 for connection to the hand piece's drive unit. The first set of bevel gears 228 connect to a linkage assembly 232 that is similar to a "locomotive linkage." The linkage assembly 232 converts the orthogonal rotational motion of the conventional hand piece 222 to orthogonal reciprocating motion. As can be seen in Figure 64, the linkage assembly 232 includes an input drive wheel 234, an output drive wheel 236, and at least one coupling rod 238. As can also be seen in Figure 64, a first end of the coupling rod 238 attaches to an edge portion of the input drive wheel 234 and the second end of the coupling rod 238 attaches to an edge portion of the output drive wheel 236.

As depicted in Figure 64, in the linkage assembly 232, the input drive wheel 234 rotates through a radius that is smaller than the diameter of the output drive wheel 236. Therefore, as the input drive wheel 234 completes 360 degrees of rotation, the output drive wheel 236 reciprocates through a motion of less than +/- 180 degrees. That is, the output drive wheel 234 never completes a complete 360 degree rotation and instead reciprocates

204
back in forth as indicated by arrow 242. By changing the diameters of the input drive wheel 234 and output drive wheel 236 of the linkage assembly 232, one can adjust the reciprocating swing downward from +/- 180 degrees. In an embodiment of the present invention, the input drive wheel 234 is approximately 6.35 mm inches in diameter and the output drive wheel 236 is approximately 9.53 mm in diameter and the coupling rod 238 is approximately 12.7 mm in length. Preferably, this provides for approximately +/- 45 degrees of reciprocating output. However, as will be readily apparent to those skilled in the art, the diameters of the input and output drive wheels, 234, 236, and/or the length of the coupling rod 238, can be changed in order to change the degree of reciprocating motion, which can range anywhere from 1 degree to 179 degrees. The linkage assembly 232 can be constructed, for example, of Nylon, Acetal, or other suitable durable low-friction plastics.

Referring again to Figure 63, the reciprocating output drive wheel 236 of the linkage assembly 232 connects to a second pair of similar (size, material, pitch, etc.) bevel miter gears 244 that convert the reciprocating output of the linkage assembly 232 to a reciprocating output (that is, in the present embodiment, at an angle of approximately 45 degrees to the elongated axis of the hand piece 222). The 45 degree reciprocating output connects to a second drive shaft 246, which, for example, can be made of stainless steel. This second drive shaft 246 is connected to a circular pad 248, which can be made, for example, of polypropylene, and which can have a diameter of approximately 12.7 mm. Supported by pad 248 is an abrasive disk 250, which may be composed of bonded aluminum oxide particles with a course CAMI grit of 24, 30, or 36. In addition, Johnson Abrasives' Jaffery NH Wet-Kut waterproof abrasive backed clothed may be adequately secured to the support pad with very-high-bond tape such as 3M-4952. However, one skilled in the art would readily understand that other materials and sizes may be used for the abrasive disk 250 and abrasive pad 248 depending upon the desired purpose of the device. Thus, as can be seen in Figures 65 and 66, the reciprocating motion of the second drive shaft 246 is transferred to the abrasive disk 250. In all of the gearing described herein, the diameters can be varied to achieve the desired transmission speed and torque conversions. Additionally, all of the materials described herein may be varied.

All of the transmission components described above may be housed in, for example, a polypropylene housing or a housing made of other suitable materials. The housing 251 can be approximately 63.5 mm in length with an external surface contoured to achieve maximum ergonomics. The inside proximal diameter is designed to be approximately 15 mm, which allows the housing 251 and hence, the entire dermabrasion tip
220 to be inserted onto the front end portion 225 of a conventional dermabrasion hand piece 222 as depicted in Figure 61. In order to provide securement to the hand piece 222, a co-molded low durometer thermoplastic rubber (TPR) or thermoplastic elastomer (TPE) insert 252 having an inside diameter of approximately 14.4 mm, is included on the inside of the dermabrasion tip 220. As can be seen in Figure 63, the insert 252 is designed to engage the distal flats 254 of a conventional dermabrasion hand piece 222, thereby forming a friction fit between the dermabrasion tip 220 and the hand piece 222. Thus, the housing 251 can slip over the front end portion 225 of a standard dermabrasion hand piece 222 and be held by the friction fit. One skilled in the art would readily understand that other materials and sizes may be used for the housing and insert 252 depending upon the desired purpose of the device.

Furthermore, as can be seen in Figures 63 and 67, an embodiment of the present invention includes an angle 256 of approximately 45 degrees between the longitudinal axis of the hand piece and the end effector. As previously discussed, such an angle improves ergonomics and allows the hand piece to be held more like a pen or artist’s paint brush. The added angle also improves clinician comfort and overall control. As will be readily apparent to those skilled in the art, different angles may be used to change the ergonomics of the dermabrasion tip. Furthermore, in another embodiment, the dermabrasion tip 220 can include an adjustable end effector such that the angle between the longitudinal axis of the hand piece 222 and the end effector can be adjusted by the clinician in order to better adapt the dermabrasion tip 220 to the clinician and/or patient and/or procedure being performed.

Moreover, in order to allow the end effector, which includes the abrasive disk 250, to better conform to the skin surface, as depicted in Figure 68, the second drive shaft 246 can include a plurality of notches 258. These notches 258 permit the second drive shaft 244 to flex, thereby allowing the abrasive disk 250 to conform to the skin surface. Other ways to achieve conformability of the abrasive pad 250 to the skin surface include, but are not limited to, use of a ball and socket joint or a universal joint.

In another embodiment of the dermabrasion tip, all the inexpensive plastic transmission parts previously described for use in a single use disposable dermabrasion tip can be made from stainless steel and incorporated into the body of a dermabrasion tip that can be reusable. Essentially, all the parts of the reciprocating dermabrasion tip would then be reusable with the exception of the abrasive disk, which could be peeled off after use and discarded. Thus, after the reusable dermabrasion tip is cleaned, a new abrasive disk could be attached prior to use.
Linear Reciprocating Converting Dermabrasion Tip

[00725] The reciprocating converting dermabrasion tip 220 previously described provides for hair-sparing, less blood splatter, and better clinician control by converting the rotational output of the dermabrasion hand piece to radial reciprocating motion. However, alternative embodiments using other forms of motion would also provide the same benefits as radial motion.

[00726] For example, linear reciprocation motion would also spare hair in a similar manner to radial motion. Therefore, an embodiment of the present invention is directed to a linear reciprocating converting dermabrasion tip. One way to achieve a linear reciprocating abrasive surface is to add an abrasive surface to the sides of the 12.7 mm (0.500”) abrasive pad 248 of the radial reciprocating converting dermabrasion tip 220. With the addition of these abrasive surfaces, when the side edge of the abrasive pad is contacted to the surface of the skin the resulting motion is perpendicular to the original radial reciprocating motion and is a "pendulum like" linear reciprocating motion. Additionally, by having abrasive surfaces on both the bottom and sides of the abrasive pad 248, the clinician would have contacting surfaces that include two different cross sectional areas. This can be advantageous for several reasons. For example, the larger bottom of the pad may be used for treating larger areas of skin while the smaller side surfaces may be used in areas of skin requiring greater precision.

[00727] Alternate embodiments of the present invention are directed to linear reciprocating converting dermabrasion tips 320, an example of which is shown in Figure 73, that include a transmission 326 to directly convert the rotational output of a standard dermabrader to linear reciprocating motion. For example, as depicted in Figure 73, a rectangular abrasive pad 348 is contacted to the skin and the abrasive pad 348 vibrates in a linear reciprocating manner.

[00728] In an embodiment of the present invention, a pair of bevel miter gears 328 converts the rotational output of the dermabrasion hand piece 322 to rotational output that is essentially orthogonal to the direction of rotation of the dermabrasion hand piece 322. The bevel miter gears 328 may be constructed of Nylon, Acetal, or any other suitable durable low-friction plastics. The bevel miter gears 328 may be approximately 6.35 mm (0.250”) in diameter, and may comprise 19 teeth per mm (48 teeth per inch). However, one skilled in the art would readily understand that the embodiments of the present invention are not limited by the stated or shown size or teeth arrangements of the elements of the linear reciprocating converting dermabrasion tip 320.
The bevel miter gears 328 are driven by a drive shaft 330 that may be constructed of, e.g., stainless steel. The drive shaft 330 may be approximately 2.36 mm (0.093") in diameter, which is a size that is suitable for connection to a standard dermabrasion hand piece 322.

In an embodiment of the present invention, the bevel miter gears 328 drive a linkage assembly 332 that is similar to a "locomotive linkage" that converts orthogonal rotational movement to linear reciprocating movement. The linkage assembly 332 comprises an input drive wheel 334 and at least one linkage/coupling rod 338 that is capable of pivoting. The coupling rod 338 may connect to the abrasive pad 348 or an extension 349 therefrom (or the like). The linkage assembly 332 may be constructed of Nylon, Acetal, or other suitable durable low-friction plastics. Alternatively, the linkage assembly 332 may be constructed of metal or any other suitable material. The total linear reciprocating displacement may be approximately +/- 6.35 mm ( +/- 0.250") (or a total travel of 12.7 mm (0.500")) in the embodiment shown in Figure 73, by pinning the linkage to an abrasive pad 348, the pad 348 also moves back and forth by approximately +/- 6.35 mm ( +/- 0.250")

In an embodiment of the present invention, the abrasive pad 348 supports an abrasive surface 350. The pad may be in the form of a polypropylene pad that is approximately 12.7 mm (0.500") in length and 6.35 mm (0.250") in width. The rectangular abrasive surface 350 formed therein may comprise bonded aluminum oxide particles with a course CAMI grit of 24, 30, or 36. In an embodiment of the present invention, Johnson Abrasives’ Jaffery NH Wet-Kut waterproof abrasive backed clothed may be adequately secured to the abrasive pad 348 with very-high-bond tape such as 3M-4952. However, one skilled in the art would readily understand that the embodiments of the present invention may include pads or abrasive surfaces of different sizes and materials from that which are outlined herein.

In embodiments of the present invention, all of the transmission components described herein may be housed in a housing 351, which may be constructed of polypropylene or any other suitable material. The housing 351 may be approximately 63.5 mm (2.500") long with an external surface contoured for maximal ergonomics. The inside proximal diameter is designed to be approximately 15 mm, which allows the housing 351 and hence, the entire dermabrasion tip 320, to be inserted onto the front end portion 325 of a conventional dermabrasion hand piece 322. The housing 351 of the linear reciprocating converting dermabrasion tip 320 may be the same or similar to the housing 251 depicted in Figures 69-72B for the radial reciprocating converting dermabrasion tip 220. In another
embodiment of the present invention, the housing 351 may be the smaller housing 351 depicted in Figure 73. One skilled in the art will understand that the housings described herein may exist in many different configurations, and the dermabrasion tips of the embodiments of the present invention are not limited to the housings depicted in the figures included herein. In fact, the embodiments of the present invention may not include a housing at all. In order to provide securement to the hand piece 322, a co-molded low durometer thermoplastic rubber (TPR) or thermoplastic elastomer (TPE) insert 352 having an inside diameter of approximately 14.4 mm, is included on the inside of the dermabrasion tip 320. The insert 352 is designed to engage the distal flats 354 of a conventional dermabrasion hand piece 322, thereby forming a friction fit between the dermabrasion tip 320 and the hand piece 322. The insert 352 of the linear reciprocating converting dermabrasion tip 320 may be the same or similar to the insert 252 depicted in, for example, Figure 71 of the radial reciprocating converting dermabrasion tip 220. One skilled in the art will understand that the inserts described herein may exist in many different configurations, and the dermabrasion tips of the embodiments of the present invention are not limited to the inserts depicted in the figures included herein. In fact, the embodiments of the present invention may not include an insert at all. Thus, the housing 351 can slip over the front end portion 325 of a standard dermabrasion hand piece 322 and be held by the friction fit. One skilled in the art would readily understand that other materials and sizes may be used for the housing and insert 352 depending upon the desired purpose of the device.

[00733] In alternate embodiments of the present invention, all of the inexpensive plastic transmission parts described above, which are primarily intended for use in a disposable transmission 326, can be made of, e.g., stainless steel and incorporated into the body of a dermabrasion hand piece 322 as a reusable transmission. Essentially, all the parts of the linear reciprocating dermabrasion system described herein would then be reusable with the exception of the abrasive surface 350, which could be peeled off after use and discarded. After cleaning the instrument, a new abrasive surface 350 could be attached prior to use.

[00734] Additionally, alternate embodiments of the linear reciprocating converting dermabrasion tip include a device that creates linear reciprocating motion via a linear actuator, oscillating solenoid, magnetostrictive transducer, ultrasound transducer, and/or ultrasound bender/bimorph. These embodiments of the present invention directly convert electrical energy into linear reciprocating motion. All of these embodiments of the present invention can be coupled to an abrasive pad with an abrasive surface that contacts a scalp.
Combination Radial and Linear Reciprocating Converting Dermabrasion Tip

[00735] The reciprocating converting dermabrasion tips 220, 320 previously described provide for hair-sparing, less blood splatter, and better clinician control by converting the rotational output of the dermabrasion hand piece to radial or linear reciprocating motion. However, alternative embodiments using other forms of motion would also provide the same benefits as radial or linear motion.

[00736] For example, combining radial reciprocating motion with linear reciprocating motion in one abrasive pad/disc would also spare hair in a similar manner to radial and linear motion alone. Therefore, an embodiment of the present invention is directed to a combination radial and linear reciprocating converting dermabrasion tip 420, as depicted in Figure 74. The combination radial and linear reciprocating converting dermabrasion tip embodiments of the present invention may provide for a faster procedure while still sparing hair.

[00737] In an embodiment of the present invention, the combination radial and linear reciprocating converting dermabrasion tip 420 includes a transmission 426 to directly convert the rotational output of a standard dermabrader to a combination of radial and linear reciprocating motion. In an embodiment of the present invention, similar to the radial and linear reciprocating converting dermabrasion tip embodiments 220, 320 described above, a first pair of bevel miter gears (not shown) converts the rotational output of the standard dermabrasion hand piece to rotational output that is essentially orthogonal to the direction of rotation of the drive unit of the hand piece. The gears can be constructed of Nylon, Acetal, or other suitable durable low-friction plastic, can be approximately 6.35 mm in diameter, and will typically have approximately 1.9 teeth/mm. However, one skilled in the art would readily understand that the embodiments of the present invention are not limited by the stated or shown size or teeth arrangements of the elements of the reciprocating converting dermabrasion tip 420. The first set of bevel gears is driven by a drive shaft that can be made, for example, from stainless steel and is approximately 2.36 mm in diameter. Such a drive shaft is suitable for insertion into the front end of the hand piece for connection to the hand piece’s drive unit. The first set of bevel gears connect to a linkage assembly 432. The linkage assembly 432 converts the orthogonal rotational motion of the conventional hand piece to a combination radial and linear reciprocating motion. As can be seen in Figure 74, the linkage assembly 432 includes an input drive wheel 434 and at least one coupling rod 438.
The transmission 426 of the combination radial and linear reciprocating converting dermabrasion tip 420 includes a piston crank, which is formed by the input drive wheel 434 and the coupling rod 438. The coupling rod 438 is pivotally connected to a second linkage rod 439, which is connected to and cooperates with a sled 447, which may include a substantially rectangular shape as depicted in Figure 74. The piston crank mechanism drives the rectangular sled 447 in a linear motion. The linear motion of the sled is represented by the arrow 443 depicted in Figure 74. In an embodiment of the present invention, the rectangular sled 447 rides in slots that are formed within the housing. These slots act as bearing tracks that accommodate the linear motion of the rectangular sled 447.

The combination radial and linear reciprocating converting dermabrasion tip 420 further includes a pad 448 located below the rectangular sled 447. The pad 448 is coupled to the rectangular sled 447 via a substantially central pivot point 460, which is represented by a dotted line that resembles a hidden pin in Figure 74. This pivotal connection at the central pivot point 460 may be any kind known in the art. In the embodiment shown in Figure 74, the pivotal connection utilizes a first vertical pin 461. The combination radial and linear reciprocating converting dermabrasion tip 420 further includes a second vertical pin 462 that is rigidly attached to the pad 448. The second vertical pin 462 passes up through the rectangular sled 447 through a substantially crescent shaped clearance slot 464 formed in the sled 447. In an embodiment of the present invention, the housing includes a cam slot 466 that runs substantially diagonally across the width of the sled 447. In Figure 74, the cam slot or channel 466 is depicted as a hidden element above the sled 447. The cam slot 466 allows for the movement of the second vertical pin 462.

Supported by pad 448 is an abrasive disk 450, which may be composed of bonded aluminum oxide particles with a course CAMI grit of 24, 30, or 36. In addition, Johnson Abrasives’ Jaffery NH Wet-Kut waterproof abrasive backed clothed may be adequately secured to the support pad with very-high-bond tape such as 3M-4952. However, one skilled in the art would readily understand that other materials and sizes may be used for the abrasive disk 450 and abrasive pad 448 depending upon the desired purpose of the device. Thus, the reciprocating motion caused by the elements of the transmission 426 is transferred to the abrasive disk 450. In all of the gearing described herein, the diameters can be varied to achieve the desired transmission speed and torque conversions. Additionally, all of the materials described herein may be varied.

In the embodiments of the present invention comprising the combination radial and linear reciprocating converting dermabrasion tip 420, when the sled 447 moves forward,
the second pin 462 follows the slot 464 rotating the pad 448 along with it. As the sled 447 then moves backwards, the pad 448 reciprocates in the opposite direction. Thus, the combination radial and linear reciprocating motion of the abrasive disk 450 is created. This motion is represented by the arrow 442 shown in Figure 74.

[00742] In embodiments of the present invention, all of the transmission components described herein may be housed in a housing, which may be constructed of polypropylene or any other suitable material. The inside proximal diameter of the housing may be designed to be approximately 15 mm, which allows the housing and hence, the entire dermabrasion tip 420, to be inserted onto the front end portion of a conventional dermabrasion hand piece. The housing of the combination radial and linear reciprocating converting dermabrasion tip 420 may be the same or similar to the housing 251 depicted in Figures 69-72B for the radial reciprocating converting dermabrasion tip 220. In another embodiment of the present invention, the housing may be the smaller housing 351 depicted in Figure 73. One skilled in the art will understand that the housings described herein may exist in many different configurations, and the dermabrasion tips of the embodiments of the present invention are not limited to the housings depicted in the figures included herein. In fact, the embodiments of the present invention may not include a housing at all. Additionally, in order to provide securement to the standard dermabrasion hand piece, a co-molded low durometer thermoplastic rubber (TPR) or thermoplastic elastomer (TPE) insert having an inside diameter of approximately 14.4 mm, may be included on the inside of the dermabrasion tip 420. The insert of the combination radial and linear reciprocating converting dermabrasion tip 420 may be the same or similar to the insert 252 depicted in, for example, Figure 71 of the radial reciprocating converting dermabrasion tip 220. One skilled in the art will understand that the inserts described herein may exist in many different configurations, and the dermabrasion tips of the embodiments of the present invention are not limited to the inserts depicted in the figures included herein. In fact, the embodiments of the present invention may not include an insert at all. Thus, the housing can slip over the front end portion of a standard dermabrasion hand piece and be held by the friction fit. One skilled in the art would readily understand that other materials and sizes may be used for the housing and insert depending upon the desired purpose of the device.

[00743] In alternate embodiments of the present invention, all of the inexpensive plastic transmission parts described above, which are primarily intended for use in a disposable transmission 426, can be made of, e.g., stainless steel and incorporated into the body of a dermabrasion hand piece as a reusable transmission. Essentially, all the parts of the
combination radial and linear reciprocating dermabrasion system described herein would then
be reusable with the exception of the abrasive surface 450, which could be peeled off after
use and discarded. After cleaning the instrument, a new abrasive surface 450 could be
attached prior to use.

Accessories

[00744] As an added convenience to the clinician, any of the dermabrasion tips of the
present invention can be packaged in a kit with a tube (or other similar container) containing
a specific composition/formulation or compositions/formulations. The
compositions/formulations may be in the form of a drug including a pharmaceutically active
ingredient, or may be in the form of a composition/formulation without a pharmaceutically
active ingredient (e.g., a lotion or cream). The compositions/formulations may include, for
example, minoxidil (an antihypertensive drug that opens the K+ channel), antiandrogens
(such as finasteride, dutasteride, ketoconazole or the like), kopenixl (for example, the product
Keranique™), finasteride, bimatoprost (Latisse), CaCl₂, adenosine, diazoxide, phentoytn, eflornithine, or the like. The compositions/formulations may also include chemicals that
inhibit the growth of hair. The kit would then include all the necessary consumables to
perform a follicular procedure in the clinic. One skilled in the art would readily understand
that the dermabrasion tips according to the embodiments of the present invention may be
used with any compositions/formulations whether or not they are listed herein. Further, the
embodiments of the present invention may be used with any compositions/formulations,
regardless of whether they are supplied in a kit.

[00745] The embodiments of the present invention may also be used as part of a system or
method utilizing a drug that promotes neogenic hair follicle formation, examples of which
include the lithium compositions or lithium formulations described herein.

Accessories Continued

[00746] In another embodiment, the present invention is directed to a method of inducing
hair growth that comprises disrupting a skin surface with a dermabrasion tip disclosed herein
and then applying a lithium composition or a lithium formulation as described above.

[00747] In some embodiments, topical administration comprises administration of lithium-
containing particles. The particles can be delivered to the skin in combination with any of the
means above and described elsewhere in US Provisional Patent Application No. 60/985,612.
Additionally, the particles can be designed for intermittent or pulse delivery of lithium. In
one embodiment, particles with different release properties are be delivered simultaneously to achieve pulse delivery.

[00748] In another embodiment, topical administration comprises administration of a lithium-containing formulation that is delivered through channels that are created by the use of micro-needle technology. The formulation can be, e.g., a liquid, a gel or a dry spray. In another variation, topical administration may be through delivery of a lithium-containing formulation through hollow needles.

[00749] In another embodiment, topical administration comprises administration of a lithium-containing formulation that is delivered into the skin by an iontophoretic patch. In one example of this embodiment, a patch can be developed in which the lithium-containing formulation is incorporated.

[00750] In another embodiment, topical administration comprises administration of a lithium-containing formulation that is incorporated into micro-needle shaped biodegradable polymers. In one such embodiment, the biodegradable microneedles penetrate the targeted skin tissue, and are optionally left in place to deliver the lithium ions in a sustained fashion over time.

Skin Condition Treatment

[00751] As stated above, the embodiments of the present invention may involve mechanical or physical treatments that cause dermabrasion which may be used as part of a treatment for different skin conditions. For example, the following is a non-exclusive list of skin conditions that could be treated with the embodiments of the present invention: viral warts, Bowen's disease (in situ squamous cell carcinoma), pyogenic granuloma, solar keratoses, basal cell carcinomas, keratoacanthoma, and skin tags. Additionally, the embodiments of the present invention could treat, for example, benign epidermal tumors including: seborrhoic keratosis (Melanoacanthoma Dermatosis papulosa nigra), lentigo Solaris (PUVA lentigo), Melanosis of Becker (Becker's nevus), stucco keratosis, Acrokeratosis verruciformis Hopf, hyperkeratosis lenticularis perstans of Flegel, confluent and reticulated papillomatosis, acanthosis nigricans, keratotic processes (multiple minute digitate keratosis, postirradiation hyperkeratosis, minute aggregate keratosis, waxy dermatosis), warty dyskeratoma (solitary Darier's disease), acantholytic acanthoma, verrucous affections caused by papillomavirus (verruca vulgaris, verruca filiformis, verruca plantaris, verruca plana, epidermodysplasia verruciformis, Condyloma accuminatum, focal epithelial hyperplasia Heck, multifocal papillomavirus epithelial hyperplasia, Hirsutes
papillares, Bowenoid papulosis of the genitalia), inverted follicular keratosis, molluscum contagiosum, arsenical keratosis, large cell acanthoma, epidermolytic acanthoma, knuckle pads, keratoacanthoma, pale cell acanthoma, pale cell papulosis, pseudoepitheliomatous hyperplasia, and follicular induction (sebaceous induction). One skilled in the art would understand that additional skin conditions and diseases not listed above may also be treated with the embodiments of the present invention.

Alternate Embodiments

[00752] An alternate embodiment of the present invention utilizes positive air pressure to "blow" the existing hair out of the way in advance of the dermabrasion tips according to the embodiments of the present invention. Also, positive air pressure could be delivered with standard dermabrasion tips to blow the existing hair out of the way in advance of the rotating tip.

[00753] Additional alternate embodiments of the present invention include: utilizing a chemical skin peel that could spare hair; hair-sparing laser skin ablation; coring needles or a coring needle roller that may cleave some hairs off but spares the majority; and particle mediated abrasion.

[00754] Yet another alternate embodiment of the present invention includes freezing the skin and wiping off the destroyed skin while sparing the hair.

[00755] Another alternate embodiment of the present invention includes shooting a high velocity water jet or jets at the scalp while intentionally not targeting existing hair.

[00756] A person of ordinary skill in the art would recognize that any of the additional features of the embodiments of the present invention described herein related to the dermabrasion tips may be applied to any of the radial, linear, or combination radial and linear reciprocating converting dermabrasion tips, within reason. For example, most of the features of the radial reciprocating converting dermabrasion tip 220 (not related to the generation of the radial movement of the abrasive disk 250) can be applied to the linear and combination radial and linear reciprocating converting dermabrasion tips 320, 420, and vice-versa.

[00757] The embodiments of the present invention are directed to a dermabrasion tip for use on a dermabrasion hand piece, the dermabrasion tip including: a housing having a first opening substantially aligned with a longitudinal axis of the housing and a second opening disposed at an angle to the longitudinal axis; and a transmission unit disposed in the housing. The transmission unit includes: a first set of gears; a linkage assembly adjacent to the first set of gears; and a second set of gears adjacent to the linkage assembly. The dermabrasion tip
further includes a platform to receive an abrasive disk, where the transmission unit converts a
rotational motion of the dermabrasion hand piece to a radial reciprocating motion causing the
platform to reciprocate.

[00758] Another embodiment of the present invention is directed to the dermabrasion tip
described above, where the angle is approximately 45 degrees.

[00759] Yet another embodiment of the present invention is directed to any of the
dermabrasion tips described above, where the linkage assembly includes an input drive
wheel, and output drive wheel, and a coupling rod.

[00760] Another embodiment of the present invention is directed to any of the
dermabrasion tips described above, where the first set and second set of gears are bevel gears.

[00761] Yet another embodiment of the present invention is directed to any of the
dermabrasion tips described above, where the reciprocating motion ranges between +/- 45
degrees.

[00762] The embodiments of the present invention are directed to a dermabrader
including: a control unit; a hand piece having a longitudinal axis and including a housing,
and a transmission unit disposed in the housing. The transmission unit includes: a first set of
gears; a linkage assembly adjacent to the first set of gears; and a second set of gears adjacent
to the linkage assembly. The dermabrader further includes an abrasive disk and a cord that
connects the hand piece to the control unit. In the embodiments of the present invention, the
transmission unit converts a rotational motion of the dermabrasion hand piece to a radial
reciprocating motion causing the abrasive disk to reciprocate, and the abrasive disk is
disposed at an angle with the longitudinal axis of the hand piece.

[00763] Another embodiment of the present invention is directed to the dermabrader as
described above, where the angle between the abrasive disk and the longitudinal axis of the
housing is approximately 45 degrees.

[00764] Yet another embodiment of the present invention is directed to any of the
dermabraders as described above, where the linkage assembly includes an input drive wheel,
an output drive wheel, and a coupling rod.

[00765] Another embodiment of the present invention is directed to any of the
dermabraders as described above, where the first set and second set of gears are bevel gears.

[00766] Yet another embodiment of the present invention is directed to any of the
dermabraders as described above, where the reciprocating motion ranges between +/- 45
degrees.
The embodiments of the present invention are directed to a kit for use in a dermabrasion process, the kit including: a drug that promotes neogenic hair follicle formation, and a disposable dermabrasion tip. The dermabrasion tip includes: a housing having a first opening substantially aligned with a longitudinal axis of the housing and a second opening disposed at an angle to the longitudinal axis; and a transmission unit disposed in the housing, where the transmission unit includes a first set of gears, a second set of gears, and a linkage assembly disposed between the first set of gears and the second set of gears. The dermabrasion tip further includes an abrasive disk, where the transmission unit converts a rotational motion of the dermabrasion hand piece to a radial reciprocating motion causing the abrasive disk to reciprocate, and where the abrasive disk is disposed at an angle with the longitudinal axis of the housing.

Another embodiment of the present invention is directed to a kit as described above, where the drug that promotes neogenic hair follicle formation is a lithium composition as described in Section 5.1, supra.

Yet another embodiment of the present invention is directed to any of the kits as described above, where the drug that promotes neogenic hair follicle formation is a lithium formulation as described in Section 5.2, supra.

Another embodiment of the present invention is directed to any of the kits as described above, where the angle between the abrasive disk and the longitudinal axis of the housing is approximately 45 degrees.

Yet another embodiment of the present invention is directed to any of the kits as described above, where the linkage assembly includes an input drive wheel, an output drive wheel, and a coupling rod.

Another embodiment of the present invention is directed to any of the kits as described above, where the first set and second set of gears are bevel gears.

Yet another embodiment of the present invention is directed to any of the kits as described above, where the reciprocating motion ranges between +/- 45 degrees.

The embodiments of the present invention are directed to a method of inducing hair growth in a human subject, the method including: disrupting a skin surface with a dermabrader having a dermabrasion hand piece with dermabrasion tip. The dermabrasion tip includes: a housing having a first opening substantially aligned with a longitudinal axis of the housing and a second opening disposed at an angle to the longitudinal axis; and a transmission unit disposed in the housing. The transmission unit includes: a first set of gears; a second set of gears; and a linkage assembly disposed between the first set of gears.
and the second set of gears. The dermabrasion tip further includes an abrasive disk. The
method further includes applying a drug that promotes neogenic hair follicle formation,
where the transmission unit converts a rotational motion of the dermabrasion hand piece to a
radial reciprocating motion causing the abrasive disk to reciprocate, and where the abrasive
disk is disposed at an angle with the longitudinal axis of the housing.

[00775] Another embodiment of the present invention is directed to the method described
above, where the drug that promotes neogenic hair follicle formation is a lithium composition
as described in Section 5.1, supra.

[00776] Yet another embodiment of the present invention is directed to any of the methods
described above, where the drug that promotes neogenic hair follicle formation is a lithium
formulation as described in Section 5.2, supra.

[00777] Another embodiment of the present invention is directed to any of the methods
described above, where the angle between the abrasive disk and the longitudinal axis of the
housing is approximately 45 degrees.

[00778] Yet another embodiment of the present invention is directed to any of the methods
described above, where the linkage assembly comprises an input drive wheel, an output drive
wheel, and a coupling rod.

[00779] Another embodiment of the present invention is directed to any of the methods
described above, where the first set and second set of gears are bevel gears.

[00780] Yet another embodiment of the present invention is directed to any of the methods
described above, where the reciprocating motion ranges between +/- 45 degrees.

[00781] The embodiments of the present invention are directed to a dermabrasion tip for
use on a dermabrasion hand piece, the dermabrasion tip including: a housing; a transmission
unit disposed in the housing, the transmission unit including a first set of gears, a linkage
assembly adjacent to the first set of gears, and a second set of gears adjacent to the linkage
assembly. The dermabrasion tip further includes a platform to receive an abrasive pad, and at
least one abrasive side surface disposed on a side edge of the abrasive pad, where the
transmission unit converts a rotational motion of the dermabrasion hand piece to a radial
reciprocating motion causing the platform to reciprocate, and where, when the side edge
including the abrasive side surface contacts a contact surface, the resulting motion is
perpendicular to the radial reciprocating motion thereby causing linear reciprocating motion
of the abrasive side surface in relation to the contact surface.
[00782] Another embodiment of the present invention is directed to the dermabrasion tip described above, where the platform further includes at least two side surfaces, and where abrasive side surfaces are disposed on the at least two side surfaces.

[00783] Yet another embodiment of the present invention is directed to any of the dermabrasion tips described above, where the platform further includes an abrasive bottom surface with a first cross sectional area and abrasive side surfaces with a second cross sectional area, and where the first cross sectional area is different from the second cross sectional area.

[00784] Another embodiment of the present invention is directed to any of the dermabrasion tips described above, where the first cross sectional area is larger than the second cross sectional area.

[00785] Yet another embodiment of the present invention is directed to any of the dermabrasion tips described above, where the linkage assembly includes an input drive wheel, an output drive wheel, and a coupling rod.

[00786] Another embodiment of the present invention is directed to any of the dermabrasion tips described above, where the first set and second set of gears are bevel gears.

[00787] Yet another embodiment of the present invention is directed to any of the dermabrasion tips described above, where the reciprocating motion ranges between +/- 45 degrees.

[00788] The embodiments of the present invention are directed to a dermabrasion tip for use on a dermabrasion hand piece, the dermabrasion tip including: a housing; and a transmission unit disposed in the housing, the transmission unit including a set of gears and a linkage assembly adjacent to the set of gears. The dermabrasion tip further includes a platform to receive an abrasive pad, where the transmission unit converts a rotational motion of the dermabrasion hand piece to a linear reciprocating motion causing the platform to reciprocate.

[00789] Another embodiment of the present invention is directed to a dermabrasion tip as described above, where the linkage assembly includes an input drive wheel and a coupling rod.

[00790] Yet another embodiment of the present invention is directed to any of the dermabrasion tips as described above, where the coupling rod is pivotally connected to the input drive wheel and the abrasive pad.
Another embodiment of the present invention is directed to any of the dermabrasion tips as described above, where the coupling rod is pivotally connected to the input drive wheel and an extension from the abrasive pad.

Yet another embodiment of the present invention is directed to any of the dermabrasion tips as described above, where the set of gears includes bevel gears.

Another embodiment of the present invention is directed to any of the dermabrasion tips as described above, where the abrasive pad supports an abrasive surface.

The embodiments of the present invention are directed to a kit for use in a dermabrasion process, the kit including: a drug that promotes neogenic hair follicle formation; a dermabrasion hand piece; and a disposable dermabrasion tip. The dermabrasion tip includes: a housing; and a transmission unit disposed in the housing, the transmission unit including a set of gears and a linkage assembly adjacent to the set of gears. The dermabrasion tip further includes an abrasive pad, where the transmission unit converts a rotational motion of the dermabrasion hand piece to a linear reciprocating motion causing the abrasive pad to reciprocate.

Another embodiment of the present invention is directed to the kit as described above, where the drug that promotes neogenic hair follicle formation is a lithium composition as described in Section 5.1, supra.

Yet another embodiment of the present invention is directed to any of the kits as described above, where the drug that promotes neogenic hair follicle formation is a lithium formulation as described in Section 5.2, supra.

Another embodiment of the present invention is directed to any of the kits as described above, where the linkage assembly includes an input drive wheel and a coupling rod.

Yet another embodiment of the present invention is directed to any of the kits as described above, where the coupling rod is pivotally connected to the input drive wheel and the abrasive pad.

Another embodiment of the present invention is directed to any of the kits as described above, where the coupling rod is pivotally connected to the input drive wheel and an extension from the abrasive pad.

Yet another embodiment of the present invention is directed to any of the kits as described above, where the set of gears includes bevel gears.

Another embodiment of the present invention is directed to any of the kits as described above, where the abrasive pad supports an abrasive surface.
The embodiments of the present invention are directed to a method of inducing hair growth in a human subject, the method including disrupting a skin surface with a dermabrader having a dermabrasion hand piece with dermabrasion tip. The dermabrasion tip includes a housing and a transmission unit disposed in the housing. The transmission unit includes: a set of gears and a linkage assembly adjacent to the set of gears. The dermabrasion tip further includes an abrasive pad. The method further includes applying a drug that promotes neogenic hair follicle formation, where the transmission unit converts a rotational motion of the dermabrasion hand piece to a linear reciprocating motion causing the abrasive pad to reciprocate.

Another embodiment of the present invention is directed to the method described above, where the drug that promotes neogenic hair follicle formation is a lithium composition as described in Section 5.1, supra.

Yet another embodiment of the present invention is directed to any of the methods described above, where the drug that promotes neogenic hair follicle formation is a lithium formulation as described in Section 5.2, supra.

Another embodiment of the present invention is directed to any of the methods described above, where the linkage assembly includes an input drive wheel and a coupling rod.

Yet another embodiment of the present invention is directed to any of the methods described above, where the coupling rod is pivotally connected to the input drive wheel and the abrasive pad.

Another embodiment of the present invention is directed to any of the methods described above, where the coupling rod is pivotally connected to the input drive wheel and an extension from the abrasive pad.

Yet another embodiment of the present invention is directed to any of the methods described above, where the set of gears includes bevel gears. Another embodiment of the present invention is directed to any of the methods described above, where the abrasive pad supports an abrasive surface.
6. **EXAMPLES**

6.1 **EXAMPLE 1: MINIMUM INHIBITORY CONCENTRATIONS OF LITHIUM COMPOUNDS AGAINST MICROBIAL AGENTS**

[00809] Minimum inhibitory concentration (MIC) assays were prepared for six microbial species using the microplate methods described by Clinical and Laboratory Standards Institute (CLSI).

[00810] **Materials:**
- Biological Safety Cabinet, Forma 1186
- Assorted Auto-pipettes
- 96-well Microdilution plates, U-bottom, polystyrene
- Spectrophotometer, Spectronic Instruments, 20 Genesys
- Napco 5200 incubator, 35-37°C
- Fisher Isotemp incubator, 30-35°C
- Assorted sterile pipettes, pipette tips, and tubes
- Tryptic Soy Agar (TSA)
- Cation-adjusted Meuller Hinton Broth (MHB)
- RPMI-1640 Liquid Medium
- RPMI-1640 Liquid Medium and Olive Oil (RPMI +00)
- Potato dextrose Agar (PDA)
- Sabouraund Dextrose Agar (SDA)
- Sabourand Dextrose Agar + Olive Oil (SDA +00)
- Supplemented Brucella Blood Agar (SBBA)
- Supplemented Brucella Broth + Lysed Horse Blood (SBB +LHB)
- 0.85% Saline (SAL)
- Anaerobic pouches

[00811] **Methods:** Isolate IDs, incubation and growth media were as indicated in Table 1 below.

**Table 1. Growth conditions for inocula and assays of the test isolates**

<table>
<thead>
<tr>
<th>Species</th>
<th>ATCC ID(^a)</th>
<th>Conditions (Medium/Time/Temperature)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>TSA/20 hour/35°C MHB/20 h/35°C</td>
</tr>
<tr>
<td><em>Staphylococcus</em></td>
<td>29213</td>
<td>TSA/20 hour/35°C MHB/20 h/35°C</td>
</tr>
</tbody>
</table>
Discussion of Results: Lithium inhibited the growth all the organisms tested, gram positive bacteria, gram negative bacteria, and fungi (see Table 2 and Figure 1). In general, the bacteria were most sensitive to lithium carbonate, by a factor of 2 to 4 over lithium succinate and by a factor of 8 to 32 over lithium gluconate (see Table 2 and Figure 1). Lithium Carbonate shows the highest activity against MRSA, with approximately 10 fold better activity that Lithium Gluconate. The activity of lithium succinate was intermediate between lithium carbonate and lithium gluconate. Lithium Gluconate and Lithium Succinate have higher activity than Lithium Carbonate against fungi, M. furfur and A. fumigatus. The standards had much lower MIC because they are all IV drugs, which typically have much lower MIC than topical drugs.

Table 2. Minimum inhibitory concentrations (MIC) of Lithium compounds against six species (lithium compounds in μg Li+/mL; standards in μg/mL)

<table>
<thead>
<tr>
<th>Species</th>
<th>ID</th>
<th>Lithium Gluconate</th>
<th>Lithium Succinate</th>
<th>Lithium Carbonate</th>
<th>Standarda</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>25922</td>
<td>10976</td>
<td>2744</td>
<td>1372</td>
<td>2-4</td>
</tr>
<tr>
<td>S. aureus</td>
<td>29213</td>
<td>10976</td>
<td>5488</td>
<td>2744</td>
<td>1</td>
</tr>
<tr>
<td>S. aureus (MRSA)</td>
<td>33591</td>
<td>10976</td>
<td>5488</td>
<td>1372</td>
<td>1</td>
</tr>
<tr>
<td>P. acnes</td>
<td>11827</td>
<td>10976</td>
<td>686</td>
<td>343</td>
<td>0.12</td>
</tr>
<tr>
<td>M. furfur</td>
<td>14521</td>
<td>686</td>
<td>686</td>
<td>1372</td>
<td>4</td>
</tr>
</tbody>
</table>

a American Type Culture Collection
C. albicans | 90028 | 1372 | 1372 | 686-1372 | 1
A. fumigatus | 204305 | 686 | 686 | 1372 | 2

*a*Standard anti-microbials: *E. coli*: tobramycin; *S. aureus*: vancomycin; *P. acnes*: clindamycin; *C. albicans*, *A. fumigatus* and *M. furfur*: amphotericin B

[00813] The fungal responses were similar for the three lithium compounds. MICs ranged from 686-1372 µg Li+/mL. While the lithium gluconate was soluble at the greatest concentration tested, the other two lithium compounds were not. This was determined by observing the bottoms of the assay wells for precipitated compound. Lithium succinate was consistently soluble at 2744 µg Li+/mL. Lithium carbonate solubility was approximately 1372 µg Li+/mL, but because it settled out of suspension rapidly there was some variability in the range of wells with precipitation. For example, *E. coli* and the two isolates of *S. aureus* were evaluated in an assay at the same time. For *E. coli* precipitate was observed in the first well only, indicating a solubility of 5488 µg Li+/mL. Precipitate was noted in the second well for *S. aureus* ATCC 29213 and in the third well for *S. aureus* ATCC 33591, indicating solubilities of 2744 and 1372 µg Li+/mL, respectively. Solubilities of lithium carbonate determined in the fungal assays were all 1372 µg Li+/mL and in the *P. acnes* assay a solubility of 343 µg Li+/mL was found.

[00814] A pink discoloration was noted at the higher concentrations of lithium succinate and lithium gluconate in the RPMI assays, indicating a pH change. The pHs of the stocks of lithium gluconate, lithium succinate and lithium carbonate were 7.49, 7.56 and 9.56, respectively. It is possible that at the higher concentrations pH may have played a role in the MIC determinations.

### 6.2 Example 2: Minimum inhibitory concentration assays of lithium compounds against wound-associated microorganisms

[00815] Minimum inhibitory concentration (MIC) assays were prepared for nine microbial species that are associated with wounds using the microplate methods described by Clinical and Laboratory Standards Institute (CLSI). Anti-microbial testing was performed with Lithium Carbonate and Lithium Gluconate in either tryptic soy broth (no serum) or tryptic soy broth plus 10% serum (with serum). Testing with serum is important because many drugs fail under these conditions.
Lithium inhibited the growth of all the organisms tested (see Figure 2A-D). Lithium Carbonate is a more potent anti-microbial than the gluconate salt form with respect to *P. aeruginosa* and *S. aureus* (see Figure 2A-D).

Table 2A below shows the *in vitro* MIC of lithium cation, lithium carbonate, and ciprofloxacin against drug-resistant clinical isolates of the following gram negative bacteria in the presence of serum: *E. Coli, K. pneumoniae, A. baumannii, and P. aeruginosa*. Table 2B below shows the *in vitro* MIC of lithium cation, lithium carbonate, mupirocin, and vancomycin against *S. aureus* drug-resistant clinical isolates. Table 2C below shows the *in vitro* MIC of lithium carbonate and Amphotericin B against various fungal organisms that cause onychomycosis.

### Table 2A: Minimum inhibitory concentrations (MIC) of Lithium compounds and Ciprofloxacin against various gram negative bacteria.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Phenotype</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>[Li+] mg/mL</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 102 (ATCC 25922)</td>
<td>Wild type</td>
<td>5.48</td>
</tr>
<tr>
<td>CLSI QC range ATCC 25922</td>
<td></td>
<td>---</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 1241</td>
<td>Ciprofloxacin resistant</td>
<td>5.48</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 1391</td>
<td>Gentamicin resistant</td>
<td>2.74</td>
</tr>
<tr>
<td><em>Escherichia coli</em> 2276</td>
<td>MDR^1/ESBL^2</td>
<td>5.48</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> 1339</td>
<td>Wild type</td>
<td>5.48</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> 1232</td>
<td>Ciprofloxacin resistant</td>
<td>5.48</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> 1461</td>
<td>Gentamicin resistant</td>
<td>2.74</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> 4385</td>
<td>MDR/ESBL</td>
<td>5.48</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> 1630 (ATCC 19606)</td>
<td>Wild type</td>
<td>5.48</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> 2240</td>
<td>Ciprofloxacin resistant</td>
<td>2.74</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em> 4491</td>
<td>Gentamicin resistant</td>
<td>5.48</td>
</tr>
</tbody>
</table>
### Table 2B: Minimum inhibitory concentrations (MIC) of Lithium compounds, Mupirocin, and Vancomycin against various *S. aureus* drug-resistant clinical isolates.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Li+ (mg/mL)</th>
<th>Lithium Carbonate (mg/mL)</th>
<th>Mupirocin (µg/mL)</th>
<th>Vancomycin (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> 3083 (MRSA) (methicillin-susceptible)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> 3084 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. aureus</em> 3085 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. aureus</em> 3086 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. aureus</em> 3087 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> 3088 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>0.12</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> 3269 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>0.12</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. aureus</em> 3270 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. aureus</em> 3271 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>0.12</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. aureus</em> 3272 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>0.12</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> 3273 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>0.12</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> 3274 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. aureus</em> 3876 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. aureus</em> 3877 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> 3878 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> 3880 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>&gt;512</td>
<td>1</td>
</tr>
<tr>
<td><em>S. aureus</em> 3881 (MRSA)</td>
<td>1.35</td>
<td>7.01</td>
<td>≤0.06</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. aureus</em> 1723 (VISA) (vancomycin-Intermediate)</td>
<td>0.68</td>
<td>3.57</td>
<td>≤0.06</td>
<td>4</td>
</tr>
<tr>
<td><em>S. aureus</em> 2012 (VISA) (vancomycin-Intermediate)</td>
<td>0.68</td>
<td>3.57</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td><em>S. aureus</em> 4661 (VISA) (vancomycin-Intermediate)</td>
<td>0.68</td>
<td>3.57</td>
<td>≤0.06</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 2C: Minimum inhibitory concentrations (MIC) of Lithium Carbonate and Amphotericin B against various fungal organisms that cause onychomycosis.

<table>
<thead>
<tr>
<th>Organism/Micromyx Number</th>
<th>ATCC Number</th>
<th>Incubation time (hours)</th>
<th>Minimum Inhibitory Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lithium carbonate (mg/mL)</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes 5278</td>
<td>MYA-4439</td>
<td>96</td>
<td>14.5</td>
</tr>
<tr>
<td>Trichophyton mentagrophytes 640</td>
<td>28185</td>
<td>96</td>
<td>7.25</td>
</tr>
<tr>
<td>Trichophyton rubrum 5279</td>
<td>MYA-4438</td>
<td>96</td>
<td>14.5</td>
</tr>
<tr>
<td>Trichophyton rubrum 5723</td>
<td>10218</td>
<td>96</td>
<td>14.5</td>
</tr>
<tr>
<td>Microsporum gypseum 5720</td>
<td>24102</td>
<td>96</td>
<td>14.5</td>
</tr>
<tr>
<td>Microsporum gypseum 5719</td>
<td>14683</td>
<td>96</td>
<td>14.5</td>
</tr>
<tr>
<td>Epidermophyton floccosum 5721</td>
<td>18397</td>
<td>96</td>
<td>NG</td>
</tr>
<tr>
<td>Epidermophyton floccosum</td>
<td>52066</td>
<td>96</td>
<td>NG</td>
</tr>
</tbody>
</table>
6.3 **EXAMPLE 3: KILLING ACTIVITY OF A LITHIUM CARBONATE SOLUTION AGAINST THREE MEDICALLY-RELEVANT BACTERIA STRAINS**

This study was undertaken to evaluate the killing activity of a lithium carbonate solution against three different, medically-relevant bacteria strains: Multiply Resistant Staph aureus (MRSA), *E. coli* and *Pseudomonas* over a 4 hour exposure. The log reduction capacity for the product tested was compared to a neutralized product control.

**Materials:**

**Microorganisms**
- *E. coli* (ATCC 25922)
- MRSA (ATCC 33591)
- *Pseudomonas aeruginosa* (ATCC 27317)

**Test Articles:**
- Lithium carbonate; 146mg of Lithium carbonate dissolved into 20mL PBS for a 7.3mg/mL [196mM Li+] solution.

**Control Articles**: Phosphate buffered saline

**Neutralizing buffer**: This buffer was used to eliminate possible carry over antimicrobial agents: 8.5g NaCl, 2.5 mL Tween 80, 0.35 gm Lecithn, 997.5mL demineralized water. Mix and autoclave 30 to 35 minutes at 121°C

**Non-Selective Media**
- Tryptic Soy Broth (TSB), prepared according to manufacturer's directions and stored in room temperature.
• Tryptic Soy Agar (TSA), prepared according to manufacturer’s directions. Approximately 400 mL volumes was poured into 500 mL glass bottles and autoclave 30 to 35 minutes at 121°C. Stored in room temperature.
• Phosphate Buffered Saline (PBS). 100 mL of 10 X Phosphate Buffered Saline (Sigma), 900mL demineralized water, autoclaved 30 to 35 minutes at 121°C

[00826] Two days prior to the experiment, a small loopful of each test bacteria, MRSA, P. aeruginosa, and E. coli, was streaked into a non-selective agar plate (e.g. TSA). The plate was incubated overnight at 37°C ± 1°C and the culture examined for purity prior to use. The day prior to the experiment, a small loopful of each bacteria from the above-mentioned plates was inoculated into TSB, vortexed, and incubated at 37°C ± 1°C for approximately eighteen hours. The O.D. was adjusted to 0.1 to 0.16 at 625 nm against sterile water as "zero." This was done to provide a bacteria number about 10^7 CFU per mL. The suspension was diluted with sterile TSB if the dilution was too high. If the bacteria suspension was too low the test organisms from the overnight liquid culture was added and vortexed. This was repeated until the O.D. was correct.
[00827] Procedure for Time Kill of lithium carbonate: 1.8mL of test article solution was added into a 15-ml conical sterile centrifuge tube. For control, Phosphate Buffered Saline was used without the active ingredient. 200 µL of inoculum was added into the centrifuge tubes, vortexed vigorously for 30 seconds and incubated at 37°C ± 1°C. The final concentration of the bacteria and the test mixture was approximately 10^6 CFU/mL (1:10 dilution.). At three different time points, 30 minutes, 2 hours and 4 hours, 0.2 mL of the test solution/ control with test bacteria from reaction tube (4.4.2.1-4.4.2.2) was transferred to 1.8ml Neutralizing Buffer (3.2.3). Since 0.2 mL of bacteria was diluted to 2.0ml in eluate, this becomes a 1:10 dilution. Serial ten-fold dilutions were prepared as follows: the test drug product was vigorously vortexed with the eluate. 100 µL of the 1:10 dilution was transferred to 0.9 mL PBS to produce a 1:100 dilution. This dilution was vortexed vigorously. 100 µL of the 1:100 (10^-2) dilution was transferred to 0.9 mL of PBS to obtain a 1:1000 (10^-3) dilution and so on. The serial dilutions were drop plated and incubated to determine the bacterial counts. The samples were plated on Tryptic Soy Agar (TSA) to determine the total bacterial counts. Bacterial counts were expressed as logio (CFU/mL). The plates were
incubated for 24 hours at 37°C ± 1°C and count colonies. Controls were run with all tests. All samples were done in triplicate and the plating was done in duplicate.

[00828] **Results:** Results are reported as order of magnitude reductions in viable organism counts, relative to the non-treated control (e.g., a four-log reduction is equivalent to 10^3 CFU/mL recovered from the test article when the non-treated control plate count indicated 10^7 CFU/mL).

[00829] MRSA demonstrated a time-dependent kill curve with minimal bacteria death (0.2 log reductions) at 30 minutes, 3.3 log reductions at 2 hrs, and greater than 7 log reductions at 4 hours (see Figure 3). *E. coli* demonstrated a time-dependent kill curve with 6.9 log reductions at 30 minutes, 7.2 log reductions at 2 hrs, and greater than 8 log reductions at 4 hours (see Figure 4). *Pseudomonas aeruginosa* demonstrated a time-dependent kill curve 6.8 log reductions at 30 minutes, 7.1 log reductions at 2 hrs, and greater than 7.5 log reductions at 4 hours (see Figure 5).

[00830] Thus, the lithium carbonate was highly effective (greater than 3 logs of kill) at destroying both gram positive and gram negative bacteria when exposure times were 2 hours or greater. The lithium carbonate was more effective against gram negative bacteria (*E. coli* and *Pseudomonas*) than gram positive (*Staph aureus*), reaching nearly total kill (greater than 7 logs of kill) within 30 minutes of exposure. All of the bacteria tested in this study demonstrated a time-dependent susceptibility to lithium carbonate.

### 6.4 EXAMPLE 4: EVALUATION OF LITHIUM CARBONATE FORMULATIONS FOR ANTI-MICROBIAL ACTIVITY IN A MOUSE SKIN ABARASION INFECTION MODEL

[00831] Lithium Carbonate formulations were evaluated for anti-microbial activity in a mouse skin abrasion infection model against *Methicillin Resistant S. aureus* (MRSA) and *E.coli*. The formulations contained varying concentrations of lithium carbonate. The study groups were: (a) vehicle, (b) Lithium Carbonate 2.74 mg Li+/g (1.46% w/w Li₂CO₃), (c) Lithium Carbonate 5.48 mg Li+/g (2.91% w/w Li₂CO₃), (d) 10.64 mg Li+/g (5.66% w/w Li₂CO₃) and (e) LithioDerm 2.74 mg Li+/g (8% Lithium Gluconate). Mice in the negative control group received no treatment, also called the “infection control” group. Mice in the positive control group received 2% Mupirocin for MRSA and Polymyxin for *E.coli*. It was previously determined that the in vitro minimum inhibitory concentration (MIC) for lithium carbonate was 1.372 mg Li+/g. Thus, the in vivo test groups contained ionized lithium concentrations that were approximately 2-10 times the *in vitro* MIC.
The pathogens selected were Methicillin-Resistant *Staphylococcus aureus* (MRSA) and wild type *E. coli*. The *in vivo* infective model was a CD-1 mouse infection, dermabrasion model. Lithium Carbonate vehicle was used as one of the test groups. Each group had 10 mice wounded at day 0 using a dermabrader. The wounds were infected with MRSA or *E. coli*. 24 hours after infection, the mice were dosed twice daily for three days with 0.1 mL of each formulation, using a calibrated wiretrol pipette. The infection control group did not receive treatment. Enumeration of the bio-load on the collected abrasions was assessed five days post infection.

**Materials and Methods:**

**Bacterial strains:**
- *Methicillin-resistant S. aureus* ATCC 33591
- *E. coli* ATCC25922

**Test articles pre-formulated:**
- Lithium Carbonate 2.74 mg Li+/g
- Lithium Carbonate 5.48 mg Li+/g
- Lithium Carbonate 10.73 mg Li+/g
- Lithium Carbonate Placebo (Vehicle Control)
- LithioDerm 2.74 mg Li+/g (Lot# HC730)
- Comparator antibiotic Bactroban Cream (Mupirocin 2%) (22 g) (MRSA Positive Control) Taro Pharmaceuticals, Hawthorne, NY; Exp: 12/2011; Lot# 10055
- Comparator antibiotic Polymyxin (1 oz) (E. coli Positive Control); Taro Pharmaceuticals, Hawthorne, NY; Exp: 11/2013; Lot# K0039

**Bacterial Growth Media and Vehicles:**
- Trypticase Soy Agar plates - BBL, Franklin Lakes, NJ
- Brain Heart Infusion (BHI) Broth - BBL, Franklin Lakes, NJ
- Sterile Saline - 0.9% prepared from powder as needed at ViviSource Laboratories, Inc
- MacConkey Agar plates - BBL, Franklin Lakes, NJ

**Animals:** Species: mouse, Strain: CD-1(ICR), Source: Harlan Laboratories, Weight: 20 to 30 grams, Number: N=10 per group

All procedures in this protocol were in compliance with the ViviSource Laboratories IACUC protocol VVSIO-016. Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.
Protocol/ Study Design:

Bacteria preparation: Methicillin-resistant *S. aureus* ATCC 33591 and *E. coli* ATCC 25922 were grown at 37°C under 5% CO₂ on tryptasesoy agar (TSA) plates. The bacterial concentration was prepared by re-suspending colonies from the overnight growth on the TSA plate in brain heart infusion (BHI) broth and adjusting a 1:20 dilution of the suspension to achieve an OD (optimal density) of 0.3 at 625nm. The bacterial suspension was then diluted in saline to achieve an inoculum of approximately in 1x10⁸ CFU/mL (Colony Forming Units).

Bacterial counts were performed to determine actual inoculum concentration; 100 µL of the bacterial suspension was serially diluted (1:10) in saline 7 times. Then 100 µL of the 10⁵, 10⁶, and 10⁷ was pipetted in duplicate on to TSA pates with glass beads, then shaken to spread bacteria on plates, then the beads were removed and plates were placed at 37°C with 5% CO₂ overnight, and then counted 24 hours later to determine bacterial concentration of the inoculum the mice received.

Animal Preparation: The right rear flank of all mice were clipped to 1.5 cm x 1.5 cm size removing fur and then cleaned with 70% alcohol. Mice were anesthetized with an inhalation mixture of 4% Isoflurane and 1.5% oxygen prior to abrasion.

Dermabrasion Procedure: The skin was abraded to a width/length of 13 mm with a sandpapered dowel to abrade the skin without causing any bleeding, by applying pressure and using a constant motion back forth for up to 15 times. This procedure results in a partial thickness wound (Vivisource data).

Infection establishment: Mice (ten per group) of weight approximately 25g and age 8-10 weeks were anesthetized with an inhalation of 4% Isoflurane with 1.5% oxygen and fur clipped. The mice were dermabraded using a dermabrader as described in the previous section. The bacterial suspension was pipetted on to the abrasion at a volume of 100µL. After the inoculum was pipetted on the skin, a sterile non-absorbent 13mm disc was placed on the wound then taped in place with dermal cloth tape. This maintains the suspension in direct contact with the wound. Animals were observed frequently after the infection procedure to confirm they were awake from the anesthesia and showing no signs of discomfort or pain from the procedure. No analgesic was provided as the abrasion wound will go no deeper than the dermal layer; literature research confirmed that analgesic was unnecessary for this procedure with an anesthetic, however if at any time during the study an animal had shown signs of serious injury or illness that was unlikely to be the result of the experimental procedures, the affected mice would have been immediately euthanized. Animals were
monitored daily for any signs of animals exhibiting severe dyspnea (labored breathing), severe depression, weight loss or other neurological signs. Animals with weight loss exceeding 20% from the time 0, body weight and other signs of obvious distress would have been immediately euthanized. Twenty-four hours post infection the tape and disc was removed, and wound area measured. Animals were weighed daily to ensure the animals were not in pain.

[00845] **Treatment:** There were 14 groups in total, with 10 mice per group. Table 3 below details the study design and group identifiers. Groups 1-7 received MRSA. Groups 8-14 received E.coli. At 24 hours post infection the tape and disc was removed. Mice were weighed once daily prior to first dose. The abrasion/dose sites were scored twice daily using the Skin Irritation Draize Scoring system prior to dose. Mice were dosed a set volume of 0.100 ml (using a wiretrol device) twice daily for three days. The dosing per day was spaced 6 hours apart. If the hydrogel did not spread well on the wound, the tip of the wiretrol device was used to spread the formulation on the wound.

Table 3: Study Design and Dose Groups/ Identifiers

<table>
<thead>
<tr>
<th>Group #</th>
<th>Lot #</th>
<th>Group Identifier</th>
<th>Bacterial Strain</th>
<th>Dose (ml)</th>
<th># of mice</th>
<th>Route</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lot# TH-002-061</td>
<td>Lithium Carbonate 2.74 mg Li+/g</td>
<td>MRSA ATCC 33591</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>2</td>
<td>Lot# TH-002-062</td>
<td>Lithium Carbonate 5.48 mg Li+/g</td>
<td>MRSA ATCC 33591</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>3</td>
<td>Lot# TH-002-062</td>
<td>Lithium Carbonate 10.73 mg Li+/g</td>
<td>MRSA ATCC 33591</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>4</td>
<td>HC-730</td>
<td>LithioDerm 2.74 mg Li+/g</td>
<td>MRSA ATCC 33591</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>5</td>
<td>Lot # TH-002-059</td>
<td>Lithium Carbonate Vehicle</td>
<td>MRSA ATCC 33591</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>6</td>
<td>Lot# 10055</td>
<td>Mupirocin 2% (+ control)</td>
<td>MRSA ATCC 33591</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>7</td>
<td>N/A</td>
<td>No treatment group</td>
<td>MRSA ATCC 33591</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>8</td>
<td>Lot# TH-002-061</td>
<td>Lithium Carbonate 2.74 mg Li+/g</td>
<td><em>E. coli</em> ATCC25922</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>9</td>
<td>Lot# TH-002-062</td>
<td>Lithium Carbonate 5.48 mg Li+/g</td>
<td><em>E. coli</em> ATCC25922</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>10</td>
<td>Lot# TH-002-089</td>
<td>Lithium Carbonate 10.73 mg Li+/g</td>
<td><em>E. coli</em> ATCC25922</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>11</td>
<td>HC-730</td>
<td>Lithium Carbonate 2.74 mg Li+/g</td>
<td><em>E. coli</em> ATCC25922</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>12</td>
<td>Lot # TH-002-059</td>
<td>Lithium Carbonate Vehicle</td>
<td><em>E. coli</em> ATCC25922</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>13</td>
<td>Lot# K0039</td>
<td>Polymyxin (+ control)</td>
<td><em>E. coli</em> ATCC25922</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
<tr>
<td>14</td>
<td>N/A</td>
<td>No treatment group</td>
<td><em>E. coli</em> ATCC25922</td>
<td>0.100</td>
<td>10</td>
<td>Topically</td>
<td>BID</td>
</tr>
</tbody>
</table>

[00846] **Tissue Processing:** Approximately sixteen hours after the last treatment, all mice were euthanized via an inhalation of C02. The skin of the animals was aseptically removed,
weighed, homogenized in 1 mL of sterile saline and serially diluted in sterile saline five times at 1:10. The five diluted samples were plated on TSA medium for bacterial quantification. After 18 hours of incubation the dilution with countable bacterial colonies was recorded for each animal and entered into raw data with weight of excised abrasion for calculation for logioCFU.

[00847] **Data End-Points:** The average logio CFU per gram of skin was calculated for each group and standard deviations determined. The logio CFU per gram of skin versus control and the logio reductions from the untreated controls were calculated.

[00848] Twenty-four hours after infection was established, erythema and edema in each dose group was assessed twice daily prior to each dose. Erythema and edema were assessed based on the Draize scoring system provided in Table 4 below.

<table>
<thead>
<tr>
<th>Table 4: Primary Skin Irritation Draize Scoring System</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary Skin Irritation Draize Scoring System</strong></td>
</tr>
<tr>
<td>Evaluation of Skin Reactions Value</td>
</tr>
<tr>
<td>(0,0)</td>
</tr>
<tr>
<td>1st-Erythema (redness) and Eschar (scabbing) formation:</td>
</tr>
<tr>
<td>No erythema ................................................................. 0</td>
</tr>
<tr>
<td>Very slight erythema (barely perceptible) ......................... 1</td>
</tr>
<tr>
<td>Well-defined erythema .................................................. 2</td>
</tr>
<tr>
<td>Moderate to severe erythema .......................................... 3</td>
</tr>
<tr>
<td>Severe erythema (beet red) to slight eschar formation .......... 4</td>
</tr>
<tr>
<td>2nd-Edema (swelling) formation:</td>
</tr>
<tr>
<td>No edema ........................................................................... 0</td>
</tr>
<tr>
<td>Very slight edema (barely perceptible) ............................... 1</td>
</tr>
<tr>
<td>Slight edema (edges well defined by definite raising) ............ 2</td>
</tr>
<tr>
<td>Moderate edema (raised approximately 1 mm) ....................... 3</td>
</tr>
<tr>
<td>Severe edema (raised &gt;1 mm and extending beyond exposure) .... 4</td>
</tr>
</tbody>
</table>

[00849] **Results:**

[00850] **Anti-microbial Activity:** As shown in Table 5, topical administration of Lithium Carbonate at 2.74 mg, 5.48 mg, and 10.73 mg Li+/g in a mouse skin abrasion infection model against MRSA (ATCC 33591) resulted in a reduction of bacterial load at -0.70, -0.74, and -1.54 CFU per gram of skin. Topically administered LithioDerm at 2.74 mg Li+/g resulted in a reduction of bacterial load at -0.10 from the control mice. Reference compound 2% Mupirocin Cream was also evaluated in the same model and generated bacterial reduction from control mice at -2.17 CFU per gram of skin, respectively. Infection controls demonstrated in a manner consistent with this model. The reference P-value for Mupirocin cream against MRSA was <0.05. The P-values for Lithium Carbonate vehicle, 2.74, 5.48,
and 10.73 mg Li+/g against MRSA were 0.5, 0.03, and <0.05. The P-value of LithioDerm at 2.74 mg Li+/g was 0.5.

As shown in Table 6, the 10.73 mg dose of Lithium Carbonate decreased the bacterial load by almost half a log (.40), the two lower doses did not decrease bacterial load. LithioDerm did not lower the bacterial load. Polymyxin (positive control) decreased the bacterial load by over half a log (0.58) from vehicle dosed which is standard with this Polymyxin ointment. There was no CNS, adverse effects seen at any dose. The 2.74 mg Li+/g Lithium Carbonate dose reduced erythema and edema more than any other treatment. The reference P-value for the positive control Polymyxin against E.coli is <0.05. The P-values for Lithium Carbonate vehicle, 2.74, 5.48, and 10.73 mg Li+/g against E.coli were all 0.5. The P-value of LithioDerm at 2.74 mg Li+/g was 0.5.

Table 5: Anti-microbial activity of Lithium Carbonate compared to LithioDerm against MRSA

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>BID Dose (mL)</th>
<th>CFU/g of skin</th>
<th>SD</th>
<th>A of CFU from Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1- 2.74mg Li+/g</td>
<td>0.1</td>
<td>8.94</td>
<td>0.92</td>
<td>-0.70</td>
</tr>
<tr>
<td>LithioDerm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2- 5.48mg Li+/g</td>
<td>0.1</td>
<td>8.29</td>
<td>0.43</td>
<td>-0.74</td>
</tr>
<tr>
<td>LithioDerm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3- 10.73mg Li+/g</td>
<td>0.1</td>
<td>7.49</td>
<td>0.57</td>
<td>-1.54</td>
</tr>
<tr>
<td>LithioDerm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4- 2.74mg Li+/g</td>
<td>0.1</td>
<td>8.94</td>
<td>0.14</td>
<td>-0.10</td>
</tr>
<tr>
<td>LithioDerm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5- Vehicle</td>
<td>0.1</td>
<td>8.84</td>
<td>0.33</td>
<td>-0.39</td>
</tr>
<tr>
<td>LithioDerm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6- 2% Mupirocin</td>
<td>0.1</td>
<td>6.87</td>
<td>0.96</td>
<td>-2.17</td>
</tr>
<tr>
<td>LithioDerm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7- Untreated</td>
<td>0.1</td>
<td>9.04</td>
<td>0.22</td>
<td></td>
</tr>
</tbody>
</table>

Bacteria: MRSA (ATCC-33591)
Infection: 5.0 x 10^7 cfu/mouse
Treatment 3 Days BID
Removal 4 Days post infection
Table 6: Anti-microbial activity of Lithium Carbonate compared to LithioDerm against E.Coli

**Mouse Skin Infection Model**

VVS 111-0223 11-01

**Bacteria**: *E. coli* (ATCC-25922)  
**Infection**: $2.65 \times 10^7$ cfu/mouse  
**Treatment**: 3 Days BID  
**Removal**: 4 Days post infection

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Dose (mg/L)</th>
<th>CFU/g of skin</th>
<th>SD</th>
<th>Δ of CFU from Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. LithioDerm</td>
<td>2.74mg Li+/g</td>
<td>0.1</td>
<td>0.29</td>
<td>0.06</td>
</tr>
<tr>
<td>2. LithioDerm</td>
<td>5.48mg Li+/g</td>
<td>0.1</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>3. LithioDerm</td>
<td>10.73mg Li+/g</td>
<td>0.1</td>
<td>0.65</td>
<td>-0.16</td>
</tr>
<tr>
<td>4. LithioDerm</td>
<td>2.74mg Li+/g</td>
<td>0.1</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>5. Vehicle</td>
<td>Follica Derm</td>
<td>0.1</td>
<td>0.21</td>
<td>0.25</td>
</tr>
<tr>
<td>6. Polymyxin</td>
<td>Follica Derm</td>
<td>0.1</td>
<td>0.59</td>
<td>-0.33</td>
</tr>
<tr>
<td>7. Untreated</td>
<td>Follica Derm</td>
<td>0.1</td>
<td>0.27</td>
<td>-</td>
</tr>
</tbody>
</table>

[00852] Erythema/Edema:

[00853] Ecoli. The wounds from each group were assessed twice daily, prior to administration of each dose, using the Draize scoring scheme described in Table 4. Table 7 summarizes the average scores of each group on Day 1-Day 3. For the E.Coli-infected mice, each of the groups had well-defined erythema (score=2-2.5) as per the erythema scale, with no major differences between the treatment groups. As per Table 4, the erythema score chart is as follows: (a) no erythema = 0; (b) very slight erythema =1, (c) well defined erythema = 2, (d) moderate to severe erythema = 3, (e) severe erythema =4.

[00854] In terms of edema, lithium treated groups appeared to have lower edema (1.1-2.4) than vehicle (1.4-2.6), untreated (1.6-2.6) or positive control animals (1.7-2.5), as shown in Table 8. Lithium treated animals appeared to be healing better, as compared to the non-lithium groups. It was observed that the wound of the Lithium Carbonate 2.74 mg Li+/g and 5.48 mg Li+/g treated mice was visibly defined by erythema, where as the Lithium Carbonate 10.73 mg Li+/g, LithioDerm, Lithium Carbonate Vehicle, and the untreated wounds were well defined by erythema and edema.

Table 7: Kinetic Assessment of Average Erythema as a Function of Treatment Group on E.coli-infected wounds

<table>
<thead>
<tr>
<th>ID</th>
<th>Group</th>
<th>Pre-1st Dose, Day 1</th>
<th>Pre-2nd dose, Day 2</th>
<th>Pre-1st Dose, Day 2</th>
<th>Pre-2nd dose, Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
</tr>
<tr>
<td>01</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
</tr>
<tr>
<td>02</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
</tr>
</tbody>
</table>

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Table 8: Kinetic Assessment of Edema as a function of treatment on E.coli-infected wounds

<table>
<thead>
<tr>
<th>ID</th>
<th>Group</th>
<th>Pre-1st Dose, Day 1</th>
<th>Pre-2nd dose, Day 1</th>
<th>Pre-1st Dose, Day 2</th>
<th>Pre-2nd dose, Day 2</th>
<th>Pre-1st Dose, Day 3</th>
<th>Pre-2nd dose, Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.74 mg Li+/g Lithium Carbonate</td>
<td>1 1.9</td>
<td>2.0</td>
<td>2.0</td>
<td>2.2</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>5.48 mg Li+/g Lithium Carbonate</td>
<td>2 2.1</td>
<td>2.2</td>
<td>2.2</td>
<td>2.6</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>10.73 mg Li+/g Lithium Carbonate</td>
<td>3 2.1</td>
<td>2.1</td>
<td>2.1</td>
<td>2.2</td>
<td>2.2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>2.74 mg Li+/g LithioDerm</td>
<td>4 2.0</td>
<td>2.1</td>
<td>2.1</td>
<td>2.2</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Lithium Carbonate Vehicle</td>
<td>5 1.8</td>
<td>2.0</td>
<td>2.3</td>
<td>2.5</td>
<td>2.2</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Polymyxin</td>
<td>6 1.9</td>
<td>2.0</td>
<td>2.3</td>
<td>2.4</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>7 2.0</td>
<td>1.9</td>
<td>2.3</td>
<td>2.4</td>
<td>2.2</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The wounds from each group were assessed twice daily, prior to administration of each dose, using the Draize scoring scheme described in Table 4. Table 9 summarizes the average scores of each group on Day 1-Day 3. For the MRSA-infected mice, each of the groups had varying erythema (score=1.2-2.4) as per the erythema scale, the Lithium treated mice had a slight increase in erythema, where as the Lithium Carbonate and 2% Mupirocin treated mice had a slightly decreased erythema after 3 days of dosing.

In terms of edema, lithium treated groups and the vehicle treated group appeared to have lower edema (1.0-2.1, vehicle (1.0-2.1), than untreated (1.0-2.7) or positive control animals (1.0-2.3), as shown in Table 10. Lithium treated animals appeared to be healing better, as compared to the non-lithium groups. The raw score for each individual wound assessment is provided in Appendix 4. It was observed by the that the wounds of the Lithium treated mice was visibly defined by erythema, where as the wounds of the vehicle, positive control and the untreated animals were well defined by erythema and edema.
Table 9: Kinetic Assessment of Erythema as a function of treatment on MRSA-infected wounds

<table>
<thead>
<tr>
<th>ID</th>
<th>Group</th>
<th>Pre-1st Dose, Day 1</th>
<th>Pre-2nd dose, Day 2</th>
<th>Pre-1st Dose, Day 2</th>
<th>Pre-2nd dose, Day 3</th>
<th>Pre-1st Dose, Day 3</th>
<th>Pre-2nd dose, Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
<td>Erythema</td>
</tr>
<tr>
<td>1</td>
<td>2.74 mg Li+/g Lithium Carbonate</td>
<td>1.4</td>
<td>1.3</td>
<td>1.2</td>
<td>1.2</td>
<td>1.6</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td>5.48 mg Li+/g Lithium Carbonate</td>
<td>1.5</td>
<td>1.6</td>
<td>1.9</td>
<td>1.7</td>
<td>2.1</td>
<td>2.2</td>
</tr>
<tr>
<td>3</td>
<td>10.73 mg Li+/g Lithium Carbonate</td>
<td>1.6</td>
<td>1.5</td>
<td>1.7</td>
<td>1.6</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>2.74 mg Li+/g LithioDerm</td>
<td>1.4</td>
<td>1.6</td>
<td>1.7</td>
<td>1.4</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>Lithium Carbonate Vehicle</td>
<td>1.5</td>
<td>1.7</td>
<td>1.6</td>
<td>1.6</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>6</td>
<td>2% Mupirocin</td>
<td>1.6</td>
<td>1.8</td>
<td>1.8</td>
<td>1.6</td>
<td>2.0</td>
<td>1.8</td>
</tr>
<tr>
<td>7</td>
<td>Untreated</td>
<td>1.3</td>
<td>1.8</td>
<td>1.9</td>
<td>1.7</td>
<td>2.4</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Table 10: Kinetic Assessment of Edema as a function of treatment on MRSA-infected wounds

<table>
<thead>
<tr>
<th>ID</th>
<th>Group</th>
<th>Pre-1st Dose, Day 1</th>
<th>Pre-2nd dose, Day 2</th>
<th>Pre-1st Dose, Day 2</th>
<th>Pre-2nd dose, Day 3</th>
<th>Pre-1st Dose, Day 3</th>
<th>Pre-2nd dose, Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Edema</td>
<td>Edema</td>
<td>Edema</td>
<td>Edema</td>
<td>Edema</td>
<td>Edema</td>
</tr>
<tr>
<td>1</td>
<td>2.74 mg Li+/g Lithium Carbonate</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.4</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>2</td>
<td>5.48 mg Li+/g Lithium Carbonate</td>
<td>1.0</td>
<td>1.3</td>
<td>1.8</td>
<td>1.6</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>10.73 mg Li+/g Lithium Carbonate</td>
<td>1.0</td>
<td>1.3</td>
<td>2.0</td>
<td>2.0</td>
<td>1.7</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>2.74 mg Li+/g LithioDerm</td>
<td>1.0</td>
<td>1.3</td>
<td>1.9</td>
<td>2.1</td>
<td>2.1</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>Lithium Carbonate Vehicle</td>
<td>1.0</td>
<td>1.3</td>
<td>2.1</td>
<td>1.9</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>2% Mupirocin</td>
<td>1.0</td>
<td>1.7</td>
<td>2.3</td>
<td>2.1</td>
<td>2.3</td>
<td>2.0</td>
</tr>
<tr>
<td>7</td>
<td>Untreated</td>
<td>1.0</td>
<td>1.8</td>
<td>2.6</td>
<td>2.7</td>
<td>2.4</td>
<td>2.6</td>
</tr>
</tbody>
</table>

[00857] **Conclusions.**

[00858] For the groups that were infected with MRSA, 2% Mupirocin significantly reduced the microbial load by 2.17 CFU/g of skin which is the standard for this treatment protocol. Similarly, Lithium Carbonate at 10.73 mg Li+/g also significantly reduced the MRSA microbial load by 1.54 CFU/g of skin. Lower concentrations of Lithium Carbonate at 2.74 and 5.48 mg Li+/g reduced the MRSA microbial infection by 0.70 and 0.74 CFU/g of skin. The Lithium Carbonate Vehicle and commercially manufactured 2.74 mg Li+/g
LithioDerm were not efficacious against the microbial MRSA infection with a 0.39 and 0.10 CFU/g of skin, respectively. In the MRSA infection the untreated group demonstrated a greater increase in erythema and edema. Whereas all the treated groups demonstrated lower erythema and edema scores. The reduction was expected due in part to the decrease in microbial load.

[00859] For groups that were infected with E.coli, Polymyxin did reduce the infection but was limited by 0.33 CFU/g of skin. Lithium Carbonate at all concentrations, the Lithium Carbonate vehicle, and the LithioDerm did not meaningfully reduce the E.coli microbial load. Lithium Carbonate at 10.73 mg Li+/g had a reduction of 0.16 logioCFU from the untreated mice. Lithium Carbonate at 2.74 mg Li+/g, and 5.48 mg Li+/g had an increase of 0.08 and 0.01 logioCFU, respectively. LithioDerm (8% lithium gluconate gel) had an increase of 0.20 logioCFU, and Lithium Carbonate vehicle had an increase of 0.25 logioCFU from the untreated mice. 5.48 and 10.73 mg Li+/g of Lithium Carbonate groups demonstrated a greater increase in erythema and edema compared to the untreated group, Polymyxin group, and LithioDerm group. All the 2.74 mg Li+/g of Lithium Carbonate group demonstrated lower erythema and edema scores. The edema and erythema did not correlate with microbial load.

[00860] References:

6.5 EXAMPLE 5: DELIVERY OF IONIZED LITHIUM AND MICRONIZED LITHIUM CARBONATE FORMULATIONS FROM A TWO CHAMBER LIQUID-LIQUID SPRAY DELIVERY SYSTEM

[00861] This example illustrates a complete spray delivery system that delivers both a cleansing agent (to clean and debride an infected wound) and an in-situ "gelling" antimicrobial gel that also accomplishes scarless wound healing. The two-chamber spraying system would contain a liquid in each compartment. The spraying mechanism of each of the chambers would be deployed separately, as opposed to simultaneously. In this concept, the spraying mechanism for chamber I would deploy the spray stream at high energy, creating a highly effective cleansing method that delivers a sterile liquid to physically debride the infected wound. The sterile liquid delivered as a high energy spray would be comprised of
ionized lithium at a concentration that is microbiocidal, combined with a surfactant molecule like benzalkonium chloride or cetyl Pyridinium chloride. Both molecules are FDA-approved as preservatives and also as potent antimicrobials. It is surmised that a high energy spray of a sterile, cleansing liquid prepares the wound tissue, for delivery of the antimicrobial gel.

**Materials:**

- Pharmaceutical grade Lithium Carbonate from Spectrum chemicals, Inc.
- Micronized lithium carbonate (particle size 1-3 microns) from FMC Corporation, Inc.
- Cetyl Pyridinium chloride from Sigma Aldrich.
- PEO-PPO-PEO polymer (F127) from BASF Corporation. 12,600 daltons in molecular weight, with each polymer segment approximately 4000 daltons.
- Benzyl alcohol, used as a preservative.
- Allantoin and sodium alginate, used as wound healing aids.

Lithium is contained in both the cleansing solution and the antimicrobial sustained release system.

Solution 1 is contained in chamber 1 of the spray system. Solution 1 contains 1% benzyl alcohol, 1% cetyl Pyridinium chloride, lithium dissolved in a concentration range (1-8% w/w lithium carbonate) and water. The solution is pH adjusted to 7.

Solution 2 is contained in chamber 2 of the spray system. Solution 2 contains 3% allantoin, 1% sodium alginate, 20% F127, 2% glycerin, 0.5-1% w/w Tween 20, micronized lithium carbonate in the concentration range 1-8% w/w lithium carbonate) and water (Q.S.). Solution 2 is pH adjusted to 7.

Both solutions 1 and 2 have viscosities less than 200 cP at temperatures of 2-8 °C. At 32 °C, solution 2 attains a viscosity that is approximately 3000 cP, creating a thick viscous gel that would stay on the wound surface. Both solutions 1 and 2 are sterile.

**6.6 EXAMPLE 6: ONE CHAMBER SPRAY SYSTEM**

This example describes the use of a single chamber spray system to debride/cleanse a wound, followed by application of a dry pliable, lithium-containing film as an antimicrobial. This example accomplishes the goals of Example 5 above, by application
of a liquid cleanser followed by application of a dry, thin adhesive film that contains lithium. Solution 1 will contain identical ingredients as solution 1 in Example 5 above.

[00868] After the wound is cleansed, a sterile film containing micronized lithium carbonate will be applied to the wound.

[00869] Films containing Li+ (2.68 mg Li+/g) were prepared using lithium carbonate as the API. The API used in those formulations is lithium carbonate from Riedel-deHaen with lot # 670740. Citric acid from KIC Chemicals with lot # 200203 was used for helping dissolve lithium carbonate in water. Polyvinyl alcohol (PVA) low Mw (~10 k), medium Mw (57–66k), and high Mw (88–97k) from Alfa-Aesar, with lot # K20U039, 27R04, and D025018 were used for forming the films. Carragenan from Kappa was used as a thickener and propylene glycol from Sigma-aldrich with lot # 111K1658 was used for increase the flexibility of the film. Calcium Chloride from Sigma-aldrich with lot # was used as a solidifier. The formulations all contained at least 2.68 mg Li+/g or 1.43 % w/w lithium carbonate. The formulations were characterized for Li+ content using flame photometry and dissolution release.

[00870] PVA film Formulations PVA is a water-soluble polymer, forming a pliable, integral film after casting and drying. Different molecular weights of PVA were selected for hoping that the formed film cannot easily be dissolved in water. Water soluble biopolymers like carragenan was added for further reinforcing the dragging effect, thus controlling the release rate of Li+. Below are the detailed the experimental results of preparing PVA films and the effect on Li+ release.

[00871] Formulation Preparation Method 0.12 g citric acid was weighed and dissolved in water with ultrasonication (about 3 min.). Water amounts varied due to the different concentrations of other excipients and are shown in Tables 11-14. The solution was heated up to 90°C in a water bath and stirred with magnetic bar. 6g of PVA was weighed and slowly added in the solution or suspension. The mixture was continuously stirred and heated until PVA was dissolved. When the solutions were still hot, they were cast on a polycarbonate film surface. Those films were transferred into 100°C for drying overnight. The amount of the excipients needed for 30 g batch formulations are listed in Tables 11-14 below and the percentages of each excipient in the film after dry are also listed in the tables.
Table 11-14 Film formulations with lot # TH-001-051, TH-001-052a, TH-001-052b, TH-001-052c (30g batch)

**Table 11: TH-001-051**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Target Amount (g)</th>
<th>Percentage %</th>
<th>Actual amount (g)</th>
<th>Actual Percentage %</th>
<th>Percentage after dry %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium carbonate</td>
<td>0.3</td>
<td>1</td>
<td>0.31</td>
<td>1.03</td>
<td>4.79</td>
</tr>
<tr>
<td>Distilled water</td>
<td>23.58</td>
<td>78.6</td>
<td>23.58</td>
<td>78.57</td>
<td>0.00</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.12</td>
<td>0.4</td>
<td>0.12</td>
<td>0.40</td>
<td>1.88</td>
</tr>
<tr>
<td>PVA-Medium Mw</td>
<td>6</td>
<td>20</td>
<td>6.00</td>
<td>20.00</td>
<td>93.34</td>
</tr>
<tr>
<td>total</td>
<td>30</td>
<td>100</td>
<td>30.01</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Table 12: TH-001-052a**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g)</th>
<th>Percentage %</th>
<th>Actual amount (g)</th>
<th>Actual Percentage %</th>
<th>Percentage after dry %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium carbonate</td>
<td>0.3</td>
<td>1</td>
<td>0.30</td>
<td>1.01</td>
<td>8.81</td>
</tr>
<tr>
<td>Distilled water</td>
<td>26.58</td>
<td>88.6</td>
<td>26.58</td>
<td>88.58</td>
<td>0.00</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.12</td>
<td>0.4</td>
<td>0.12</td>
<td>0.41</td>
<td>3.57</td>
</tr>
<tr>
<td>PVA-Medium Mw</td>
<td>3</td>
<td>10</td>
<td>3.00</td>
<td>10.01</td>
<td>87.62</td>
</tr>
<tr>
<td>total</td>
<td>30</td>
<td>100</td>
<td>30.01</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Table 13: TH-001-052b**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g)</th>
<th>Percentage %</th>
<th>Actual amount (g)</th>
<th>Actual Percentage (%)</th>
<th>Percentage after dry (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium carbonate</td>
<td>0.3</td>
<td>1</td>
<td>0.30</td>
<td>1.00</td>
<td>8.76</td>
</tr>
<tr>
<td>Distilled water</td>
<td>26.58</td>
<td>88.6</td>
<td>26.58</td>
<td>88.58</td>
<td>0.00</td>
</tr>
<tr>
<td>Citric acid</td>
<td>0.12</td>
<td>0.4</td>
<td>0.12</td>
<td>0.41</td>
<td>3.61</td>
</tr>
<tr>
<td>PVA-High Mw</td>
<td>3</td>
<td>10</td>
<td>3.00</td>
<td>10.01</td>
<td>87.63</td>
</tr>
<tr>
<td>total</td>
<td>30</td>
<td>100</td>
<td>30.01</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Table 14: TH-001-052c**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g)</th>
<th>Percentage %</th>
<th>Actual amount (g)</th>
<th>Actual Percentage %</th>
<th>Percentage after dry (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium carbonate (micronized)</td>
<td>0.6</td>
<td>2</td>
<td>0.61</td>
<td>2.00</td>
<td>16.77</td>
</tr>
<tr>
<td>Distilled water</td>
<td>26.4</td>
<td>88</td>
<td>26.61</td>
<td>88.05</td>
<td>0.00</td>
</tr>
<tr>
<td>PVA-medium Mw</td>
<td>3</td>
<td>10</td>
<td>3.00</td>
<td>9.94</td>
<td>83.23</td>
</tr>
<tr>
<td>---------------</td>
<td>----</td>
<td>----</td>
<td>------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>total</td>
<td>30</td>
<td>100</td>
<td>30.21</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

[00872] **Measurement of Li+ Content** The films were dissolved in water and measured for Li content by Atomic absorption spectroscopy (AAS) (Varian SpectrAA-20 plus). AAS was used for analyze lithium ion in the dissolution bath. 5 lithium standards with concentrations from 0.01, 0.05, 0.2, 1 and 2 ppm were used for generating calibration curve before samples were measured. The concentration of lithium ion was directly read from instrument in ppm. Typical standards curve is shown in Figure 6.

[00873] **Determination of Release Rates** Less than 1 g film was used for dissolution test in 500 ml water. The films made from formulation lot# TH-00 1-051 and Th-001-052a that have medium molecular weight (50~60k) PVA were dissolved in water within 30 min. However, the film made from formulation TH-00 1-052c that has medium molecular weight PVA too but contains micronized lithium carbonate particles was found to release Li+ for more than 3 days. The films made from formulation TH-00 1-052b of high molecular weight (88~97k) PVA and formulations Lot# TH-00 1-53a, TH-00 1-053b, TH-00 1-054 with Carrageenan and low molecular weight of PVA survived in dissolution bath for more than three days in water.

### 6.7 EXAMPLE 7: PETROLATUM-BASED FORMULATIONS WITH LITHIUM GLUCONATE

[00874] In this example it is shown that the rate of lithium release from different ointment formulations can be varied by varying the molecular fluidity of the carrier without changing its hydrophobicity. This was accomplished by varying the petrolatum to mineral oil ratio. Petrolatum Formulations 1-4 (Table 15) had mineral oil/ petrolatum in the following ratios: (1) 8.6; (2) 2.85; (3) 0.925; and (4) 0.283. The scale of the formulations was 10 grams. Each formulation had 8% lithium gluconate (2.74 mg Li+/g). Each formulation had 15% lanolin alcohol as an emollient and emulsifier. Lithium gluconate was incorporated into a petrolatum formulation in the suspension form, to hinder release of Li+. Four 20 ml scintillation vials with caps and spin bars were labeled 1-4 and were each charged with 0.800 grams of lithium gluconate and 1.500 grams of lanolin alcohol. This mixture was heated to 80 °C until it melted into a fluid melt that stirred easily. The fluid melt was alternately sonicated and stirred until a homogeneous suspension of lithium gluconate was obtained. The mineral oil and petrolatum components were combined into four more vials ("Petrol. Form 1-4") and heated
to 80 °C. The contents of the vials labeled 1-4 were combined with the contents of the vials labeled Petrol. Form 1-4 and stirred at 80 °C for approximately 15 minutes. The vials were then removed from heat and placed on a roller and rolled at medium speed until the mixture thickened. The formulation was left to equilibrate overnight at 25 °C. The following morning, it was observed that the formulations had gelled into semi-solid fluids. A portion of the formulation was retrieved by a spatula and felt between two fingers for skin feel and "spreading ease." The formulations were smooth to the touch. The formulations were placed on stability. In vitro release experiments were performed in a dissolution chamber set at 32 °C and pH 7.4 to simulate the temperature and pH of a topical wound.

Table 15:

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Lithium Gluconate (g)</th>
<th>Lanolin Alcohol (g)</th>
<th>Petrolatum (g)</th>
<th>Mineral Oil (Drapeol 350)(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petrol. Form 1</td>
<td>0.8</td>
<td>1.5</td>
<td>0.8</td>
<td>6.9</td>
</tr>
<tr>
<td>Petrol. Form 2</td>
<td>0.8</td>
<td>1.5</td>
<td>2.0</td>
<td>5.7</td>
</tr>
<tr>
<td>Petrol. Form 3</td>
<td>0.8</td>
<td>1.5</td>
<td>4.0</td>
<td>3.7</td>
</tr>
<tr>
<td>Petrol. Form 4</td>
<td>0.8</td>
<td>1.5</td>
<td>6.0</td>
<td>1.7</td>
</tr>
</tbody>
</table>

[00875] As shown in Figure 7, greater fluidity of the formulation caused by greater amounts of mineral oil in the formulation led to a higher rate of diffusion of water into the formulation. Thus, the release rate of Li+ from the formulations followed the following trend: Petrol. Form #1>Petrol. Form#2>Petrol. Form#3>Petrol. Form. #4. These formulations were determined to be occlusive, and stable for 3 months at all tested temperatures (4, 25, and 40 °C).

6.8 EXAMPLE 8: PETROLATUM/WATER EMULSIONS WITH LITHIUM GLUCONATE

[00876] This example demonstrates that Li+ release can be modulated by varying the ratio of hydrophobic and hydrophilic components in an emulsion cream formulation. Petrolatum Formulation #2 from Example 7 above was selected as the most hydrophobic formulation, with 8% lithium gluconate, 15% lanolin alcohol and a mineral oil/petrolatum ratio of 2.85. The second formulation (60% hydrophilic/40% hydrophobic) had 60% of a water phase (containing 2% Carbopol 980) emulsified into a 40% petrolatum/mineral oil/lanolin alcohol phase. The lithium gluconate 8% was dissolved into the water phase. The third formulation (100%, hydrophilic) was comprised of a 100%, aqueous gel containing 8% lithium gluconate, 1.5% Carbopol 980, 10% glycerol, and water. In an in vitro release experiment, it was demonstrated that release rates of Li+ from a formulation can be modulated by varying the
hydrophobic/hydrophilic ratio of the carrier (Figure 8). All formulations were stable for 3 months at all temperatures tested (4, 25, and 40 °C). The formulations ranged from non-occlusive (hydrophilic) to semi-occlusive (60/40) to occlusive (hydrophobic).

6.9 EXAMPLE 9: AQUEOUS FORMULATIONS WITH LITHIUM CARBONATE, LITHIUM CITRATE, LITHIUM CHLORIDE, AND LITHIUM SUCCINATE

This example demonstrates that various salt forms of lithium can be utilized to prepare stable, aqueous topical formulations. Aqueous formulations were prepared with the following lithium salts: lithium carbonate, lithium citrate, lithium chloride, and lithium succinate, each with a concentration of 2.74 mg Li+/g gel (see Tables 16-19 below). All formulations contained the lithium salt form of choice, Carbopol 980, propylene glycol, glycerin, distilled water, sodium hydroxide, methyl paraben, and propyl paraben. The lithium salt was first dissolved in an aqueous solution containing Carbopol 980 and propylene glycol at 35 °C. The solutions took 4 hours to completely dissolve into a homogeneous hydrogel. The parabens were first dissolved in glycerin and added to the hydrogel solution. The solutions were cooled down to room temperature and pH-adjusted to pH 6.5-7.5 with 10% sodium hydroxide.

**Table 16. Lithium Carbonate hydrogel, 2.74 mg Li+/g (scale = 12.5 g)**

<table>
<thead>
<tr>
<th>Excipients and Active</th>
<th>Weight in grams</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Carbonate</td>
<td>0.182</td>
<td>1.46</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>0.188</td>
<td>1.50</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.013</td>
<td>0.10</td>
</tr>
<tr>
<td>Propyl Paraben</td>
<td>0.006</td>
<td>0.048</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>9.266</td>
<td>74.13</td>
</tr>
<tr>
<td>10% NaOH</td>
<td>0.970</td>
<td>pH adjust</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>0.625</td>
<td>5</td>
</tr>
<tr>
<td>Glycerine</td>
<td>1.250</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 17. Lithium Citrate hydrogel, 2.74 mg Li+/G (scale = 12.5 g)**

<table>
<thead>
<tr>
<th>Excipients and Active and Active</th>
<th>Citrate Product</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Citrate</td>
<td>0.345</td>
<td>2.76</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>0.188</td>
<td>1.5</td>
</tr>
</tbody>
</table>
Table 18. Lithium Succinate hydrogel, 2.74 mg Li+/G (scale = 12.5 g)

<table>
<thead>
<tr>
<th>Excipients and Active</th>
<th>Succinate Product</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Succinate</td>
<td>0.325</td>
<td>2.6</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>0.188</td>
<td>1.5</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.013</td>
<td>0.1</td>
</tr>
<tr>
<td>Propyl Paraben</td>
<td>0.006</td>
<td>0.050</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>9.123</td>
<td>72.98</td>
</tr>
<tr>
<td>10% NaOH</td>
<td>0.970</td>
<td>pH adjust</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>0.625</td>
<td>5</td>
</tr>
<tr>
<td>Glycerine</td>
<td>1.250</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 19. Lithium Chloride hydrogel, 2.74 mg Li+/g (scale = 250 g)

<table>
<thead>
<tr>
<th>Excipients and Active</th>
<th>Lithium Chloride Product (g)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Chloride</td>
<td>4.200</td>
<td>1.68</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>3.750</td>
<td>1.5</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.250</td>
<td>0.1</td>
</tr>
<tr>
<td>Propyl Paraben</td>
<td>0.000</td>
<td>0</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>184.900</td>
<td>73.96</td>
</tr>
<tr>
<td>10% NaOH</td>
<td>19.400</td>
<td>7.76</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>12.500</td>
<td>5</td>
</tr>
<tr>
<td>Glycerine</td>
<td>25.000</td>
<td>10</td>
</tr>
</tbody>
</table>

[00878] In vitro release experiments were performed at 32°C and pH 7.4 in phosphate buffered saline in a 4-compartment dissolution chamber. One gram of each formulation was loaded in 1.5 ml slide-lyzer cassettes and mounted in each compartment. The cassette containing the formulation was rotated at 5 rotations/minute. 900 mL of phosphate buffered saline was used in the receiving compartment. Cellulose membrane with a molecular weight cutoff of 3500 Daltons (Da) was selected as the membrane for the slide-lyzer cassette. This cutoff was selected to measure the diffusion of ionic, unbound Li+ from the donor compartment into the aqueous receiving compartment. Since Carbopol 980 is an anionic polymer with a high molecular weight (>100KDa), the concentration of Li+ measured in the receiving chamber over time denotes the diffusion kinetics of ionic, unbound Li+ from the
formulation into an aqueous space. Figure 9 shows the comparative cumulative release rates of Li+ from formulations prepared with each salt form. The release profiles among the salt forms were similar, with no statistically significant variation between the groups, possibly due to the fact that all of the lithium salt forms were dissolved and existed in the ionic form.

6.10  EXAMPLE 10: GENERATION AND CHARACTERIZATION OF LITHIUM CARBONATE HYDROGEL FORMULATION A

[00879]  This example describes the generation and characterization of a topical lithium carbonate formulation, referred to herein as "lithium carbonate hydrogel Formulation A."

6.10.1  FORMULATION

[00880]  Lithium carbonate hydrogel Formulation A was formulated with approximately 90% water, using excipients that are classified as GRAS (generally recognized as safe) in the concentration range used in the formulation and for topical use, as per the FDA Inactive Ingredients Database. Different strengths of this gel-based formulation were generated, containing lithium carbonate at concentrations from 0.18% (0.34 mg Li+/g) - 5.66% (10.64 mg Li+/g). The lithium carbonate hydrogel Formulation A contains lithium carbonate (strengths from 0.18%->5.66 % w/w; which corresponds to 0.34 mg Li+/g -10.64 mg Li+/g, respectively), glycerol (10.4%), carboxymethyl cellulose (2% w/w), allantoin (0.16%), sodium alginate (0.12%), methyl paraben (0.1%), propyl paraben (0.048%), water (Q.S.), and NaOH (pH adjusted to 6.5-7.5), and was prepared as follows.

[00881]  Ingredients were obtained from the manufacturers listed in Table 20 below.

<table>
<thead>
<tr>
<th>Excipient/drug</th>
<th>Manufacturer</th>
<th>Lot#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate</td>
<td>FMC Biopolymer</td>
<td>S19626</td>
</tr>
<tr>
<td>Allantoin</td>
<td>Spectrum</td>
<td>YT0711</td>
</tr>
<tr>
<td>Citric acid</td>
<td>Roche</td>
<td>UJ247</td>
</tr>
<tr>
<td>Carboxymethyl cellulose (MW X)</td>
<td>Hercules</td>
<td>91157</td>
</tr>
<tr>
<td>Distilled water</td>
<td>VWR</td>
<td></td>
</tr>
<tr>
<td>Glycerin</td>
<td>Spectrum</td>
<td>TD0414</td>
</tr>
<tr>
<td>Li₂CO₃</td>
<td>FMC Biopolymer</td>
<td>1121</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>Spectrum</td>
<td>TN1071</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>Spectrum</td>
<td>TN1074</td>
</tr>
<tr>
<td>NaOH</td>
<td>Alfa Aesar</td>
<td>F015028</td>
</tr>
<tr>
<td>HCl</td>
<td>Alfa Aesar</td>
<td>C04025</td>
</tr>
</tbody>
</table>
The 0.34 mg Li+/g strength of lithium carbonate hydrogel Formulation A was prepared as follows.

For a 100g batch, 4g citric acid was added to 59.7g of distilled water at room temperature. 0.18g of lithium carbonate was gradually poured in the solution at 25 °C, with vigorous stirring with a stir bar. The stirring was conducted at 25 °C. During the process of dissolution, bubbles of carbon dioxide were generated. The solution was stirred until all the bubbles were dissipated from the system (Solution 1). Next, 0.104g of methyl paraben and 0.049g of propyl paraben were added to Solution 1, with vigorous stirring at room temperature. 0.165g of allantoin, 0.12g of sodium alginate, and 2g of carboxymethyl cellulose were added to the solution and stirred vigorously. The temperature of the solution was raised to 80 °C and stirred. Approximately 4 hours was needed for dissolution. The clear solution was cooled down to room temperature and 10.46g of glycerin was added to the solution. The solution was stirred at room temperature for 2 hours to achieve a homogeneous solution. 23.42g of 10% NaOH solution was added to the solution to adjust the pH to 6.5-7.5.

The 2.74 mg Li+/g strength (1.46% w/w Li₂C₀₃) lithium carbonate hydrogel Formulation A was prepared as follows.

For a 100g batch, 4g citric acid was added to 71.61g of distilled water at room temperature. 1.456 of Lithium Carbonate was gradually poured in the solution at 25 °C, with vigorous stirring with a stir bar. The stirring was conducted at 25 °C. During the process of dissolution, bubbles of carbon dioxide were generated. The solution was stirred until all the bubbles were dissipated from the system (Solution 1). Next, 0.104g of methyl paraben and 0.049g of propyl paraben were added to Solution 1, with vigorous stirring at room temperature. 0.165g of allantoin, 0.12g of sodium alginate and 2g of carboxymethyl cellulose were added to the solution and stirred vigorously. The temperature of the solution was raised to 80 °C and stirred. Approximately 4 hours of dissolution time were needed. The clear solution was cooled down to room temperature and 10.46g of glycerin was added to the solution. The solution was stirred at room temperature for 2 hours to achieve a homogeneous solution. 10.1g of 10% NaOH solution was added to the solution to adjust the pH to 6.5-7.5.

The 5.48 mg Li+/g (2.91% w/w Li₂C₀₃) strength lithium carbonate hydrogel Formulation A was prepared as follows.

For a 100g batch, 4g citric acid was added to 71.61g of distilled water at room temperature. 2.91g of Lithium Carbonate was poured in the solution at 25 °C, with vigorous stirring with a stir bar. The stirring was conducted at 25 °C. During the process of dissolution, bubbles of carbon dioxide were generated. The solution was stirred until all the
bubbles were dissipated from the system (Solution 1). Next, 0.104g of methyl paraben and 0.049g of propyl paraben were added to Solution 1, with vigorous stirring at room temperature. 0.165g of allantoin, 0.12g of sodium alginate, and 2g of carboxymethyl cellulose were added to the solution and stirred vigorously. The temperature of the solution was raised to 80 °C and stirred. Approximately 4 hours of dissolution time were needed. The clear solution was cooled down to room temperature and 10.46g of glycerin was added to the solution. The solution was stirred at room temperature for 2 hours to achieve a homogeneous solution. 1.234g of 10% HCl solution was added to the solution to adjust the pH to 6.5-7.5.

For a 20g batch, 1.93g citric acid was added to 14.43g of distilled water at room temperature. 1.164g of Lithium Carbonate was poured in the solution at 25°C, with vigorous stirring with a stir bar. The stirring was conducted at 25 °C. During the process of dissolution, bubbles of carbon dioxide were generated. The solution was stirred until all the bubbles were dissipated from the system (Solution 1). Next, 0.022g of methyl paraben and 0.011g of propyl paraben were added to Solution 1, with vigorous stirring at room temperature. 0.032g of allantoin, 0.026g of sodium alginate, and 0.4g of carboxymethyl cellulose were added to the solution and stirred vigorously. The temperature of the solution was raised to 80 °C and stirred. Approximately 4 hours of dissolution time were needed. The clear solution was cooled down to room temperature and 2.091g of glycerin was added to the solution. The solution was stirred at room temperature for 2 hours to achieve a homogeneous solution. 0.347g of 10% HCl solution was added to the solution to adjust the pH to 6.5-7.5.

6.10.2 CHARACTERIZATION

Multiple 100g batches of three strengths (0.34 mg Li+/g, 2.74 mg Li+/g, and 5.48 mg Li+/g) were prepared using the process described above. The batches were characterized for the following attributes: lithium content (mg Li+/g); dose uniformity (mg Li+/g), measured by flame photometry; pH (target: 7.0 ± 0.5 °C); viscosity (cP, 25 °C) (target range 6,000-10,000 cP); and appearance (target: transparency). Characterization data is summarized in Table 21.

**Table 21. Batch-to-Batch Characterization**

<table>
<thead>
<tr>
<th>Target (mg Li+/g)</th>
<th>Assay Li+ Content (mg Li+/g)</th>
<th>Dose Uniformity (mg Li+/g) (top, middle, bottom)</th>
<th>pH</th>
<th>Viscosity (cP) (25 °C) (RPM 16-25, 98% torque)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0</td>
<td>0,0,0</td>
<td>6.96</td>
<td>6059</td>
</tr>
</tbody>
</table>
Determination of Li+ Content (mg Li+/g) was as follows. Assay and dosage uniformity of lithium-based dosage forms that are aqueous solutions /gels were expressed as mg Li+/g gel. Assay and dose uniformity of batches of lithium carbonate hydrogel Formulation A were measured utilizing atomic adsorption spectrometry (AAS) method. The operating conditions on SpectrAA-20 plus, range of linearity, specificity, matrix effects, and limit of quantitation (LOQ) of Li+ were determined as follows.

Chemicals

- Lithium atomic absorption standards contained 1000 µg/mL of Li+ ion in 1 weight% HC1, Fluka, Lot#MKBD3873
- Deionized water
- Sodium phosphate, Injection USP, 45mM, single use, HOSPIRA, INC, Lot#45-454-DK

Instruments/equipment

- Varian SpectrAA-20 plus Atomic Adsorption Spectrometer
- Micro-liter pipettes
- Analytical balance

Procedures

Preliminary analytical methods to characterize the Li+ content in the batches were developed using a modification of the atomic adsorption described in USP 26, by Neo-Advent Technologies. Suitable calibration curves with an adequate range of linearity, accuracy and precision were obtained using reference lithium standards, as described in detail below. The formulations were diluted with water to fall within the calibration curve for determination of their Li+ content.

The manual "The Operating Procedure of Varian SpectrAA-20 Plus Atomic Adsorption Spectrometer, was followed. 45mM sodium phosphate was diluted 900 times to yield a concentration of 0.05 mM and pH was adjusted using 10 M NaOH to 7.4, before use.
**Range of Linearity and Calibration Curve**

[00900] A calibration curve, shown in Figure 10 in the range from 0.01 ppm to 0.2 ppm of Li+ prepared in distilled water demonstrates linearity within the range of concentrations measured.

[00901] Of the solutions tested, the solution containing 2 ppm Li+ seemed to be the highest whole number ppm concentration the instrument can accommodate (Figure 11). When 3 ppm standard was measured, the adsorption was more than 1 (the instrument displays an "over range" message). This is also evidenced by a calibration curve obtained from 0.5 ppm to 10 ppm, shown in Figure 12. The response demonstrated a trend to reach a plateau.

[00902] The Varian SpectrAA-20 Plus provides 5 levels of standards when preparing a calibration curve. Considering both the low and high ends of concentration range, a calibration curve, shown in Figure 11 in the range from 0.01 ppm to 2.0 ppm of lithium ion, was selected.

**Limit of Quantitation**

[00903] Based on the calibration curve shown in Figure 11, the limit of quantitation (LOQ) was set at 0.01 ppm of Li.

**Determination of Li+ content in Formulations**

[00904] The lithium ion content in four samples was measured as shown in Table 22. The samples were gels and had label concentrations of Li+ at 2700 ppm. Samples were diluted in two steps for a total of approximately 3000 times to bring the concentration levels within the calibration curve range. Each sample was prepared in duplicate.

**Table 22. Determination of Lithium Ion Content in Lithium Carbonate Hydrogels**

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Lithium Carbonate mg Li+/G</th>
<th>Weight [mg]</th>
<th>Li+ [ppm] Theoretical</th>
<th>Li+ (ppm) Measured (AA)</th>
<th>Measured/theoretical %</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>10.1</td>
<td>0.000</td>
<td>0.01</td>
<td>0.00</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>9.9</td>
<td>0.000</td>
<td>0.01</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>9.8</td>
<td>0.000</td>
<td>0.01</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.34</td>
<td>81.3</td>
<td>0.340</td>
<td>0.341</td>
<td>100.29</td>
<td>100.1</td>
</tr>
<tr>
<td>2</td>
<td>0.34</td>
<td>82.3</td>
<td>0.341</td>
<td>0.34</td>
<td>99.71</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.34</td>
<td>79.9</td>
<td>0.340</td>
<td>0.341</td>
<td>100.29</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.74</td>
<td>10.6</td>
<td>0.279</td>
<td>0.27</td>
<td>96.77</td>
<td>95.3</td>
</tr>
<tr>
<td>2</td>
<td>2.74</td>
<td>10.4</td>
<td>0.284</td>
<td>0.27</td>
<td>94.97</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.74</td>
<td>10.5</td>
<td>0.287</td>
<td>0.27</td>
<td>94.07</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5.48</td>
<td>10.5</td>
<td>0.574</td>
<td>0.57</td>
<td>99.23</td>
<td>97.4</td>
</tr>
</tbody>
</table>
Assay (mg Li+/g) was calculated using the equation generated by the calibration curve using five Li+ standards.

**Measurement of Dose Uniformity**

Dose uniformity was measured by retrieving 10 mg of gel from the top, middle and bottom of the vial containing the formulation. The gel was placed in a 100 ml volumetric flask and filled to mark with distilled water, to generate samples containing Li+ in the measurable range of 0-2 ppm.

**Measurement of Viscosity**

The shear viscosities of the formulations were measured using a Brookefield DV-III Ultra Rheometer IV, using the spindle X. Silicone oil, (12,400 cP at 25 C) was used as the standard. Samples were run at 25 C, with maximum torque (> 98%).

The viscosity of the formulations at 25 °C was measured as between 2,000-8,000 centipoise (cP). Stability studies on 100 gram batches conducted under ICH temperature conditions of 4 °C, 25 °C, and 40 °C were stable at 8 weeks with respect to strength, viscosity and homogeneity.

Three 2.5L batches of each of the three strengths (0.34 mg Li+/g, 2.74 mg Li+/g, and 5.48 mg Li+/g) were also prepared, using the process described above, with scaled-up measures of all ingredients, as set forth in Table 23.

**Table 23.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>0</th>
<th>0.34 mg Li+/g</th>
<th>2.74 mg Li+/g</th>
<th>5.48 mg Li+/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Carbonate (g)</td>
<td>0</td>
<td>4.5</td>
<td>36.399</td>
<td>72.801</td>
</tr>
<tr>
<td>Citric Acid (g)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Sodium Alginate (g)</td>
<td>3.007</td>
<td>2.999</td>
<td>3.00</td>
<td>2.999</td>
</tr>
<tr>
<td>Carboxymethyl Cellulose Na salt (g)</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Allantoin (g)</td>
<td>4.00</td>
<td>4.0</td>
<td>3.99</td>
<td>4.002</td>
</tr>
<tr>
<td>Glycerin (g)</td>
<td>260</td>
<td>260</td>
<td>260</td>
<td>260</td>
</tr>
<tr>
<td>Methyl paraben (g)</td>
<td>2.599</td>
<td>2.599</td>
<td>2.602</td>
<td>2.599</td>
</tr>
<tr>
<td>Propyl paraben (g)</td>
<td>1.199</td>
<td>1.205</td>
<td>1.2</td>
<td>1.199</td>
</tr>
<tr>
<td>Deionized Water (g)</td>
<td>1448</td>
<td>1490</td>
<td>1790</td>
<td>1976</td>
</tr>
<tr>
<td>Sodium Hydroxide 10%</td>
<td>629.6</td>
<td>581.9</td>
<td>250.26</td>
<td></td>
</tr>
<tr>
<td>Hydrochloric Acid 10%</td>
<td>N/A*</td>
<td>N/A</td>
<td>N/A</td>
<td>27.3</td>
</tr>
</tbody>
</table>

*N/A = not applicable

The attributes of the scaled-up batches are set forth in Table 24.
Table 24. Characterization of Scaled-Up Batches

<table>
<thead>
<tr>
<th>Lot #</th>
<th>Target Li+/g (mg)</th>
<th>Assay Li+ Content (mg Li+/g)</th>
<th>Dose Uniformity (mg Li+/g)</th>
<th>pH</th>
<th>Viscosity (cP) (25 °C) 98% torque</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH-002-080A</td>
<td>0.00</td>
<td>0</td>
<td>0.0, 0.0</td>
<td>6.99</td>
<td>2859</td>
</tr>
<tr>
<td>TH-002-080B</td>
<td>0.34</td>
<td>0.30</td>
<td>0.30, 0.40, 0.40</td>
<td>7.34</td>
<td>3586</td>
</tr>
<tr>
<td>TH-002-080C</td>
<td>2.74</td>
<td>3.07±</td>
<td>3.3, 2.9, 3.0</td>
<td>7.15</td>
<td>7528</td>
</tr>
<tr>
<td>TH-002-080D</td>
<td>5.48</td>
<td>5.17</td>
<td>5.7, 5.2, 4.6</td>
<td>6.98</td>
<td>2046</td>
</tr>
</tbody>
</table>

Other properties

[00911] Lithium carbonate hydrogel Formulation A is a transparent, odorless, colorless formulation that readily spreads over a surface. The migration to surrounding sites and run off was minimal. The pH of the gel was adjusted to neutral (7.0 ± 0.5 °C) for skin and wound compatibility. Lithium carbonate hydrogel Formulation A may be stored at room temperature.

In Vitro Release

[00912] In vitro release experiments to determine the release of Li+ from lithium carbonate hydrogel Formulation A at various strengths were performed. As a comparison, the in vitro release rate of Li+ from lithium gluconate 8% gel (2.74 mg Li+/g) was also determined.

[00913] The experiments were performed in a dissolution chamber (Franz cell with a cellulose membrane), with 900 mL of distilled water in each of the dissolution buckets, maintained at 32 °C. 5 mL of the gel formulations were loaded in 15mL slide-lyzer cassettes (manufacturer). The drug-loaded cassettes were mounted on the rotating mandrell that rotated the cassettes at a rpm of 5.

[00914] Figure 13 shows the cumulative percent Li+ released over time in hours. The lithium carbonate hydrogel Formulation A at 2.74 mg Li+/g and 5.48 mg Li+/g had equivalent release kinetics of Li+, which were comparable to ionized lithium released from 8% lithium gluconate, and demonstrated an in vitro release profile that releases 100% of incorporated Li+ within 12 hours. The lowest dose concentration, at 0.34 mg Li+/g, demonstrated a slower rate of release, possibly due to a lower concentration gradient compared to the other groups.
6.11  **EXAMPLE 11: PHARMACOKINETIC PROFILES OF TOPICAL LITHIUM CARBONATE HYDROGEL OBTAINED IN A MOUSE DERMABRASION MODEL.**

[00915] In this example, pharmacokinetics studies with different concentrations of lithium carbonate hydrogel Formulation A were undertaken in a mouse dermabrasion wounding model. The study design is shown in Figure 14.

6.11.1 **STUDY PROTOCOL**

[00916] 86 C57BL6/J female, non-pregnant and nulliparous mice were selected for use in the study (purchased from Jackson Laboratories, Bar Harbor, ME). The weight range of the mice was 17.4 - 21.9 grams (weighed to the nearest 0.1 g), with an age-range of 9 weeks + 5 days old. The mice were healthy, and not previously used in any experimental procedures. The mice were allowed to acclimate for a minimum of 5 days, under the same conditions as for the actual study, as follows:

- Animal Room Target Temperature: 68 ± 5 °F
- Animal Room Target Relative Humidity: 30-70%
- Air Exchanges per Hour: a minimum of 10 changes per hour
- Lights: 12-hour light/dark cycle, full spectrum fluorescent lights
- Housing: Group housed
- Cages: polycarbonate
- Bedding: PWI Industries, St-Hyacinthe, Quebec, Canada (contact)
- Animal Rations: TEK 7012 Rodent Diet, Harlan Laboratories, Madison, WI, ad libitum
- Water: tap water, ad libitum

[00917] There were no known contaminants present in the feed, water, or bedding expected to interfere with the test data. The laboratory and animal rooms were maintained as limited access facilities.

[00918] The study was designed with the guidance of the following references: OECD 407, Organization for Economic Co-operation and Development (OECD), Guideline for the Testing of Chemicals, "Acute Oral Toxicity- Acute Class Method", as modified for repeat dermal administration to mice, adopted 17 December 2001; and ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories.

[00919] No evidence of pain and distress to the animals was reported to the Veterinarian and/or Study Director during the course of the study. The following standards were adhered to, where applicable, in maintaining the animal care and use program: United States

[00920] Animals were assigned to the study and distributed into groups. There were 20 female animals assigned to each dose group, i.e., for dermal administration of either the 0.343, 2.74, or 5.48 mg Li+/g lithium carbonate hydrogel Formulation A (test article groups) or 2.74 mg Li+/g of Lithioderm™ (comparator group). See Table 25.

Table 25. Group Assignments

<table>
<thead>
<tr>
<th>Group</th>
<th>Test article</th>
<th>Number of Animals/Sex</th>
<th>Dose Volume (mL)</th>
<th>Dose Frequency</th>
<th>Formulation Concentration (mg Li/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 Low-dose</td>
<td>Lithium carbonate</td>
<td>20 F</td>
<td>0.1 mL</td>
<td>Twice per day, at least 4 hours apart</td>
<td>0.343</td>
</tr>
<tr>
<td>Group 2 Mid-dose</td>
<td>Lithium carbonate</td>
<td>20 F</td>
<td></td>
<td></td>
<td>2.74</td>
</tr>
<tr>
<td>Group 3 High- dose</td>
<td>Lithium carbonate</td>
<td>20 F</td>
<td></td>
<td></td>
<td>5.48</td>
</tr>
<tr>
<td>Group 4 Comparator</td>
<td>Lithioderm®</td>
<td>20 F</td>
<td></td>
<td></td>
<td>2.74</td>
</tr>
</tbody>
</table>

[00921] Dermabrasion (DA) was performed on all animals on the first dosing day as follows.

[00922] 1. Mice were weighed and treated with buprenorphine (single IP injection, 0.05 mg/kg, 50 µL per 20 g mouse using a dosing solution of 0.02 mg/ml) 60 minutes before the procedure, on the day following DA (two doses, ~ 8 hr apart), and as needed the second day after DA.

[00923] 2. After one hour has passed mice were anesthetized with ketamine (80 mg/kg)/xylazine (8 mg/kg). Mice were given an eye ointment to keep their eyes from drying out during their immobilization.

[00924] 3. Once the mice ceased being mobile, both the left and right sides of the dorsal rear back skin were clipped, and Nair was applied for 1 min to right and left flank, hair wiped off with wet paper towel, and dried with paper towel.
4. The mice were dermabraded once they are out to toe-pinch. Dermabrasion (DA) was carried out using a microdermabrasion device. The machine settings (Advanced Microderm, DX model) were set to max vacuum, large tip, and max mixture. Aluminum oxide crystals were used for the DA. Ten passes on the right dorsal rear flank were carried out (each pass was a single movement from cranial to caudal direction; skin was held taught). The left side was not dermabraded.

5. After DA, the 4 corners and the mid points along the edge of the wound were tattooed with an injection of India ink (using a tuberculin syringe). There were 8 total tattoo marks made.

6. The mice were then placed back into their respective cages which were pre-warmed on low-heat heating pads prior to surgery and kept on heating pads (under cage) until they woke up.

7. Upon waking, mice were dosed with a treatment according to the label on their cage. Treatment groups and dosage method were pre-determined before the date of the actual DA.

After DA, the test article or the comparator was placed into the wound, using a capillary microdispenser (wiretrol), and spread over the wound. The first day of dosing for each animal was designated as Day 1. Three concentrations of lithium carbonate hydrogel Formulation A (0.34 mg Li+/G (0.18% w/w Li₂C₅O₃); 2.74 mg Li+/G (1.46% w/w Li₂C₅O₃) and 5.48 mg Li+/G (2.91% w/w Li₂C₅O₃)) were administered at a volume of 0.1 mL twice daily to the wound site for 7 consecutive days immediately after wounding by DA. Lithium gluconate 8% gel (2.74 mg Li+/g, Lithioderm®) was enrolled as a comparator group.

The wound sites were loosely covered by a tent-like bandage created to protect the wound, prevent occlusion and allow ease of dosing. Body weights were recorded daily for all animals and each animal was observed daily for clinical signs of toxicity. Weight variation of the mice was within normal daily variation and no clinical symptoms of toxicity were observed in mice in any of the groups. Clinical observations included, but were not limited to, changes in the skin, fur, eyes, and mucous membranes, respiratory system, circulatory system, autonomic central nervous system, somatomotor activity, locomotor activity, hypersensitivity and behavior pattern. Particular attention was given to local clinical signs at the administration site. The animals were also separately observed for moribundity/mortality daily, at least three hours apart from the clinical observation.

Two mice were sacrificed at each time point to collect skin (wound sites) and blood. Blood samples (approximately 0.2 mL each) from all animals were collected via
cheek puncture. If blood collection by cheek puncture was not possible, it was collected from the abdominal vena cava (terminal blood collection). Blood was collected in tubes containing K2EDTA as the anticoagulant. The blood was not centrifuged. Tubes were placed on dry ice immediately following collection. Pharmacokinetic blood collection was performed on Days 1, 2, 3, and 8 as described in Table 26. The wound area was harvested from all animals at the same time points indicated for blood collection. The wound sites were "cleaned" off with wet alcohol gauze before sacrifice, to remove residual drug on the surface. The wound sites were collected into Eppendorf microcentrifuge tubes. Tubes were placed on dry ice immediately following collection. Blood and tissue samples were stored at -80 ± 12 °C. The wound sites were homogenized and processed for analysis of Li+ by a validated ICP-MS method.

Table 26. Pharmacokinetic Group Assignment - Blood and Tissue Collection

<table>
<thead>
<tr>
<th>Group Number</th>
<th>Pre-dose</th>
<th>1 h (after 1st dose)</th>
<th>4 h (before 2nd dose)</th>
<th>24 h (before 3rd dose)</th>
<th>25 h (one hour after 3rd dose)</th>
<th>28-30 h (before 4th dose)</th>
<th>48 h (before 5th dose)</th>
<th>49 h (one hour after 5th dose)</th>
<th>52-54 h (before 6th dose)</th>
<th>24 h after last dose on Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Group 2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Group 3</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Group 4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

[00932] All animals survived the course of the study. The only abnormal clinical sign observed was lethargy. Lethargy was observed in animals G3 and G4 (mid-dose) and L4 (high-dose) on Day 2. These animals were assigned to the 24 hour time point. No other adverse clinical signs were noted during the course of the study.

6.11.2 RESULTS

[00933] In the current study, lithium carbonate hydrogel Formulation A was administered twice daily for 7 days. Skin and Blood samples were collected at the 8th day (or -18 hours after the last dose) and analyzed for Li+ content by ICP-MS. Li+ concentrations in skin and blood at this time-point were comparable to "trough" Li+ levels obtained for other earlier
time points. See Figure 15 and Figure 16, which show the pharmacokinetic profiles of Li+ concentrations in skin and blood.

[00934] As shown in Figure 15, skin concentrations increased with increasing dose; the Cmax was achieved between 0-1 hour after dosing; the kinetic profile for lithium carbonate concentration in skin is similar to that of lithium gluconate; and rapid clearance from the site of delivery was observed, i.e., Li+ does not build up in skin (steady state = 0.33 mg Li+/g after 6 days administration, twice/day). The peak, or "Cmax" values for skin for lithium carbonate were in the following ranges: 0.96-1.18 mM Li+ (lowest dose, 0.34 mg Li+/g); 3.44-6.78 mM Li+ (mid dose, 2.74 mg Li+/g); and 6-8.95 mM Li+ (highest dose, 5.48 mg Li+/g), as shown in Figure 17. Cmax levels decreased with each progressive day of dosing as the wound healed, and were Cmax approximately dose-responsive.

[00935] Peak or "Cmax" values for blood for lithium carbonate were in the following ranges: 0.06 - 0.12 mM Li+ (lowest dose, 0.34 mg Li+/g); 0.39-0.6 mM Li+ (mid dose, 2.74 mg Li+/g); and 0.85-0.89 mM Li+ (highest dose, 5.48 mg Li+/g) (Figure 18). As shown in Figure 18, Cmax levels at all doses were below levels of systemic toxicity, which may be important since the drug formulation is administered to wounded skin immediately after wounding.

[00936] Figure 19 shows a comparison of blood and skin levels for lithium carbonate and lithium gluconate (comparator group in this study) at the same concentration (2.74 mg Li+/g); skin and blood levels of Li+ from the two lithium salts were, generally, in agreement (comparable Cmax levels; comparable trough levels; comparable kinetics of clearance from blood and skin compartments).

[00937] Lithium concentrations in skin were higher than Li+ concentrations in blood at all dose levels. Without being bound by theory, it is postulated that the desired higher concentrations of Li+ at the targeted skin site with relatively low systemic levels is due to an ionic interaction of the positively charged lithium ions with the negatively charged polymeric excipient, Carbomer 50 000 (Carbopol 980), contained in the formulation used (manufactured according to Good Laboratory Practice [GLP]) standards. The rate of Li+ clearance from both skin and blood was rapid, with Li+ concentrations at 4 h post dosing half those at 1 h post-dosing. Lithium did not build up at the site of administration after repeated dosing. Without being bound by theory, this may be due to the rapid clearance kinetics of Li+ from both skin and blood.
6.12 EXAMPLE 12: LI+ CREAM FORMULATIONS FOR SUSTAINED RELEASE DELIVERY

[00938] This example reports the generation of lithium carbonate cream formulations for sustained release delivery.

[00939] Formulations containing at least 3.38 mg/g Li+ (1.80% w/w Li+ lithium carbonate) as the salt form, were generated. The formulations described in this example are all oil/water emulsions. Three types of formulations with the following Li+ release rates were generated by varying the formulation excipients: Immediate Release (< 1 day); Intermediate Release (1-3 days); and Sustained Release (3-7 days).

[00940] The formulations were characterized for Li+ content using flame photometry, dissolution release and preliminary stability.

6.12.1 IMMEDIATE RELEASE FORMULATIONS

[00941] Immediate release formulations were generated using a two-phase system: one aqueous phase for dissolving lithium carbonate and hydrophilic excipients and another non-aqueous phase for dissolving hydrophobic polymers.

[00942] Formulation preparation method. Lithium carbonate for the immediate release formulations was obtained from Riedel-deHaen, lot # 670740. Citric acid from KIC Chemicals (lot # 200203) was used as a solubilizing agent and buffering agent. Carbopol from Lubrizol (lot# 100655645) was used as a thickener for the aqueous phase. For the non-aqueous phase, a mixture of two silicon oils from Dow Corning Chemicals (Silicone oil 350 cts, lot # 6080660, and silicone oil 12500 cts, lot # 6019987, were mixed at a ratio 25:75, referred to herein as "S25") was used as a medium-viscosity carrier, and cetearyl alcohol (Croda Lot#334447) and lanolin alcohol (Croda, lot#2691 13) were used as thickeners/emulsifiers. Span 80 (Sigma, lot# 114k0137) and tween 20 (Spectrum Chem., lot# TO0434) were used as surfactants for the non-aqueous and aqueous phases, respectively. Emulsifier 10 from Dow Corning (lot# 5864667) was used as an additional emulsifier for the formulations. See Table 27.

Table 27. Excipients/drug used in a lithium carbonate immediate release formulation (designated as lot #TH-00 1-081)

<table>
<thead>
<tr>
<th>Excipients/drug</th>
<th>Manufacturer</th>
<th>Lot#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brij 721</td>
<td>Uniqema</td>
<td>52022</td>
</tr>
<tr>
<td>Carbopol</td>
<td>Lubrizol</td>
<td>1.01E+08</td>
</tr>
<tr>
<td>Citric acid</td>
<td>KIC chem</td>
<td>200203</td>
</tr>
</tbody>
</table>
The immediate release formulation was prepared using three steps, as follows:

i) Aqueous phase (phase I, 24G): 0.72g of lithium carbonate was gradually added into a solution containing 1.2 g of citric acid and 21.3g of deionized water. The mixture was vortexed and ultrasonicated until all of the lithium carbonate went into solution. 0.72g of Carbopol and 0.048g of Tween 20 were added into the solution. The resultant system under vigorous stirring was heated up to 90°C in a water bath until the Carbopol was fully swollen and dispersed into water, to yield a single liquid phase of translucent appearance.

ii) Non-aqueous phase (phase II, 16G): 1.6g of cetearyl alcohol and 3.2g of lanolin alcohol were added into a silicone oil mixture (S25, Silicone oil 350 cts, lot # 6080660, and silicone oil 12500 cts, lot # 6019987, were mixed at a ratio 25:75). After addition of 0.032g of span 80 and 0.32g of emulsifier 10, the mixture was heated up to 90°C in a water bath until all ingredients were dissolved, resulting in a clear, single phase solution (while still hot).

c) Homogenization: All 16 g of phase II (while still hot) was added into 24 g of Phase I and the two phases were mixed together using a high speed homogenizer to obtain a single phase cream. The ratio of aqueous (Phase I) to non-aqueous (Phase II) was 60:40.

Immediate release formulation ("lot # Th-00 1-081") was generated according to these methods, as summarized in Table 28. The aqueous phase, non-aqueous phase, and the final phase of the mixture are shown in the table, and the amount of the excipients needed in each phase are listed for a 40g batch formulation.
The Li+ content in the immediate release formulation was measured as follows.

**Sample preparation.** About 10 mg samples were taken from the top, medium, and bottom of the container (named "1," "2," and "3," respectively) and then mixed with ~2-3 ml DMSO until there were no particles in the solution, ultrasonicated for about 3 min, and then diluted with water into a 100 ml flask.

**Standards and matrix effect.** Lithium ion concentration was determined with atomic spectrum instrument (AA 20+). Five lithium ion standard solutions were used for
calibration purposes and the influence of sodium ion on the adsorption of lithium ion was also evaluated. It was determined that, in the cream formulation methods described in this example, sodium ions do not pose a large enough impact to affect the accuracy of the lithium ion determination.

[00951] The standard calibration curves are shown in Figure 20. For demonstrating sodium ion suppression effect, a placebo formulation was prepared and two series of lithium ion standard solutions, one in water and another in placebo solution, were prepared and lithium ion concentrations measured on the basis of these two standards were plotted together in Figure 21. Figure 21 demonstrates that the suppression effect exists, but is insignificant at the concentrations used.

[00952] **Determination of Li+ release rates.** About 3.3235 g cream was loaded into a slide-A-lyzer cassette (Thermo Scientific, Lot# LD144491, membrane molecular weight cutoff 3500). 900ml water of 5 mM sodium phosphate buffer was used as the receiving medium. 200 µL samples were retrieved at different time points. The samples were diluted with water in a 10 ml flask for absorption measurement using the atomic absorption instrument, AA 20+. The time points are shown in Table 29 and the measured release profile plotted as the Li+ relative percentage vs. release time for the cream Lot# TH-001-081 is shown in Figure 22. Figure 22 shows that this cream releases Li+ to more than 90% within 10 hours.

**Table 29. Time points for experiment to assess release of Li+ from immediate formulation lot # TH-001-081**

<table>
<thead>
<tr>
<th>Day</th>
<th>Time (in hours)</th>
<th>% Cumulative Li+ Release</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 DAY PERIOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>40.00</td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>56.00</td>
<td></td>
</tr>
<tr>
<td>3.0</td>
<td>64.00</td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>80.00</td>
<td></td>
</tr>
<tr>
<td>6.0</td>
<td>96.00</td>
<td></td>
</tr>
<tr>
<td>25.0</td>
<td>96.00</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>96.00</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>104.00</td>
<td></td>
</tr>
</tbody>
</table>

[00953] **Results and discussion.** The immediate release formulation described above releases ~100% of Li+ content within 10 hours. The formulation has an emollient "smooth-feel" texture, and can be easily applied to skin.
6.12.2. INTERMEDIATE RELEASE FORMULATIONS

[00954] An intermediate release emulsion cream formulation of lithium carbonate was developed. The emulsion was prepared by homogenization of two phases, as described above for the immediate release formulation.

[00955] **Formulation preparation method.** The raw materials used in this formulation were identical to those used in the immediate release formulation described above, except that mineral oil instead of silicone oil was used. The lot #s of the excipients for this intermediate release formulation are listed in Table 30 below.

Table 30. Excipients/drug used in intermediate release formulation "Lot# TH-001-084b"

<table>
<thead>
<tr>
<th>Excipients/drug</th>
<th>Manufacturer</th>
<th>Lot#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetearyl alcohol</td>
<td>Croda</td>
<td>334447</td>
</tr>
<tr>
<td>Carbopol</td>
<td>Lubrizol</td>
<td>100655645</td>
</tr>
<tr>
<td>Citric acid</td>
<td>KIC chem</td>
<td>200203</td>
</tr>
<tr>
<td>Drakeol 350 Mineral oil</td>
<td>Penreco</td>
<td>K8061</td>
</tr>
<tr>
<td>Emulsifier 10</td>
<td>Dow Corning</td>
<td>5864667</td>
</tr>
<tr>
<td>Lanolin alcohol</td>
<td>Croda</td>
<td>269113</td>
</tr>
<tr>
<td>Leцитhin</td>
<td>Spectrum Chemicals</td>
<td>UK0763</td>
</tr>
<tr>
<td>Lithium carbonate</td>
<td>Riedel-deHaen</td>
<td>670740</td>
</tr>
<tr>
<td>Span 80</td>
<td>Sigma</td>
<td>114k0137</td>
</tr>
<tr>
<td>Tween 20</td>
<td>Spectrum Chemicals</td>
<td>T00434</td>
</tr>
</tbody>
</table>

[00956] For a 40g batch, the following preparation method was used.

[00957] i) **Aqueous phase (Phase I, 24G):** 0.72 g of lithium carbonate was gradually added into a solution containing 1.2 g of citric acid and 21.32 g of water. The mixture was vortexed and ultrasonicated until all of the ingredients were dissolved. 0.72 g of Carbopol 980 and 0.05 g of Tween 20 were then added into above solution. The resultant system under vigorous stirring was heated up to 90°C in a water bath until the Carbopol was fully swollen and dispersed into water. Phase I is a single liquid phase of translucent appearance.

[00958] ii) **Non-aqueous phase (Phase II, 16G):** 3.23 g of cetearyl alcohol and 2.59 g of lanolin alcohol were added into 9.89 g of Drakeol 350 mineral oil. After 0.04 g of span 80 and 0.33 g of emulsifier 10 was added into the above system, the whole mixture was heated up to 90 °C in a water bath until all polymers were dissolved. Phase II is a clear single phase solution.

[00959] ii) **Homogenization:** The phases were mixed together with homogenization. The ratio of aqueous to non-aqueous is 60:40.
An example of intermediate release formulation with lot # Th-001-084b is shown in Table 31, with detailed amounts of excipients at each phase formulation.

**Table 31. Intermediate release formulation with lot # Th-001-084-b**

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Phase 1</th>
<th>Total wt for 24g</th>
<th>Actual amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg/g</td>
<td>(g)</td>
</tr>
<tr>
<td>Lithium Carbonate</td>
<td>3</td>
<td>30</td>
<td>0.72</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>5</td>
<td>50</td>
<td>1.2</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>3</td>
<td>30</td>
<td>0.72</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.2</td>
<td>2</td>
<td>0.048</td>
</tr>
<tr>
<td>10% NaOH</td>
<td>use to neutralize to 6.5</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>88.8</td>
<td>888</td>
<td>21.312</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 2</th>
<th>Phase II (#24)</th>
<th>16 g batch</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetearyl alcohol</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>Drakeol 350 Mineral Oil</td>
<td>61.8</td>
<td>618</td>
</tr>
<tr>
<td>Span 80</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>Lanolin alcohol</td>
<td>16</td>
<td>160</td>
</tr>
<tr>
<td>Emulsifier 10</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 3</th>
<th>Mix &amp; Homogenize</th>
</tr>
</thead>
</table>

**Final Composition**

<table>
<thead>
<tr>
<th></th>
<th>%</th>
<th>mg/g</th>
<th>(g)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Carbonate</td>
<td>1.8</td>
<td>18</td>
<td>0.72</td>
<td>1.80</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>3</td>
<td>30</td>
<td>1.20</td>
<td>2.99</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>1.8</td>
<td>18</td>
<td>0.72</td>
<td>1.80</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.12</td>
<td>1.2</td>
<td>0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>53.28</td>
<td>532.8</td>
<td>21.32</td>
<td>53.16</td>
</tr>
<tr>
<td>Cetearyl alcohol</td>
<td>8</td>
<td>80</td>
<td>3.23</td>
<td>8.05</td>
</tr>
<tr>
<td>D350 Mineral Oil</td>
<td>24.72</td>
<td>247.2</td>
<td>9.89</td>
<td>24.66</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Span 80</td>
<td>0.08</td>
<td>0.8</td>
<td>0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>Lanolin alcohol</td>
<td>6.4</td>
<td>64</td>
<td>2.59</td>
<td>6.47</td>
</tr>
<tr>
<td>Emulsifier 10</td>
<td>0.8</td>
<td>8</td>
<td>0.32</td>
<td>0.82</td>
</tr>
<tr>
<td>total</td>
<td>40.10</td>
<td>100.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Measurement of Li+ content.** The same method used for measuring Li+ in the immediate release formulation above was used for this intermediate release formulation. To determine the Li+ release profile, the cream was assayed using the following method. About 10 mg samples were taken from the top, medium, and bottom of the container and then mixed with ~2-3 ml DMSO until there were no particles in the solution, ultrasonicated for about 3 min, and then diluted with water into a 100 ml flask. The obtained solution was measured with AA 20+ for Li+. The assay result for Lot# Th-001-084b is provided in Table 32 below, with sample notation as "1," "2," and "3," respectively.

**Table 32.** Samples used for assaying Li+ for intermediate release formulation lot # TH-001-084

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Weight (mg)</th>
<th>Li+ (ppm) Theoretical</th>
<th>Li+ (ppm) Measured (AA)</th>
<th>Measured/theoretical %</th>
<th>Average %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH-001-084b1</td>
<td>10.2</td>
<td>0.345168</td>
<td>0.34</td>
<td>98.50</td>
<td>92.88</td>
</tr>
<tr>
<td>TH-001-084b2</td>
<td>10.4</td>
<td>0.351936</td>
<td>0.32</td>
<td>90.93</td>
<td></td>
</tr>
<tr>
<td>TH-001-084b3</td>
<td>10.6</td>
<td>0.358704</td>
<td>0.32</td>
<td>89.21</td>
<td></td>
</tr>
</tbody>
</table>

**Determination of Li+ release rates.** A 4.7747 gram sample was injected into a slide-A-lyzer cassette (thermo Scientific, lot# LD 144491, membrane molecular weight cutoff 3500). 900 mL of 5 mM phosphate buffer, pH 7.4, was used as a medium for dissolution. 200 μL samples were taken by micropipette at various time points; the original data are shown in Table 33 below. The collected samples were diluted with water in a 10 ml flask for absorption measurement using the atomic absorption instrument, AA 20+. The measured release profile for the cream Lot# TH-001-084b is shown in Figure 23.
Table 33. Time points for experiment to assess release of Li+ from intermediate release formulation lot # TH-001-084b

<table>
<thead>
<tr>
<th>Day</th>
<th>Time in Hours</th>
<th>% Cumulative Rel of Li+ (assayed)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 DAY PERIOD</td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>29.99</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td>44.99</td>
</tr>
<tr>
<td>3.0</td>
<td></td>
<td>53.98</td>
</tr>
<tr>
<td>5.0</td>
<td></td>
<td>68.98</td>
</tr>
<tr>
<td>6.0</td>
<td></td>
<td>71.98</td>
</tr>
<tr>
<td>8.0</td>
<td></td>
<td>80.97</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>89.97</td>
</tr>
<tr>
<td>28</td>
<td></td>
<td>89.97</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>89.97</td>
</tr>
<tr>
<td>48</td>
<td></td>
<td>92.97</td>
</tr>
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<td>96</td>
<td></td>
<td>104.97</td>
</tr>
<tr>
<td>110</td>
<td></td>
<td>101.97</td>
</tr>
<tr>
<td>118</td>
<td></td>
<td>104.97</td>
</tr>
</tbody>
</table>

6.12.3 SUSTAINED RELEASE FORMULATIONS

[00964] The sustained release formulations were expected to release 90-100% of Li+ from 3-7 days. As for the immediate and intermediate release formulations described above, two phases, the first an aqueous phase for dissolving lithium carbonate and the second a non-aqueous phase for dissolving hydrophobic polymers, was used for preparing the formulation. Those two phases were prepared individually and mixed together using a homogenizer.

[00965] Formulation preparation method. The raw materials used in this sustained release formulation are the same as those used in the intermediate release formulation described above. Materials used in the generation of sustained release formulation Lot# TH-001-084a are the same as those listed in Table 30 above.

[00966] The following three-step procedure was followed in order to prepare a 40 g batch of the sustained release formulation:
[00967] i) Aqueous phase (phase I): 0.72 g of lithium carbonate was gradually added into a solution containing 1.2 g of citric acid and 21.32 g of water. The mixture was vortexed and ultrasonicated until all lithium carbonate powder went into solution. 0.72 g of carbopol 980 and 0.05 g of tween 20 were then added into above solution. The resultant system under vigorous stirring is heated up to 90 °C in a water bath until the Carbopol was fully swollen and dispersed into water. The obtained system was a single liquid phase of translucent appearance.

[00968] ii) Non-aqueous phase (phase II): 1.61 g of cetearyl alcohol and 2.59 g of lanolin alcohol were added into 11.89 g of Drakeol 350 mineral oil. After 0.03 g of span 80 and 0.33 g of emulsifier 10 was added into the above system, the whole mixture was heated up to 90 °C in a water bath until all polymers were dissolved. The obtained system was a clear single phase solution.

[00969] iii) Homogenization: 16 g of phase II was added into 24 g of Phase I, which were mixed together using a high speed homogenizer for obtaining a single phase cream. The ratio of aqueous to non-aqueous is 60:40.

[00970] The steps used for generation of sustained release formulation lot # TH-001-084a is are summarized in Table 34, including the amounts of excipients in each phase.

Table 34. Sustained release formulation "lot # TH-001-084a"

<table>
<thead>
<tr>
<th>Step 1</th>
<th>Phase 1</th>
<th>Total wt for 24G</th>
<th>Actual amount</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg/g</td>
<td>(g)</td>
</tr>
<tr>
<td>Lithium Carbonate</td>
<td>3</td>
<td>30</td>
<td>0.72</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>5</td>
<td>50</td>
<td>1.2</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>3</td>
<td>30</td>
<td>0.72</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.2</td>
<td>2</td>
<td>0.048</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>88.8</td>
<td>888</td>
<td>21.312</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Step 2</th>
<th>Phase II</th>
<th>16 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecithin</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>D350 Mineral Oil:Silicone 350</td>
<td>67.8</td>
<td>678</td>
</tr>
<tr>
<td>Span 80</td>
<td>0.2</td>
<td>2</td>
</tr>
<tr>
<td>Lanolin alcohol</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>Emulsifier 10</td>
<td>2</td>
<td>20</td>
</tr>
</tbody>
</table>

<p>| Step 3 | Mix &amp; |</p>
<table>
<thead>
<tr>
<th>Homogenized Composition</th>
<th>m</th>
<th>t 1/1</th>
<th>(g)</th>
<th>m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium Carbonate</td>
<td>1.8</td>
<td>18</td>
<td>0.72</td>
<td>1.80</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>3</td>
<td>30</td>
<td>1.20</td>
<td>2.99</td>
</tr>
<tr>
<td>Carbopol 980</td>
<td>1.8</td>
<td>18</td>
<td>0.72</td>
<td>1.80</td>
</tr>
<tr>
<td>Tween 20</td>
<td>0.12</td>
<td>1.2</td>
<td>0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>Deionized Water</td>
<td>53.28</td>
<td>532.8</td>
<td>21.32</td>
<td>53.05</td>
</tr>
<tr>
<td>Lecithin</td>
<td>4</td>
<td>40</td>
<td>1.60</td>
<td>3.98</td>
</tr>
<tr>
<td>D350 Mineral Oil Silicone 350</td>
<td>27.12</td>
<td>271.2</td>
<td>10.92</td>
<td>27.15</td>
</tr>
<tr>
<td>Span 80</td>
<td>0.08</td>
<td>0.8</td>
<td>0.04</td>
<td>0.11</td>
</tr>
<tr>
<td>Lanolin Alcohol</td>
<td>8</td>
<td>80</td>
<td>3.28</td>
<td>8.16</td>
</tr>
<tr>
<td>Emulsifier 10</td>
<td>0.8</td>
<td>8</td>
<td>0.34</td>
<td>0.84</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>40.20</td>
<td>100.00</td>
</tr>
</tbody>
</table>

[00971] **Measurement of Li+ content.** The sustained release cream was assayed using the same method as employed for assaying the intermediate release formulation, as described above. The results are shown in Table 35 below. The average recovery measured vs. theoretical is 105.35%.

[00972] **Table 35. Samples used to assay Li+ content of sustained release formulation lot# Th-001-084a**

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Weight (mg)</th>
<th>Li+ (ppm) Theoretical</th>
<th>Li+ (ppm) Measured (AA)</th>
<th>Measured/theoretical %</th>
<th>Average %</th>
</tr>
</thead>
<tbody>
<tr>
<td>TH-001-084a1</td>
<td>10.5</td>
<td>0.35532</td>
<td>0.43</td>
<td>121.02</td>
<td>105.35</td>
</tr>
<tr>
<td>TH-001-084a2</td>
<td>9.7</td>
<td>0.328248</td>
<td>0.32</td>
<td>97.49</td>
<td></td>
</tr>
<tr>
<td>TH-001-084a3</td>
<td>10.3</td>
<td>0.348552</td>
<td>0.34</td>
<td>97.55</td>
<td></td>
</tr>
</tbody>
</table>

[00973] **Determination of Li+ release rates.** 4.1558 g of cream was injected into a slide-A-lyzer cassette (thermo Scientific, Lot# LD 144491, membrane molecular weight cutoff 3500). 900 mL of 5 mM phosphate buffer, pH 7.4, was used as a medium for dissolution. 200 μL samples were taken by micropipette at various time points (data shown Table 36 below).
The collected samples were diluted with water in a 10 ml flask for absorption measurement using the atomic absorption instrument, AA 20+. The measured Li+ release profile for the cream Lot# TH-001-084a is shown in Figure 24.

Table 36. Time points for experiment to assess release of Li+ from sustained release formulation lot # TH-001-084a

<table>
<thead>
<tr>
<th>Day</th>
<th>Time in Hours</th>
<th>% Cumulative Rel of Li+ (assayed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 DAY PERIOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td></td>
<td>0.00</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>9.11</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td>18.22</td>
</tr>
<tr>
<td>3.0</td>
<td></td>
<td>21.26</td>
</tr>
<tr>
<td>5.0</td>
<td></td>
<td>27.33</td>
</tr>
<tr>
<td>6.0</td>
<td></td>
<td>33.40</td>
</tr>
<tr>
<td>8.0</td>
<td></td>
<td>39.47</td>
</tr>
<tr>
<td>24</td>
<td></td>
<td>51.62</td>
</tr>
<tr>
<td>28</td>
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<td>54.66</td>
</tr>
<tr>
<td>32</td>
<td></td>
<td>57.69</td>
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<tr>
<td>48</td>
<td></td>
<td>60.73</td>
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<tr>
<td>96</td>
<td></td>
<td>72.88</td>
</tr>
<tr>
<td>110</td>
<td></td>
<td>85.02</td>
</tr>
<tr>
<td>118</td>
<td></td>
<td>88.06</td>
</tr>
</tbody>
</table>

6.12.4 STABILITY OF CREAM FORMULATIONS AND CONCLUSION

[00974] About 10 ml of the cream formulations described above were each transferred into a 25 ml vial with closed cap and put into three different chambers: 4 °C/10%RH, 25 °C/50% RH, and 40 °C/75%RH. All creams survived at 4 °C/10%RH for more than 6 months, but underwent a phase separation at 40 °C/75%RH within a day. At 25 °C/50% RH, all creams stayed intact for about a month, and then spotty oil droplets were observed.

[00975] This example demonstrates that the release rate of lithium ion can be controlled in cream formulations.

6.13 EXAMPLE 13: LITHIUM HYDROXIDE HYDROGELS

[00976] This example describes the formulation of hydrogels using lithium hydroxide as a source of Li+. Hydrogel formulations of lithium hydroxide at 0.34 mg Li+/g, 2.74 mg Li+/g, 5.48 mg Li+/g and 10.73 mg Li+/g were generated in accordance with the Table 37 below.
Table 37. Formulations of lithium hydroxide at 0.34 mg Li+/g, 2.74 mg Li+/g, 5.48 mg Li+/g and 10.73 mg Li+/g

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>0.34 mg Li+/g</th>
<th>2.74 mg Li+/g</th>
<th>5.48 mg Li+/g</th>
<th>10.73 mg Li+/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g</td>
<td>%</td>
<td>g</td>
<td>%</td>
</tr>
<tr>
<td>Lithium Hydroxide monohydrate</td>
<td>0.041</td>
<td>2.06</td>
<td>0.332</td>
<td>1.660</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>1.600</td>
<td>8.000</td>
<td>1.600</td>
<td>8.000</td>
</tr>
<tr>
<td>CMC</td>
<td>0.400</td>
<td>2.000</td>
<td>0.400</td>
<td>2.000</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>0.021</td>
<td>0.104</td>
<td>0.021</td>
<td>0.104</td>
</tr>
<tr>
<td>Propyl Paraben</td>
<td>0.010</td>
<td>0.048</td>
<td>0.010</td>
<td>0.048</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>15.792</td>
<td>78.962</td>
<td>15.502</td>
<td>77.508</td>
</tr>
<tr>
<td>10% NaOH</td>
<td>PH adjust</td>
<td>PH adjust</td>
<td>PH adjust</td>
<td>PH adjust</td>
</tr>
<tr>
<td>Allantoin</td>
<td>0.032</td>
<td>0.160</td>
<td>0.032</td>
<td>0.160</td>
</tr>
<tr>
<td>Alginate</td>
<td>0.024</td>
<td>0.120</td>
<td>0.024</td>
<td>0.120</td>
</tr>
</tbody>
</table>

7. **EXAMPLE 14: SOLID-LIQUID IN-SITU CROSS-LINKING SPRAY ON A WOUND**

[00977] Certain issues exist when preparing formulations that release sustained concentrations of ionized lithium (Li+), without the use of highly hydrophobic matrices that are also occlusive. Drugs that are hydrophobic (log P > 2) can be delivered to tissues in a sustained manner due to their slow dissolution in aqueous media and their subsequent extraction in cellular and tissue lipids. In the case of ionized Li, the low molecular weight of the ions (atomic weight =3) and their high water solubility, results in rapid clearance from the tissue compartment (skin or blood). One way to slow down release of ionized Li is with the use of highly hydrophobic matrices such as petrolatum/mineral oil ointments. These matrices can offer high stability in storage and they are easy to apply to skin or to a dermal wound. Furthermore, petrolatum-based ointment bases can provide 7-14 days of sustained release of Li+. However, these ointment-based formulations are occlusive. An occlusive formulation lowers the exchange of oxygen and moisture, after application to the tissue. A "breathing" surface during the process of healing of a wound and during the process of hair neogenesis is
important. Emulsions (water-oil) can be modulated for its occlusive properties by varying its hydrophilic/hydrophobic ratio, but these result in faster release of Li+.

Microsphere encapsulating drugs have been used as ways to sustain release of a molecule. Lithium salts have been encapsulated in poly (lactide-co-glycolide) (PLG) microspheres and have demonstrated the ability to modulate release of Li+. The rate of release of Li+ varies as a function of L/G of the polymer. However, particulates and microspheres of sizes < 10 microns are cleared rapidly by phagocytosis from a wound site in less than 3 days. Thus, a drug delivery system that deliver and maintain the microspheres at the wound site in order promote sustained delivery of the drug is needed.

One way to increase the residence time of the microspheres is to sequester the delivery system to the wound surface by an in-situ cross-linking hydrogel that forms molecular bonds with the tissue surface. An in-situ cross-linking hydrogel cannot be "rubbed" off like an ointment or a cream. The microspheres (that contain the lithium salt) will be sequestered in the hydrogel, releasing Li+ in a sustained manner. Thus, the issue of phagocytosis of the microspheres can be overcome.

Additionally, sequestration can be enhanced by functionalization of the surface of the microspheres with a charge that will "bind" the microspheres to the tissue and the hydrogel. The net charge of the dermis is negative. Thus, positively charged microspheres would enhance the sequestration process of the lithium-containing microspheres. PLG microspheres can be imparted a positive charge by a coating with a cationic surfactant such as cetyl pyridinium chloride, benkalkonium chloride, or cetyl tri-ammonium bromide (CTAB). Alternatively, the coating can be polymeric, such as a coating of chitosan, or polylysine, or poly(arginine), or poly(amidoamine) (PAMAM) or poly(ethylenimine)(PEI).

Formation of molecular bonds of the hydrogel with the wound surface can only be accomplished if some of the reactive groups of the hydrogel components are capable of reacting lightly with the proteins present in the dermis. The concept includes a spraying device that can deliver the hydrogel components and the microspheres onto the wound surface creating a homogeneous coating on the surface. After spraying, the liquid coating turns into a cross-linked hydrogel with the microspheres sequestered within. A solution that is sprayed has a higher energy than one that has been extruded—this assists in the mechanical interlocking of the hydrogel with the dermis as it cross-links on the tissue. The hydrogel needs to be biodegradable and needs to "slough off" the healing wound after the drug has been delivered. The characteristics of the hydrogel, such as its biodegradability, the "gel
time” of its components, and its cross-link density are important characteristics that need to be optimized to arrive at the requisite delivery system.

[00982] The lithium salt can be dissolved directly in the hydrogel components prior to formation of the cross-linked hydrogel. Dissolution of the lithium salt directly in the pre-hydrogel components would not result in sustained release of Li+, due to the size of the ion and its hydrophilicity. However, if another drug such as an anesthetic needs to be administered in combination with Li+ (such as lidocaine hydrochloride), this can be dissolved in one of the hydrogel components.

[00983] Hair growth can be achieved by epidermal/dermal laser ablation. The laser can be an Erbium 2940 nm, or a 10,400 nm CO₂ with fractional or bulk ablative function. After ablation, the clinician mixes a first polymer (Polymer 1) with a second polymer (Polymer 2) by reconstitution of the dry solid (+ Li+-microspheres) with the liquid solution and rapidly sprays the ablated area with the in-situ cross-linking hydrogel, which acts as a biocompatible, biodegradable wound dressing and delivery system. This can be achieved using a two-chamber sprayer that contains a liquid in one chamber and a lyophilized solid (± microspheres containing a lithium salt) in the other chamber. It should be noted that another drug can be dissolved in the chamber containing the liquid. One drug or a combination of drugs can be administered in this way.

7.1 Two-Chamber Sprayer with a lyophilized solid in one chamber and a liquid in the other chamber

[00984] The sprayer design incorporates homogeneous mixing of the liquid component with the lyophilized solid component. The sprayer design also incorporates protection of each of the components from moisture. The sprayer materials are selected from those that allow sterilization.

[00985] The lyophilized solid component contained in chamber 1, is comprised of a polymer macromonomer (Polymer 1) (a polymer that can further crosslink with another component). It is necessary for this polymer to be lyophilized due to its hydrolytic labile bonds. Thus, this component cannot be stored in water. The component in the other chamber (chamber 2) contains another polymer macromonomer (Polymer 2) that is capable of reacting with the lyophilized polymer (Polymer 1). Polymer 2 is dissolved in a phosphate buffer of pH 6-8. Polymer 2 does not contain hydrolytically labile linkages and is stable in water. Thus, Polymer 2 can be stored in water. In this concept, it is envisioned that the solution containing Polymer 2 reconstitutes the lyophilized Polymer 1 through mixing that occurs within the sprayer. The mixed solution is then rapidly sprayed on the site of administration. Upon
spraying, the solution cross-links, or forms a hydrogel. The cross-linking reaction of the mutually reacting polymers increases the viscosity of the solution to a critical point of gelation, at which time the solution is a cross-linked, solid hydrogel. The polymers need to be formulated in such a manner, that the mixed solution does not prematurely gel, or crosslink in the spraying chamber, before spraying.

[00986] Polymer 2 dissolved in phosphate buffer is the reconstitution solution for Polymer 1. In preparation of spraying, the components of chamber 2 are mixed with the solid in chamber 1, to create a homogeneous solution. The components (Polymer 1 + Polymer 2) are capable of mutually reacting to form a biodegradable hydrogel. There are various means by which the "time to gelation" can be varied. The cross-linking reaction time of the polymers can be modulated by pH, since the reaction is triggered by higher pH. The pH of the reconstituting solution can be such that reaction between Polymer 1 and Polymer 2 does not cross-link instantaneously forming a gel in the sprayer, prior to spraying. The rate of cross-linking can also be modulated by the number of cross-linking groups. Thus, the two polymers at higher concentrations will crosslink faster than those at lower concentrations. The number of cross-linking groups per polymer molecule is also a factor in modulation of "gel time."

Thus, 4-armed polymers with 4 reacting groups at a 3% w/w concentration will react faster than 2-armed polymers at the same concentration. The choice of buffer pH and the structure of mutually reactive polymers will control the rate of gelation.

[00987] Considering which chamber the lithium-containing microspheres reside in, it becomes evident that the PLG microspheres would need to be stored in chamber 1, which is the chamber that contains the lyophilized polymer or solid component, because both the polymer and the PLG microspheres are hydrolytically labile. Also, because the lithium contained in the microspheres is released only after the microspheres are hydrated, storage in a dry form is necessary. Thus, chamber 1 would contain Polymer 1 and the drug. Other excipients can be added to be part of the lyophilized solid to aid in the dispersion of the drug, minimize "clumping" of the solids, and minimize reconstitution time. Chamber 2 (the liquid chamber) would contain Polymer 2 in an aqueous solution, or a solution that is mostly aqueous. Other excipients may be added to Polymer 2, as needed, to impart additional properties to the resultant hydrogel. For example, a positively charged molecule may be added to aqueous solution in chamber 2, to impart additional bioadhesive properties to the hydrogel.
Examples of formulation compositions of Polymer 1 (chamber 1 or the solid chamber) and Polymer 2 (chamber 2 or the liquid chamber) are provided in the sub-sections below.

**EXAMPLE 14A: REACTION OF POLYETHYLENE GLYCOL 4-ARMED AMINE (PEG-AM) WITH POLYETHYLENE GLYCOL 4-ARMED SUCCINIMIDYL ESTER (PEG-NHS)**

PEG-AM/PEG-NHS hydrogels have been approved for adhesion prevention and each of the ingredients is available in pharmaceutical grade. Additionally, the hydrogels have high water content, functioning as a highly biocompatible wound dressing, while the wound heals underneath. The high water content of the hydrogels also keeps the wound environment moist. The PEG-AM and PEG-NHS are mutually reactive at pH 6-8, to form a biodegradable, covalently cross-linked, hydrogel network. Each component is 4-armed, with each arm capable of reaction. PEG-NHS is an electrophile in the reaction, activated by higher pH. Because PEG-NHS has a cleavable linkage, this ingredient must be maintained dry and in the solid state. PEG-NHS would be stored in a lyophilized, flocculated state in Chamber 1 of the sprayer. PEG-AM is the nucleophile in the reaction. The N-hydroxy succinimide groups of PEG-NHS are reactive to nucleophilic substitution by amine groups of PEG-AM. Additionally, PEG-NHS is reactive to amino groups present in the proteins in the dermis, thus enabling molecular interlocking of the hydrogel to tissue to occur. This will sequester the hydrogel with the microspheres (containing the lithium salt) at the site.

Because there are no cleavable linkages in PEG-AM, this component can be stored in the liquid state in Chamber 2 of the sprayer. After mixing and cross-linking into a hydrogel, ester linkages created by the reaction would render the hydrogel biodegradable by hydrolytic cleavage. The cross-linking reaction of PEG-AM (polyethylene oxide-amine) and PEG-NHS is given in Figure 25.

**Polymer 1:** Polyethylene Glycol 4-Armed Succinimidyl Ester (PEG-NHS) was purchased from Nanocs, Catalog # 4APN05 122010, Molecular weight 10,000 Daltons

**Polymer 2:** Polyethylene Glycol 4-Armed Amine (PEG-AM) was purchased from Nanocs, Catalog# PEG4A-AM- 10K, lot # 4APM09 162009, Molecular weight 10,000 Daltons

**BUFFER COMPONENTS**

- Sodium Phosphate Dibasic, Anhydrous, Sigma, Catalog# S-0876, Lot# 120K0126
- Sodium Phosphate Monobasic, Anhydrous, Sigma, Catalog# S-0751, Lot# 20K0228
• Potassium Dihydrogen Phosphate, Alfa Aesar, Catalog# 11594, Lot# B04V025
• Sodium Chloride, Aldrich, Catalog# 7647-14-5, Lot# 12516HI

EQUIPMENT
• Magnetic stirrer IKA Labortechnik, RET DVS 1 with temperature/ RPM control
• Accument pH meter 25
• Water bath

Procedures

[00993] (1) Preparation of Phosphate Buffer
[00994] Stock solutions of 0.2M Sodium Phosphate Monobasic (SPM) and 0.2M Sodium Phosphate Dibasic (SPD) were prepared by dissolving 0.139 g of SPM in 5.0 mL of water and 0.284 g SPD in 10.0 mL of water. The required volumes (Table 38) of SPM and SPD were mixed and diluted by 3.0 mL water to obtain 0.1M Phosphate at pH 7.0, pH 7.5 or pH 8. The pH values were verified by pH meter and if necessary, adjusted to the required values. The recipe for preparation of the buffers is provided in Table 38.

Table 38. Phosphate Buffer Preparation

<table>
<thead>
<tr>
<th>pH</th>
<th>Sodium Phosphate Monobasic (0.2M), ml</th>
<th>Sodium Phosphate Dibasic (0.2M), ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>1.17</td>
<td>1.83</td>
</tr>
<tr>
<td>7.5</td>
<td>0.48</td>
<td>2.52</td>
</tr>
<tr>
<td>8.0</td>
<td>0.159</td>
<td>2.84</td>
</tr>
</tbody>
</table>

[00995] (2) Preparation of Phosphate Buffered Saline (PBS)
[00996] The 1xPBS was prepared by dissolving 8.0g of Sodium Chloride, 0.2g of Potassium Chloride, 1.44 g of Sodium Phosphate Dibasic, and 0.24 g of Potassium Phosphate Monobasic in 1.0 L of water.

[00997] (3) Preparation and Characterization of PEG-AM/PEG-NHS Hydrogels
[00998] (a) Preparation of PEG-AM/PEG-NHS Hydrogels
[00999] In this experiment, the potential of storing the PEG-AM and PEG-NHS in water was examined, by testing gel time.

MIXING OF PEG-NHS AQUEOUS SOLUTION WITH A PEG-AM AQUEOUS SOLUTION AND PHOSPHATE BUFFER (CROSS-LINKING SOLUTION)

[001000] Two stock solutions of 5% w/w PEG-NHS and 5% w/w PEG-AM were prepared by dissolution of 50.0mg of each polymer in 1.0mL of water. 100 μL of each stock solution
was withdrawn and mixed together, followed by the addition of 200 µl of phosphate buffer. The phosphate was prepared by dissolution Sodium Phosphate Dibasic (5.678 g) and Sodium Borate (3.3401 g) in 200 ml of water. The pH of buffer solution was 8. Experiments 08-03-mix3 to 08-03-mix8 tests the cross-linking ability of PEG-AM and PEG-NHS after each of the individual solutions are stored in water for 3-120 minutes. As outlined in Table 39, the gel time increases with increased storage time in water, indicating hydrolytic instability of PEG-NHS.

Table 39. Effect of PEG-NHS Storage in Water on Gelation Time

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS Conc., %</th>
<th>PEG-AM Conc., %</th>
<th>Gel time (seconds)</th>
<th>Time* (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>08-03-mix3</td>
<td>2.5</td>
<td>2.5</td>
<td>3&quot;</td>
<td>3</td>
</tr>
<tr>
<td>08-03-mix4</td>
<td>2.5</td>
<td>2.5</td>
<td>15&quot;</td>
<td>5</td>
</tr>
<tr>
<td>08-03-mix5</td>
<td>2.5</td>
<td>2.5</td>
<td>23&quot;</td>
<td>10</td>
</tr>
<tr>
<td>08-03-mix6</td>
<td>2.5</td>
<td>2.5</td>
<td>37&quot;</td>
<td>15</td>
</tr>
<tr>
<td>08-03-mix7</td>
<td>2.5</td>
<td>2.5</td>
<td>60&quot;</td>
<td>22</td>
</tr>
<tr>
<td>08-03-mix8</td>
<td>2.5</td>
<td>2.5</td>
<td>no gel</td>
<td>120</td>
</tr>
</tbody>
</table>

* Time passed after PEG-NHS dissolution in water and before mixing it with PEG-AM

[001001] Because of instability, PEG-NHS cannot be stored in an aqueous solution at ambient temperatures and should be stored in a solid form.

[001002] (b) Mixing of Solid PEG-NHS with PEG-AM Solution.

**TWO STEP HYDROGEL PREPARATION**

[001003] This experiment tested if PEG-AM and PEG-NHS can be formulated together in water, even to lyophilize thereafter. The experiment is testing a product concept of both PEG-AM and PEG-NHS dissolved together in water, to be lyophilized into a single chamber. The other chamber then, would only contain the phosphate buffer as the reconstitution solution. This concept can work only if PEG-NHS and PEG-AM do not react while in water (without the buffer).

[001004] In experiments 08-04-mix1 to 08-04-mix4, a solution of 2.5% PEG-AM/2.5% PEG-NHS was prepared. The final pH of the mixture was 6.4. The mixture was stored at room temperature to establish life time of the solution. Aliquots of 100 µl after each predetermined time-point were withdrawn from this mixture and added to 100 µl of 0.1M Phosphate buffer at pH 8 to induce gelation. Thus, for experiment 08-04-mix1, the buffer was
added after 9 minutes and the gel time was measured. For experiment 08-04-mix4, the buffer was added after 23 minutes and gel time was measured. The results are included in Table 40.

Table 40. Two Step Incorporation of PEG-NHS into Hydrogel (0.1M Phosphate buffer, pH 8)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS Conc., %</th>
<th>PEG-AM Conc., %</th>
<th>Gel time</th>
<th>Time*</th>
</tr>
</thead>
<tbody>
<tr>
<td>08-04-mix1</td>
<td>2.5</td>
<td>2.5</td>
<td>3'35&quot;</td>
<td>9</td>
</tr>
<tr>
<td>08-04-mix2</td>
<td>2.5</td>
<td>2.5</td>
<td>2'55&quot;</td>
<td>13</td>
</tr>
<tr>
<td>08-04-mix3</td>
<td>2.5</td>
<td>2.5</td>
<td>2'13&quot;</td>
<td>18</td>
</tr>
<tr>
<td>08-04-mix4</td>
<td>2.5</td>
<td>2.5</td>
<td>1'47&quot;</td>
<td>23</td>
</tr>
</tbody>
</table>

* Time passed after mixing solid PEG-NHS with aqueous PEG-AM and before addition of phosphate buffer

[001005] The longer contact times of PEG-NHS with PEG-AM before addition of the phosphate buffer resulted in decreasing gel times, indicating the components had started to mutually react. The time* shown in Table 40 denotes the storage of PEG-NHS and PEG-AM together prior to the addition of the buffer. After 18 minutes of storage, there was marked increase in viscosity of the mixture. After 23 minutes of contact time, it was difficult to withdraw an aliquot from this solution. After 30 minutes, the solution formed a cross-linked hydrogel plug. The PEG-NHS and PEG-AM started to react with each other immediately after mixing and formed cross-links, even though the kinetics of cross-linking was low at pH 6.4. But, the decrease in gel time indicated that cross-linking was occurring, leading to decrease of gel times and increases in viscosity (visually).

[001006] This experiment demonstrates that the cross-linking reaction between the two components in water begins prior to addition of the buffer. In practical terms, this experiment demonstrates that these two components cannot be formulated together, even it is to lyophilize. Thus, polymer 1 (PEG-NHS) would need to be formulated and lyophilized. Polymer 2 (PEG-AM) needs to be separately formulated and can be stored in a water solution.

**ONE STEP HYDROGEL PREPARATION**

[001007] This experiment demonstrates that PEG-AM formulated in phosphate buffer at pH 8 (and contained in chamber 2 of the sprayer) can be used as a reconstitution solution for PEG-NHS (contained in chamber 1 of the sprayer).
[001008] Solutions of PEG-AM in phosphate buffer were prepared, with a final pH of 8. The stock solutions of PEG-AM at concentrations 5% (50 mg/ml) or 2.5% (25 mg/ml) were prepared by dissolution of 50.0 mg in 1.0 ml or 2.0 ml of 0.1M Phosphate buffer at pH 8.0. Buffered PEG-AM was added to solid PEG-NHS. The PEG-AM buffered solutions were added to solid PEG-NHS in the amounts required to obtain equal final concentrations of both reagents. The concentrations of each of the ingredients PEG-AM or PEG-NHS were 5% w/w or 2.5% w/w (Experiment 08-05-mix, Table 41). The moment of PEG-AM addition was used as the starting point for gel time determination. The mixture was stirred using magnetic stirrer at 300 rpm. It took around 30 seconds for the PEG-AM buffer to reconstitute the PEG-NHS to be dissolved. The dissolution of PEG-NHS was included in the total gel time. Gel time was measured in triplicates for concentration of reagents at concentrations of 5% and 2.5%.

**Table 41.** One Step Incorporation of PEG-NHS into Hydrogel (0.1M Phosphate buffer, pH 8)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS</th>
<th>PEG-AM</th>
<th>Conc.</th>
<th>Gel time</th>
</tr>
</thead>
<tbody>
<tr>
<td>08-05-mix3a</td>
<td>11.4</td>
<td>228</td>
<td>5</td>
<td>2'</td>
</tr>
<tr>
<td>08-05-mix3b</td>
<td>10.3</td>
<td>206</td>
<td>5</td>
<td>1' 56&quot;</td>
</tr>
<tr>
<td>08-05-mix3c</td>
<td>11.5</td>
<td>230</td>
<td>5</td>
<td>1' 59&quot;</td>
</tr>
<tr>
<td>08-05-mix4a</td>
<td>11.3</td>
<td>452</td>
<td>2.5</td>
<td>3' 15&quot;</td>
</tr>
<tr>
<td>08-05-mix4b</td>
<td>11.8</td>
<td>472</td>
<td>2.5</td>
<td>3' 08&quot;</td>
</tr>
<tr>
<td>08-05-mix4c</td>
<td>11.7</td>
<td>468</td>
<td>2.5</td>
<td>3' 20&quot;</td>
</tr>
</tbody>
</table>

[001009] The results of experiment 08-05-mix demonstrated that there was no interference between PEG-NHS dissolution and gelling. Therefore, the one step method was used for further studies. Gel time was faster for higher concentrations (see 08-05-mix3a-3c in Table 41).

[001010] The experiment also demonstrates that PEG-NHS can be contained as a solid in one chamber and reconstituted easily with buffered PEG-Amine, contained as the reconstitution solution in chamber 2.

[001011] **(4) Determination of Gel Time, As a Measure of Cross-linking Kinetics**

[001012] For all gel time determinations, a method was developed to standardize this test as a measure of cross-linking kinetics. Solid PEG-NHS (10-20 mg) was added to a 4.0 mL transparent glass vial with a 4 mm magnetic stir bar placed inside. The vial with solid PEG-NHS was placed in a water bath for temperature control. The stirring rate was adjusted to 300 rpm and the temperature inside the water bath was adjusted to 25-26°C. The solution of PEG-AM was prepared in a phosphate buffer at pH 7.0; 7.5 or 8.0 and added to the solid PEG-
NHS. At this point, the stopwatch was started and was stopped when the solution coalesced into a solid and continued to rotate as one piece with the magnetic stir bar imbedded inside it.

\[ \text{(A) EFFECT OF PH ON GEL TIME} \]

The effect of pH on gel times was investigated in the experiments (Experiment 08-06-mix, Table 42), where PEG-AM solutions buffered at different pH were added to solid PEG-NHS. The solutions of PEG-AM at concentration 5% (50 mg/ml) were prepared by dissolution of 50.0 mg in 1.0 ml of 0.1M phosphate buffer at pH 7.0, 7.5 and 8.0. PEG-AM solutions were added to solid PEG-NHS in the amounts required to obtain a 5% w/w concentration of each reagent. The mixture was stirred using magnetic stirrer at 300 rpm, leading to dissolution of PEG-NHS and gel formation. Gel time was measured in triplicates for each pH.

As discussed earlier, the pH of the reconstitution solution can be used to control the gel time, so that the cross-linking reaction does not occur prematurely before spraying. As shown in Table 42 and Figure 26, a pH of 7.5 provides a gelation time of 3.5 minutes. This provides ample time for the clinician to spray the wound with the solution prior to gelation.

\[ \text{(B) EFFECT OF PEG CONCENTRATION ON GEL TIME} \]

The effect of pH on gel times was investigated in the experiments (Experiment 08-23-mix, Table 43), where PEG-AM at different concentrations was added to solid PEG-NHS. The solutions of PEG-AM at concentration 2.5% (25 mg/ml), 5.0% (50 mg/ml) and 7.5% (75.0 mg/ml) were prepared by dissolution of corresponding amounts in 0.1M NHS.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
Experiment & PEG-NHS & PEG-AM & pH & Gel time  \\
& mg & \( \mu l \) & &  \\
\hline
08-06-mix1a & 11.5 & 230 & 7 & 7' 32"  \\
08-06-mix1b & 11.1 & 222 & 7 & 7' 28"  \\
08-06-mix1c & 10.2 & 222 & 7 & 7' 20"  \\
08-06-mix2a & 11.3 & 226 & 7.5 & 3' 50"  \\
08-06-mix2b & 11.7 & 234 & 7.5 & 3' 52"  \\
08-06-mix2c & 11.7 & 234 & 7.5 & 3' 37"  \\
08-06-mix3a & 11.4 & 228 & 8 & 2' 13"  \\
08-06-mix3b & 12.1 & 242 & 8 & 2' 17"  \\
08-06-mix3c & 10.2 & 204 & 8 & 2' 17"  \\
\hline
\end{tabular}
\caption{Effect of pH on Gel Time (0.1M Phosphate buffer concentration, PEG-NHS and PEG-AM concentration 5%)}
\end{table}

As can be seen in Figure 26, the increasing of pH from 7 to 8 decreases the gel time from 7'32" to 2'13".

As can be seen in Figure 26, the increasing of pH from 7 to 8 decreases the gel time from 7'32" to 2'13".

As can be seen in Figure 26, the increasing of pH from 7 to 8 decreases the gel time from 7'32" to 2'13".
Phosphate buffer at pH 7.5. The PEG-AM solutions were added to solid PEG-NHS in amounts required to obtain final concentrations of both compounds at 2.5%, 5% and 7.5%. The mixture was stirred using a magnetic stirrer at 300 rpm, resulting in a dissolution of PEG-NHS and gel formation. Gel time was measured in triplicates for each concentration.

Table 43. PEG-NHS/PEG-AM HYDROGEL. Effect of PEG Concentration on Gel Time (0.1M Phosphate buffer, pH 7.5)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS (mg)</th>
<th>PEG-AM (µl)</th>
<th>PEG-NHS, %</th>
<th>PEG-AM, %</th>
<th>Gel time</th>
</tr>
</thead>
<tbody>
<tr>
<td>08-23-mix1</td>
<td>5</td>
<td>200</td>
<td>2.5</td>
<td>2.5</td>
<td>8' 19&quot;</td>
</tr>
<tr>
<td>08-23-mix2</td>
<td>5.1</td>
<td>200</td>
<td>2.5</td>
<td>2.5</td>
<td>8' 10&quot;</td>
</tr>
<tr>
<td>08-23-mix3</td>
<td>5.1</td>
<td>200</td>
<td>2.5</td>
<td>2.5</td>
<td>8' 18&quot;</td>
</tr>
<tr>
<td>08-23-mix4</td>
<td>10</td>
<td>200</td>
<td>5</td>
<td>5</td>
<td>4' 40&quot;</td>
</tr>
<tr>
<td>08-23-mix5</td>
<td>10.1</td>
<td>200</td>
<td>5</td>
<td>5</td>
<td>4' 41&quot;</td>
</tr>
<tr>
<td>08-23-mix6</td>
<td>9.7</td>
<td>200</td>
<td>5</td>
<td>5</td>
<td>4' 24&quot;</td>
</tr>
<tr>
<td>08-23-mix7</td>
<td>15.5</td>
<td>206</td>
<td>7.5</td>
<td>7.5</td>
<td>3' 34&quot;</td>
</tr>
<tr>
<td>08-23-mix8</td>
<td>15.4</td>
<td>205</td>
<td>7.5</td>
<td>7.5</td>
<td>3' 43&quot;</td>
</tr>
<tr>
<td>08-23-mix9</td>
<td>14.3</td>
<td>190</td>
<td>7.5</td>
<td>7.5</td>
<td>3' 44&quot;</td>
</tr>
</tbody>
</table>

[001017] As can be seen in Table 43 and Figures 27 and 28, gel times decrease when the PEG concentrations are increased. This allows modulation of PEG concentrations in each spraying chamber, so that the solution does not prematurely crosslink in the sprayer.

EXAMPLE 14B: PEG-NHS/PEI HYDROGELS

[001018] The following example is directed to PEG-NHS/PEI hydrogels. These hydrogels were PEG-NHS/polyethyleneimine networks and provide an alternative to PEG-AM/PEG-NHS hydrogels. Polyethyleneimine (PEI) are branched polymers with a high density of amine groups. These polymers form light molecular bonds with the dermis, preventing delamination of the hydrogel from the tissue surface. The formation of PEG-NHS/PEI hydrogels utilize identical chemistry as PEG-NHS/PEG-AM hydrogels previously described. PEI can be added in small quantities to the PEG-AM solution contained in chamber 2 of the sprayer, or it can replace PEG-AM entirely. Using PEI instead of PEG-AM, or adding this ingredient to PEG-AM introduces highly positively charged imine groups that lead to a tighter binding of the gel to the human skin.

[001019] In the examples below, the gels were prepared by the addition of PEI solutions in phosphate buffer to solid PEG-NHS. Polyethyleneimine (PEI) was purchased as a 50 wt% solution in water with a number average molecular weight of 1200 Daltons and a weight
average molecular weight of 1300 from Aldrich, Catalog# 485595, Lot# 05329KH. The use of 5% w/w of PEI with 5% w/w PEG-NHS have led to an instantaneous precipitate formation, but 10-50 times reduction in PEI concentration resulted in an in-situ cross-linking hydrogel.

[001020] The solutions of PEI at concentrations of 0.125% w/w, 0.25% w/w and 0.5% w/w each at three concentrations of phosphate were prepared by dilution of 5% PEI stock solution with 0.1M, 0.2M and 0.5M Phosphate buffer at pH 7 (Table 44, Experiment 08-13-mix). The pH of PEI solutions was measured by pH indicator paper. The mixture was stirred using magnetic stirrer at 300 rpm, resulting in a dissolution of PEG-NHS and gel formation.

[001021] At 0.5% w/w PEI concentration, phosphate buffer capacity was not enough to maintain pH of its solution at 7.0, which can be seen in Table 44, resulted in shorter gel times in experiments 08-13-mix4, 08-13-mix5 and 08-13-mix6, than would be measured at pH 7. This, however, does not change the effect of PEI concentration—as shown in Figure 29, gel time decreased when PEI concentration increased for all three phosphate concentrations.

[001022] The concentration of phosphate appeared to have an opposite effect on gel time—gel time increased when the phosphate concentration was increased (Figure 29). In the extreme case, 0.5M phosphate at 0.125% PEI failed to produce a gel (08-13-mix9, Table 44 (0.125% PEI in 0.5M Phosphate)).

Table 44. PEG-NHS/PEI Hydrogels. Effect of PEI and Phosphate Concentration on Gel Time (Phosphate buffer 0.1M, 0.2M and 0.5M at pH 7)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS conc., %</th>
<th>PEI conc., %</th>
<th>Phosphate conc., M</th>
<th>pH*</th>
<th>Gel time</th>
</tr>
</thead>
<tbody>
<tr>
<td>08-13-mix7</td>
<td>5</td>
<td>0.125</td>
<td>0.1</td>
<td>7.0</td>
<td>18' 18&quot;</td>
</tr>
<tr>
<td>08-13-mix1</td>
<td>5</td>
<td>0.25</td>
<td>0.1</td>
<td>7.0</td>
<td>5' 07&quot;</td>
</tr>
<tr>
<td>08-13-mix4</td>
<td>5</td>
<td>0.5</td>
<td>0.1</td>
<td>9.0</td>
<td>1' 25&quot;</td>
</tr>
<tr>
<td>08-13-mix8</td>
<td>5</td>
<td>0.125</td>
<td>0.2</td>
<td>7.0</td>
<td>20' 51&quot;</td>
</tr>
<tr>
<td>08-13-mix2</td>
<td>5</td>
<td>0.25</td>
<td>0.2</td>
<td>7.0</td>
<td>7' 31&quot;</td>
</tr>
<tr>
<td>08-13-mix5</td>
<td>5</td>
<td>0.5</td>
<td>0.2</td>
<td>8.0</td>
<td>2' 22&quot;</td>
</tr>
<tr>
<td>08-13-mix9</td>
<td>5</td>
<td>0.125</td>
<td>0.5</td>
<td>7.0</td>
<td>did not gel</td>
</tr>
<tr>
<td>08-13-mix3</td>
<td>5</td>
<td>0.25</td>
<td>0.5</td>
<td>7.0</td>
<td>13' 11&quot;</td>
</tr>
<tr>
<td>08-13-mix6</td>
<td>5</td>
<td>0.5</td>
<td>0.5</td>
<td>7.5</td>
<td>4' 36&quot;</td>
</tr>
</tbody>
</table>

* pH of the PEI solutions was measured with pH indicator paper.
EXAMPLE 14C: PEG-NHS/PEG-AM/CHITOSAN HYDROGELS

The following example is directed to PEG-MA/PEG-NHS/Chitosan hydrogels. Chitosan can be added to the PEG-AM/PEG-NHS hydrogels for impartation of a positive charge to the hydrogel, for the purpose of sequestration of the hydrogel to the skin.

PEG-NHS/Chitosan gel formation was investigated to see if chitosan could replace PEG-AM. Chitosan Batch# FP-21 1-03 was purchased from NovaMatrix. Because Chitosan is not soluble at pH 7 in phosphate buffer, Chitosan solution in water at pH 5-6 has been used. The addition of Chitosan water solutions at concentrations 0.25% and 0.65% to the solid PEG-NHS failed to produce gels. The mixture of PEG-NHS with Chitosan remained in a liquid form after 20 hours from the start of the reaction. This experiment demonstrated that chitosan could not replace PEG-AM.

The next experiment was to test if chitosan could be added to PEG-AM. Therefore, a three component system was developed, a chitosan solution was added to PEG-NHS and PEG-AM.

Two methods of introducing chitosan into PEG-AM/PEG-NHS hydrogels were tested. In Method 1, a 2.5 % w/w chitosan aqueous solution was mixed with 5% w/w PEG-AM in 0.1M phosphate buffer at pH 7. This solution was added to solid PEG-NHS. In Method 2, a 2.5% w/w chitosan aqueous solution was added to solid PEG-NHS and dissolved. 5% w/w PEG-AM in 0.1M Phosphate buffer at pH 7 was added to this solution. Chitosan solutions at 0.22-0.27% and 0.57-0.64% were obtained using these methods (Table 8). The experimental conditions, final reagent concentrations, and gel times for PEG-NHS/PEG-AM/chitosan hydrogels are shown in Table 45.

In both methods, partial precipitation of chitosan was observed, but gels became transparent as the reaction proceeded. This indicated that chitosan was incorporated into the hydrogel matrix as the reaction proceeded. The gel times of the three-component system PEG-NHS/PEG-AM/chitosan hydrogel were compared with a control—the two component PEG-NHS/PEG-AM hydrogel system. As outlined in Table 45, at chitosan concentrations of 0.22-0.27%, the gel times were either longer (first method of chitosan introduction) or close to the gel time of the controls (second method of chitosan introduction). For chitosan at concentrations of 0.57-0.64%, gel times were longer than for controls independently of the way chitosan was introduced into the gel. Thus, incorporation of chitosan into PEG-AM/PEG-NHS HYDROGELS slows down the reaction significantly at higher concentrations.
Table 45. Gel times of PEG-NHS/PEG-AM/Chitosan Hydrogels (0.1M Phosphate buffer, pH 7.0)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Method*</th>
<th>PEG-NHS conc., %</th>
<th>PEG-AM conc., %</th>
<th>Chitosan conc., %</th>
<th>Gel time</th>
</tr>
</thead>
<tbody>
<tr>
<td>08-13 Mix12</td>
<td>1</td>
<td>4.6</td>
<td>4.6</td>
<td>0.22</td>
<td>13' 44&quot;</td>
</tr>
<tr>
<td>08-17 Mix8</td>
<td>1</td>
<td>4.4</td>
<td>4.5</td>
<td>0.27</td>
<td>12' 19&quot;</td>
</tr>
<tr>
<td>08-17 Mix4</td>
<td>2</td>
<td>5.0</td>
<td>4.6</td>
<td>0.22</td>
<td>9' 24&quot;</td>
</tr>
<tr>
<td>08-17 Mix7</td>
<td>2</td>
<td>4.5</td>
<td>4.5</td>
<td>0.27</td>
<td>7' 5&quot;</td>
</tr>
<tr>
<td>08-17 Mix10</td>
<td>control</td>
<td>4.6</td>
<td>4.5</td>
<td>0.00</td>
<td>8' 24&quot;</td>
</tr>
<tr>
<td>08-17 Mix5</td>
<td>control</td>
<td>5.0</td>
<td>4.5</td>
<td>0.00</td>
<td>8' 03&quot;</td>
</tr>
<tr>
<td>08-18-mix4</td>
<td>1</td>
<td>3.9</td>
<td>4.0</td>
<td>0.64</td>
<td>21' 09&quot;</td>
</tr>
<tr>
<td>08-18-mix5</td>
<td>2</td>
<td>4.1</td>
<td>4.1</td>
<td>0.57</td>
<td>21' 30&quot;</td>
</tr>
<tr>
<td>08-18-mix6</td>
<td>control</td>
<td>4.1</td>
<td>4.0</td>
<td>0.00</td>
<td>13' 11&quot;</td>
</tr>
</tbody>
</table>

[001028] The PEG-NHS/PEG-AM/Chitosan hydrogel has disadvantages compared with the PEG-NHS/PEG-AM hydrogel and PEG-NHS/PEI hydrogel. At a pH ~7, Chitosan precipitates and cannot be covalently bonded to the gel matrix.

[001029] (5) Determination of Equilibrium Swelling

[001030] Equilibrium swelling measures the crosslink density of a covalently cross-linked hydrogel. A highly cross-linked hydrogel swells less, due to its high mesh density. Hydrogels that have high equilibrium swelling can also delaminate from the tissue surface. Thus, low equilibrium swelling is desired to prevent delamination from the tissue surface.

[001031] Factors that affect equilibrium swelling include concentration of the PEGs and the number of reactive groups per molecule. Thus, a 4-armed PEG-AM reacting with a 4-armed PEG-NHS would have a lower equilibrium swelling than a 2-armed PEG-AM/PEG-NHS hydrogel due to lower crosslink density.

[001032] In this experiment, the solid PEG-NHS was mixed with solutions of PEG-AM. The mixed solutions were withdrawn by a 1.0 mL disposable syringe (Henke Sass Wolf GmbH) and gels were formed inside the syringes. This method allowed formation of hydrogel molds with a fixed geometry. The syringes were cut into small cylindrical pieces. The gel plugs were 5-6 mm in length and 5 mm in diameter. The gel plugs were weighed and placed into Falcon tubes filled with 10 mL of IxPBS at pH 7.4. The Falcon tubes were placed into a 37°C water bath for 24 hours. After 24 hours, the gel plugs were removed from the Falcon tubes and excess PBS was wiped off. The gel plugs were weighed after swelling. The
percent swell was calculated by dividing the change in weight by the original weight, and expressing the result as a percentage:

\[
\text{Percentage Swell} = \frac{\text{Weight after swelling} - \text{Weight before swelling}}{\text{Weight before swelling}} \times 100
\]

[001033] As outlined in Table 46, as a general rule, the swelling of PEG-AM/PEG-NHS hydrogels increased with increasing concentration of each PEG component, due to the high binding of polyethylene glycol polymers with water.

[001034] a. PEG-NHS/PEG-AM Hydrogels: PEG-AM at 2.5% (25 mg/ml), 5.0% (50 mg/ml) and 7.5% (75.0 mg/ml) in 0.1M Phosphate buffer at pH 7.5 was added to solid PEG-NHS to obtain equal final concentrations of both reagents 2.5%, 5% and 7.5%.

[001035] b. PEG-NHS/PEI Hydrogels: PEI at concentration 0.125%, 0.25% and 0.5% in 0.1M Phosphate buffer at pH 7.0 was added to solid PEG-NHS to obtain 5% concentration.

[001036] c. PEG-NHS/PEG-AM/Chitosan Hydrogels: Aqueous chitosan solution at concentration 2.5% was added to solid PEG-NHS. PEG-AM in 0.1M Phosphate at pH 7 was added to this solution.

[001037] d. Gel plugs were fabricated to test equilibrium swelling at room temperature.

**Table 46. Preparation of Hydrogels for Equilibrium Swelling**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS</th>
<th>PEG-AM</th>
<th>PEG-NHS</th>
<th>PEG-AM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg</td>
<td>µl</td>
<td>Conc., %</td>
<td>Conc., %</td>
</tr>
<tr>
<td>PEG-NHS/PEG-AM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08-25-mix1</td>
<td>13.2</td>
<td>528</td>
<td>2.500</td>
<td>2.5</td>
</tr>
<tr>
<td>08-25-mix2</td>
<td>25.2</td>
<td>510</td>
<td>5.000</td>
<td>5.00</td>
</tr>
<tr>
<td>08-25-mix3</td>
<td>38.2</td>
<td>509</td>
<td>7.505</td>
<td>7.5</td>
</tr>
<tr>
<td>PEG-NHS/PEI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Conc., %</td>
<td>Conc., %</td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>µl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>08-25-mix4</td>
<td>25.3</td>
<td>500</td>
<td>5.060</td>
<td>0.125</td>
</tr>
<tr>
<td>08-25-mix5</td>
<td>24.4</td>
<td>500</td>
<td>5.000</td>
<td>0.25</td>
</tr>
<tr>
<td>08-25-mix6</td>
<td>25.1</td>
<td>500</td>
<td>5.000</td>
<td>0.5</td>
</tr>
<tr>
<td>PEG-NHS/PEG-AM/Chitosan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mg</td>
<td>µl</td>
<td>µl</td>
<td>Conc., %</td>
</tr>
<tr>
<td>08-25-mix7</td>
<td>26.4</td>
<td>500</td>
<td>50</td>
<td>4.5</td>
</tr>
</tbody>
</table>
The results of equilibrium swelling for PEG-NHS/PEG-AM hydrogels, PEG-NHS/PEI hydrogels, and PEG-NHS/PEG-AM/Chitosan hydrogels are shown in Table 47 and Figure 34. The degree of swelling increased for PEG-NHS/PEG-AM hydrogels from 65.3% to 146.9% with the increasing of total PEG concentration from 5% to 15% (Table 47, Figure 34). The degree of swelling is a function of cross-linking density, with higher degrees corresponding to a higher density. In this case, the effect of higher PEG concentration overwhelms the increase of cross-linking density. This is due to the high affinity of polyethylene glycol for water.

For PEG-NHS/PEI hydrogels, increase in PEI concentration did not change the degree of swelling and was close to the degree of cross-linking of PEG-NHS/PEG-AM hydrogels, indicating similar cross-linking density in both hydrogels (Figure 34). In contrast, the degree of swelling was significantly higher for PEG-NHS/PEG-AM/Chitosan hydrogels (187%) when compared to PEG-NHS/PEG-AM hydrogels (112%) at 10% PEG concentrations. This is an indication of lower cross-linking density for the PEG-NHS/PEG-AM/chitosan hydrogels, due to the interference of chitosan in the reaction between PEG-NHS and PEG-AM.

The results of swelling test for PEG-NHS/PEG-AM hydrogels, PEG-NHS/PEI hydrogels, and PEG-NHS/PEG-AM/Chitosan hydrogels are shown in Table 47 and Figure 34. The degree of swelling increased for PEG-NHS/PEG-AM hydrogels from 65.3% to 146.9% with the increasing of total PEG concentration from 5% to 15% (Table 47, Figure 5). The degree of swelling is a function of cross-linking density, with higher degrees corresponding to a higher density. In this case, the effect of higher PEG concentration overwhelms the increase of cross-linking density.

For PEG-NHS/PEI hydrogels, increase in PEI concentration did not change the degree of swelling and was close to the degree of cross-linking in the PEG-NHS/PEG-AM hydrogel, indicating similar cross-linking density in both hydrogels (Figure 34). In contrast, the degree of swelling was significantly higher for PEG-NHS/PEG-AM/Chitosan hydrogels (187%) when compared to PEG-NHS/PEG-AM hydrogels (112%) at the 10% PEG concentration, which is an indication of lower cross-linking density for the latter system and can be explained by the interfering effect of Chitosan, which hindered the reaction between PEG-NHS and PEG-AM.
Table 47. Swelling Properties of Hydrogels.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS Conc., %</th>
<th>PEG-AM Conc., %</th>
<th>PEG, total Conc., %</th>
<th>Weight b.s. mg</th>
<th>Weight a.s. mg</th>
<th>Swell %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEG-NHS/PEG-AM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08-25-mix1a</td>
<td>2.5</td>
<td>2.5</td>
<td>5.0</td>
<td>86.4</td>
<td>142</td>
<td>64.4</td>
</tr>
<tr>
<td>08-25 -mix1b</td>
<td>2.5</td>
<td>2.5</td>
<td>5.0</td>
<td>87.9</td>
<td>148</td>
<td>68.4</td>
</tr>
<tr>
<td>08-25 -mix1c</td>
<td>2.5</td>
<td>2.5</td>
<td>5.0</td>
<td>78.5</td>
<td>128</td>
<td>63.1</td>
</tr>
<tr>
<td>08-25-mix2a</td>
<td>5.0</td>
<td>5.0</td>
<td>10.0</td>
<td>99</td>
<td>202.1</td>
<td>104.1</td>
</tr>
<tr>
<td>08-25 -mix2b</td>
<td>5.0</td>
<td>5.0</td>
<td>10.0</td>
<td>93.7</td>
<td>192.7</td>
<td>105.7</td>
</tr>
<tr>
<td>08-25 -mix2c</td>
<td>5.0</td>
<td>5.0</td>
<td>10.0</td>
<td>91.8</td>
<td>208.6</td>
<td>127.2</td>
</tr>
<tr>
<td>08-25-mix3a</td>
<td>7.5</td>
<td>7.5</td>
<td>15.0</td>
<td>102.9</td>
<td>259.6</td>
<td>152.3</td>
</tr>
<tr>
<td>08-25 -mix3b</td>
<td>7.5</td>
<td>7.5</td>
<td>15.0</td>
<td>104.1</td>
<td>258.9</td>
<td>148.7</td>
</tr>
<tr>
<td>08-25 -mix3c</td>
<td>7.5</td>
<td>7.5</td>
<td>15.0</td>
<td>102.3</td>
<td>245.1</td>
<td>139.6</td>
</tr>
<tr>
<td>PEG-NHS/PEI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08-25-mix4a</td>
<td>5.0</td>
<td>0.125</td>
<td>5.0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>08-25 -mix4b</td>
<td>5.0</td>
<td>0.125</td>
<td>5.0</td>
<td>97.5</td>
<td>134</td>
<td>37.4</td>
</tr>
<tr>
<td>08-25 -mix4c</td>
<td>5.0</td>
<td>0.125</td>
<td>5.0</td>
<td>93.9</td>
<td>128</td>
<td>36.3</td>
</tr>
<tr>
<td>08-25-mix5a</td>
<td>5.0</td>
<td>0.250</td>
<td>5.0</td>
<td>91</td>
<td>95.5</td>
<td>4.9</td>
</tr>
<tr>
<td>08-25 -mix5b</td>
<td>5.0</td>
<td>0.250</td>
<td>5.0</td>
<td>90.7</td>
<td>97.8</td>
<td>7.8</td>
</tr>
<tr>
<td>08-25 -mix5c</td>
<td>5.0</td>
<td>0.250</td>
<td>5.0</td>
<td>102.8</td>
<td>109.7</td>
<td>6.7</td>
</tr>
<tr>
<td>08-25-mix6a</td>
<td>5.0</td>
<td>0.500</td>
<td>5.0</td>
<td>99.2</td>
<td>142.1</td>
<td>43.2</td>
</tr>
<tr>
<td>08-25 -mix6b</td>
<td>5.0</td>
<td>0.500</td>
<td>5.0</td>
<td>79.5</td>
<td>115.5</td>
<td>45.3</td>
</tr>
<tr>
<td>08-25 -mix6c</td>
<td>5.0</td>
<td>0.500</td>
<td>5.0</td>
<td>n.d.</td>
<td>n.d</td>
<td>n.d</td>
</tr>
<tr>
<td>PEG-NHS/PEG-AM/Chitosan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08-25 -mix7a</td>
<td>4.5</td>
<td>4.5</td>
<td>0.23</td>
<td>9</td>
<td>101.1</td>
<td>280.8</td>
</tr>
<tr>
<td>08-25 -mix7b</td>
<td>4.5</td>
<td>4.5</td>
<td>0.23</td>
<td>9</td>
<td>98.4</td>
<td>279.9</td>
</tr>
<tr>
<td>08-25 -mix7c</td>
<td>4.5</td>
<td>4.5</td>
<td>0.23</td>
<td>9</td>
<td>100.9</td>
<td>301.6</td>
</tr>
</tbody>
</table>

* Weight b.s. - weight of gel plugs before swelling; Weight a.s. - weight of gel plugs after swelling

7.2 Hydrogel Testing on Skin.

[001042] PEG-NHS/PEG-AM Hydrogel: 5% w/w PEG-AM in 0.1M Phosphate buffer at pH 7.5 was added to solid PEG-NHS (Table 48). The mixture was applied to intact skin. The liquid formed a sticky solution that coalesced within few minutes into a thin film that adhered well to skin. The film remained attached to the skin for few hours.
PEG-NHS/PEI Hydrogel: 0.5% PEI in 0.1M Phosphate buffer at pH 7.0 was added to solid PEG-NHS. The liquid formed a sticky solution that coalesced within few minutes into a thin, skin-adherent film. The hydrogel was applied to intact skin, and remained attached to the skin for few hours. The PEG-NHS/PEI hydrogel transformed to a thin film faster and seemed to be attached more tightly to the skin than PEG-NHS/PEG-AM hydrogel.

PEG-NHS/PEG-AM/Chitosan Hydrogel: Aqueous chitosan solution (2.5% w/w) was added to solid PEG-NHS. PEG-AM (in 0.1M Phosphate at pH 7) was added to this solution. The hydrogel was applied to intact skin. The gel did not adhere well to the skin.

Table 48. Preparation of Hydrogels for Testing on Skin

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS</th>
<th>PEI</th>
<th>PEG-NHS</th>
<th>PEI</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>µl</td>
<td>Conc., %</td>
<td>Conc., %</td>
<td></td>
</tr>
<tr>
<td>PEG-NHS/PEG-AM</td>
<td>08-27-mix1</td>
<td>25.9</td>
<td>500</td>
<td>5.0</td>
</tr>
<tr>
<td>PEG-NHS/PEI</td>
<td>08-27-mix4</td>
<td>25.7</td>
<td>500</td>
<td>5.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS</th>
<th>PEG-AM</th>
<th>Chitosan</th>
<th>PEG-NHS</th>
<th>PEG-AM</th>
<th>Chitosan</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg</td>
<td>µl</td>
<td>µl</td>
<td>Conc., %</td>
<td>Conc., %</td>
<td>Conc., %</td>
<td></td>
</tr>
<tr>
<td>PEG-NHS/PEG-AM/Chitosan</td>
<td>08-27-mix3</td>
<td>24.0</td>
<td>500</td>
<td>49.6</td>
<td>4.4</td>
<td>4.5</td>
</tr>
</tbody>
</table>

EXAMPLE 14D: A SPRAYABLE LITHIUM-CONTAINING MICROSPHERES-IN-HYDROGEL WOUND DRESSING FOR TREATMENT OF WOUND-ASSOCIATED INFECTIONS

The following example is directed to a sprayable lithium-containing microspheres-in-hydrogel wound dressing for treatment of wound-associated infections.

As described herein, lithium carbonate can be used against drug-resistant strains of MRSA (methicillin-resistant staphylococcus aureus), Acinetobacter baumannii and Enterococcus, all microorganisms associated with wound-related infections. For example, as demonstrated herein, a gel formulation of 11.5 % w/w lithium carbonate was tested in a mouse infection model and significantly reduced Methicillin Resistant Staphylococcus aureus (MRSA) microbial load, as shown in Figure 30. Lithium carbonate is released from the gel as ionized Li+ and due to its highly water solubility and small size, is rapidly cleared from the site. The use of lithium carbonate as antimicrobial therapy would benefit from a sustained
drug release mechanism or repeated administrations of the drug to maintain requisite concentrations at the site. To meet this clinical need, a novel, sprayable in-situ crosslinking breathable hydrogel bandage that can deliver sustained levels of Li+ to a wound site is described.

[001047] Described herein is a two-chamber sprayer that contains (i) a liquid crosslinkable polymer (4 armed PEG3.3K-amine and branched polyethyleneimine (PEI)) in one chamber (chamber 1); and (ii) a lyophilized solid (which contains lithium carbonate-containing PLG (poly-lactide-co-glycolide) microspheres with another crosslinkable pre-polymer (4 armed PEG3.3K-n-hydroxysuccinimide (PEG3.3K-NHS)) in the other (chamber 2). The sprayer design incorporates homogeneous mixing of the liquid component with the lyophilized solid component. The solution containing polymer 1 reconstitutes the lyophilized solid in chamber 2, and is mixed in the "mixing chamber" in the sprayer. PEI is added to the formulation in the liquid in chamber 1 to enhance binding of the hydrogel to the tissue site. The polymers are formulated in such a manner that the mixed solution does not prematurely gel, or crosslink, in the spraying chamber before spraying. The rate of gelation is controlled by the pH of the liquid and concentration of the crosslinking polymers (PEG-NHS, PEI and PEG-AM). Rate of release of Li+ from microspheres can be varied by varying the composition of the PLG polymer. The mixed solution is then rapidly sprayed on the site of administration. Upon spraying the solution crosslinks, or forms a hydrogel. Figure 33 demonstrates that the gel time of the mixing components of chamber 1 and chamber 2 can be optimized to a time frame that allows the clinician to adequately spray the lithium-containing sustained release system, without premature gelation in the sprayer head.

Methods:

[001048] Feasibility of Li+ as an antimicrobial. Gel formulations used to test the feasibility of lithium carbonate as an antimicrobial in an infected mouse wound contained 1% carboxymethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and various concentrations of micronized lithium carbonate. The infected mouse model was established using a common strain of MRSA (ATCC 33591). The lithium-containing gels were applied for 3 days twice per day. Enumeration of the microbial burden on the collected abrasions was assessed five days post infection, by colony counts and calculated as log reduction from untreated (Figure 30).

[001049] Preparation/characterization of lithium carbonate PLG microspheres. PLG (poly-lactide-co-glycolide) was selected as a matrix polymer to create the sustained delivery system for Li+. PLG polymers were used. Micronized lithium carbonate was suspended in
20% PLG solution in methylene chloride. This solution was added drop-wise to a 1% solution of PVA and homogenized using a Omni Cell Ruptor 400. The solution was stirred for 3 hours, then washed with distilled water. PEG-NHS was added to this suspension and lyophilized. The rate of Li+ release from the microspheres was quantified using standard in vitro release experiments. The concentration of lithium was determined by atomic absorption spectroscopy. Microspheres in lyophilized PEG-NHS were imaged using a Scanning Electron Micrograph at a magnification of 2000X. Sizing of the microspheres were performed using a Malvern Mastersizer 2000E.

**Gelation time.** Gel time experiments were performed by mixing solutions of PEG-AM/PEI with PEG-NHS. The PEG-AM/PEI solutions were prepared in phosphate buffer, pH 7.4, at concentrations of 1%, 1.5% and 2% PEG-AM and 0.5% branched PEL. The PEG-NHS solutions were at concentrations of 1%, 1.5% and 2% w/w. After mixing, the gel time for critical gelation to occur was recorded using a timer.

**Results:**

**[001051]** Antimicrobial activity of lithium carbonate against common MRSA strain ATCC 33591 was demonstrated in a dose-dependent fashion, with 11% w/w Li₂CO₃ demonstrating activity equivalent to 2% w/w Mupirocin (standard of care) (Figure 30).

**[001052]** To demonstrate the feasibility of incorporating lithium carbonate into microspheres, the following experiment was conducted. The liquid component 4-armed PEG3.3K-amine (PEG3.3K-AM) was dissolved in phosphate buffer at pH 7. Branched polyetheleneimine (PEI) was added to a PEG-NHS solution at a concentration of 1% w/w. The PLG microspheres were prepared by a standard without emulsion method. The crosslinking reaction of the mutually reacting polymers increased the viscosity of the solution to a critical point of gelation, at which time the solution was a crosslinked, solid hydrogel.

**[001053]** A scanning electron micrograph of the microsphere-containing lyophilized polymer is shown (Figure 31). Figure 31 shows lithium carbonate-containing microspheres lyophilized with PEG-NHS. The microspheres were intact with a size distribution of 1.2-5 microns and well-distributed within the PEG-NHS polymer. Ionized lithium was released at a sustained rate from PLG microspheres, with rate of release slowest for the 75:25 PLG matrix and fastest from the 25:75 PLG matrix (Figure 32). This was expected since the 25:75 PLG (L:G: 75:25) matrix has a higher rate of hydrolytic degradation, resulting in a higher release rate of encapsulated lithium carbonate; as the encapsulated lithium carbonate dissolves within the matrix, ionized lithium (Li+) was released.
Conclusion:

[001054] An in-situ crosslinkable hydrogel bandage that releases ionized lithium in a sustained fashion is feasible as an antimicrobial wound dressing.

8. Example 15: SOLID-LIQUID IN-SITU CROSS-LINKING SPRAY ON A BIODEGRADABLE SCAFFOLD THAT RELEASES LI+

[001055] Example 14 is directed to sustained release of ionized lithium from PLG microspheres sequestered to the dermis via an in-situ cross-linking, biodegradable hydrogel. Because the drug delivery system is applied to a dynamic wound healing environment, ionic lithium uptake into the dermis may become limited as the skin heals and slowly re-establishes its barrier function. Thus, sustained transport of ionized lithium into the dermis may diminish as a thick, fibrous scab is formed on the wound. Accordingly, ionic lithium will be released from the microspheres sequestered in the hydrogel, but transport of the drug through the scab will likely be limited.

[001056] When the problem is transport of ionic lithium through a scab, a solution can be to incorporate the delivery system into the scab. Once the delivery system is incorporated into the scab, the release of ionized Li will be from the scab into the healing dermis. Thus, the scab becomes part of the delivery system.

[001057] In the present example, a lithium carbonate-containing thin, gauze-like, pliable biodegradable scaffold is placed on the fresh wound. The material properties of the scaffold can be adjusted such that the gauze is able to absorb the blood and other exudates from the wound. Thus, the biodegradable scaffold will have a high content of void space in order to absorb blood, fibrin, and fibrinogen. This incorporation of the scaffold into the fibrin clot during its formation, results in its incorporation into the fibrous network, also called a scab, after it solidifies. After placement of the drug-containing biodegradable scaffold into the wound, an in-situ cross-linking hydrogel will be applied on top to cover the entire site as a wound dressing.

[001058] In Example 14, the drug is contained within PLG microspheres co-lyophilized with Polymer 1 (PEG-NHS) in chamber 1 of the sprayer. In the present example, the sprayer contains only the in-situ cross-linking polymer components. The drug is incorporated in the biodegradable scaffold.

[001059] This concept is advantageous and important implications for wound healing. The scaffold allows close contact of the drug system with the wound. Recent studies have shown that lithium carbonate has antimicrobial properties. Close contact with the wound may
prevent infections in the wound, thus aiding in effective wound healing. The fibrinincorporated drug delivery system is an excellent "scaffold" for cells to attach to.

8.1 DESCRIPTION OF THE EXAMPLE

[001060] In this example, (a) a two-chamber sprayer that contains a liquid in one chamber and a lyophilized solid in the other and (b) a biodegradable pre-fabricated scaffold that contains the drug (lithium carbonate in this case) is used.

[001061] The biodegradable scaffold is in the form a pliable, gauze-like material that is a blend of PLG polymers. Other polymers may be added to the main component (PLG) to impart characteristics such as biodegradability, pliability, etc. Lithium Carbonate will be incorporated in the biodegradable scaffold.

[001062] The biodegradable scaffold will have an "open-cell" structure that allows cells to (a) attach themselves, (b) differentiate, and (c) proliferate. The scaffold may have other components such as RGD peptides, etc. incorporated in order to promote cell attachment. The scaffold will have bioadhesive attributes to keep it "in place."

8.2 Method of Use

[001063] After placement of the scaffold on the fresh wound, the blood oozing from the site is allowed to be soaked into the gauze. The sprayer is then engaged to mix the two components contained in chamber 1 and chamber 2.

[001064] As described in Example 14, the lyophilized solid component contained in chamber 1, is comprised of a polymer macromonomer (Polymer 1) (a polymer that can further cross-link with another component). The component in the other chamber (chamber 2) contains another polymer macromonomer (Polymer 2) that is capable of reacting with the lyophilized polymer (Polymer 1). Polymer 2 is dissolved in a phosphate buffer of pH 6-8. Polymer 2 does not contain hydrolytically labile linkages and is therefore, stable in water. Thus, Polymer 2 can be stored in the reconstitution buffer. Accordingly, the solution containing Polymer 2 reconstitutes the lyophilized Polymer 1 when the polymers are mixed together in the sprayer. The mixed solution is then rapidly sprayed onto the wound site. Upon spraying, the solution cross-links, thereby forming a hydrogel.

[001065] In this example, the scaffold in the drug delivery carrier and the hydrogel is the wound dressing. Examples of in-situ cross-linking hydrogels are provided in Example 14.
8.3 THE EXAMPLE

[001066] The objective of this experiment was to develop prototypes of biodegradable scaffold patches that could be placed on wounded tissue to deliver a drug to the wound. The "scaffold" is a three-dimensional structure that can provide a high surface area for cell attachment. The drug was lithium gluconate. Varying the polymer composition of the scaffold matrix can modulate Li+ release rates from 3 days to 14 days.

[001067] Lithium gluconate was purchased from Spectrum Chemical, Inc. Lithium carbonate was dissolved in water at a concentration of 50 mg/mL. Poly(lactide-co-glycolide) (PLG), MW 12000 g/mole, poly(lactic acid) (PLA), MW 30,000 g/mole and blends thereof, were used to prepare fibrous scaffolds. The blends of polymers were 100/0 PLA/PLG, 50/50 PLA/PLG, 25/75 PLA/PLG and 0/100 PLA/PLG, respectively. PLA and PLG were purchased from Purac, Inc.

[001068] A cotton candy machine (Gold Medal Floss, Cat# 3024) was set at a setting of 3 (there are five settings in total, ranging from temperatures of 40° C to 200° C. One gram of a blend of 100/0 PLA and 1 ml of the lithium gluconate solution was fed into the hopper, which resulted in fine fibers collecting (much like a spider web) in the collection chamber. The fibers with the incorporated drug were collected and pressed into patches of 1 g each using a low pressure Carver press. The patches were then punched out into 1-inch by 1-inch squares. A similar procedure was followed for the other blends of 50/50 PLA/PLG, 25/75 PLA/PLG and 0/100 PLA/PLG.

[001069] Scanning electron micrographs (SEM) were taken of the patches (see Figure 36). By SEM, the mesh size, or open-cell size was estimated to be approximately 100-200 microns. Estimated thickness of the fabricated patches ranged from 500-1000 microns. The patches were placed into mesh buckets in dissolution baths containing phosphate buffered saline at 37° C and a pH of 7.4, to simulate physiological conditions. Aliquots of the dissolution media were retrieved at predetermined time-points and analyzed for Li+ content by flame-emission atomic adsorption spectroscopy (AA).

8.4 Results

[001070] SEM. Scanning electron micrographs of 100/0 PLA and 0/100 PLG are shown in Figure 36. The micrographs demonstrate a fibrous texture.

[001071] Visual and Flexural Modulus. The pressed fiber patches were tested for flexural strength by a simple flex method of bending the patch between the thumb and the index
finger. The patches could be bent, but they were brittle to the touch. Future patches should incorporate some plasticizing polymers such as PEGs or silicones in order to impart flexibility to the patches. By SEM, the mesh size or open-cell size of the patches was estimated to be approximately 100-200 microns. Estimated thickness of the fabricated patches ranged from 500-1000 microns.

**Release Rates.** As shown in Figure 36, the release of Li+ can be modulated by varying the ratio of PLA to PLG in the biodegradable scaffold. As a rule of thumb, a higher crystallinity of the poly(lactide) (PLA) slows down the release of Li+ from the matrix. The amorphous nature of poly (lactide-co-glycolide) (PLG) result in higher release rates of Li+. The approach of blending various ratios of PLA:PLG can be utilized effectively to modulate the release rate of Li+ from the matrix.

**Biodegradability.** The biodegradability of the patches can be tested in vitro by incubation of pre-weighed patches in phosphate buffer saline at a pH of 7.4 and a temperature of 37° C. Over time, the patches are removed from the bath and dried in a vacuum oven maintained at 30° C. The weight of the patches at T=0 and t=t, provides a biodegradation profile. Because the polymers degrade by hydrolysis and not by enzymolysis, the degradation buffer would not contain enzymes.

**Bioadhesion.** The bioadhesiveness of the drug-loaded patches can be assessed by placing the patch of wet tissue, inverting the tissue and measuring the rate at which the patch detaches from the tissue.

**Cell Adhesion.** The propensity of the drug-loaded patches to adhere to cells is measured by in-vitro culture of COS cells or keratinocytes in the presence of the scaffolds.

The experiments were performed with lithium gluconate as the incorporated drug. Lithium carbonate can be substituted for the gluconate and used in the same manner described above. Methods that can be employed to fabricate the scaffold include electrospinning, micromachining, and others. Nano-fiber meshes fabricated by electrospinning have been utilized to create three-dimensional microstructures. In situ forming scaffolds are also contemplated.

9. **EXAMPLE 16: SIMULTANEOUS ADMINISTRATION OF TWO OR MORE DRUGS VIA A TWO CHAMBER LIQUID-LIQUID SPRAYER**

Occasionally, multiple drugs need to be administered simultaneously. For a dermal application, application of one drug followed by the other is possible, but has practical issues such as accidentally rubbing off the first drug during administration of the second. If
these drugs can be co-formulated, then a single application of a combined formulation offers ease of use and administration. An example of this could be a dermal cream with two co-formulated drugs. However, many drugs cannot be formulated together, either due to differences in solubility properties of one drug relative to another or physical/chemical incompatibilities arising from being co-formulated. For example, an excipient that stabilizes or solubilizes one of the drugs may initiate precipitation for the other. Ionic binding of drugs to each other can create additional issues such as unpredictable bioavailability, absorption and clearance. Thus, what is needed is a pharmaceutically compliant way of co-administering two drugs at the target site, without co-formulation. A precise volume of delivery and the ability to cover a large site in a homogenous fashion would be additionally desired attributes.

[001078] A drug delivery device that co-administers two separate formulations can be used to address the above-described problems associated with administering multiple drugs simultaneously. One example of a drug delivery system that can administer multiple drugs simultaneously is a co-ointment tube, through which both the formulations are extruded together. Another example of a drug delivery system that can administer multiple drugs simultaneously is a dual chamber delivery spray device that contains a formulated drug in each chamber and co-sprays the drug formulations in a precise volume. Alternatively, the spray device can be engaged for spraying each drug separately, if required. For example, an alcoholic solution (±drug) may be used to first "prepare" the wound by thorough cleansing, followed by spraying of a lithium formulation. The spraying mechanism may be at high energy or low energy, depending upon the application. In another application, both chambers could contain the same drug, but in different forms and formulated differently to achieve different release profiles. For example, chamber 1 could contain micronized lithium carbonate suspended in a FDA-approved liquid excipient. Chamber 2 could contain dissolved ionized lithium in an aqueous sprayable gel. Co-spraying both forms of lithium carbonate provides instantly-bioavailable, ionized Li+ and a sustained form of Li+ made available as the micronized lithium carbonate dissolves.

[001079] A drug sprayer that can apply drug combinations has large implications in the treatment of dermatological conditions and can be used to (a) deliver a precise combination of the drug combination and (2) provide uniform coverage over large areas. Additionally, for treatment of wounds, wound cleansing with a lithium solution can be combined with administration of a lithium-containing gel to the target site.

[001080] The solutions that are contained in chamber 1 and chamber 2 sprayer (a) must be sprayable, (b) must not "run-off" the skin, and (c) must form a uniform coating on skin. The
first two are dependent on the modulation of viscosity and the last is dependent upon the surface wettability of the formulation. In terms of a formulation being fluid enough to spray, but viscous enough to "stay on the skin", one of the formulations has the added requirement of adding viscosity to the spray. This can be accomplished by using thermo-reversible polymers that have the property of being a liquid while cold, but "gels" when the solution reaches skin temperatures. One such polymer is of the PEO-PPO-PEO (polyethylene oxide-co-polypropylene oxide-co-polyethylene oxide) structure. At low temperatures (0-15°C), both the PPO and the PEO are fully dissolved and the polymer exists in a random-coil conformation. At higher temperatures (T>15°C), the PPO segments begin to collapse, while the PEO segments are still soluble. The polymer begins to undergo a state of "critical gelation," brought on by higher temperatures. Physically, the polymer solution attains a higher viscosity like a gel. This allows the drug-containing polymer solution to be sprayed while still attaining a homogeneous gel coating on the skin. In Examples 14 and 15, the in-situ cross-linking reaction was covalent in nature and triggered by a change in pH. In this concept, the in-situ gelation is non-covalent in nature and in triggered by change in pH. The "gelation" phenomenon is physical cross-linking, caused by a collapse of polymer segments, creating a solution of higher viscosity. A physical cross-link is not covalent or permanent in nature but accomplishes the task of minimizing or preventing "run-off."

[001081] Another way to develop a sprayable formulation that does not "run off" the skin after administration is to accomplish the gelation in-situ while spraying. This can be achieved by lecithin / polyethylene glycol/ water solutions which gel instantly when mixed. The lithium carbonate can be dissolved in a polyethylene glycol/water solution and included in the first chamber. Lecithin included in the second chamber and co-sprayed with the contents of the first chamber, will result in a "gel" on the skin.

[001082] Provided below are some examples of the two-chamber liquid-liquid drug delivery system.

9.1 Delivery of ionized Li+ and micronized lithium carbonate PEO-PPO-PEO formulations from a two chamber liquid-liquid spray delivery system

[001083] This experiment is directed to delivering ionized lithium and micronized lithium carbonate for a sustained period of time from an in-situ gelling system, triggered by change in temperature. Pharmaceutical grade Lithium Carbonate was purchased from Spectrum chemicals, Inc. Micronized lithium carbonate (particle size 1-3 microns) was purchased from FMC Corporation, Inc. The PEO-PPO-PEO polymer was F127, purchased from BASF
Corporation. The block copolymer was 12,600 daltons in molecular weight, with each polymer segment approximately 4000 daltons. Benzyl alcohol was used as a preservative. Allantoin and sodium alginate was used as wound healing aids.

A solution (solution 1) was prepared that contained 5.48 mg Li+/g lithium carbonate dissolved in a solution that contained 1% benzyl alcohol, 3% allantoin, 20% F127 and 3% sodium alginate and water (Q.S). Another solution (solution 2) was prepared that contained 1% micronized lithium carbonate dispersed in a solution that contained 78% propylene glycol, 1% tween 20 and 10% polyethylene glycol 400. Solution 1 can be contained in chamber 1 of the liquid-liquid sprayer and solution 2 can be contained in chamber 2. The viscosity of each solution was measured using a Brookefield Viscometer at 4°C, 25°C and 32°C (skin temperature). Gel times were measured by mixing 1:1, 1:2 and 1:3 ratios of each solution. The gelling concept was tested on skin to estimate if the gelled formulation stayed on the surface.

Solution 2 contained excipients that are water-soluble. There was no water in solution 2 because micronized lithium carbonate would dissolve in water over time. However, the excipients are all soluble in water because the ingredients in solution 2 would need to be miscible with all ingredients in solution 1. Propylene glycol and polyethylene glycol were included as surface wetting agents. The viscosity of solution 1 did not vary with temperature, whereas the viscosity of solution 2 varied significantly with temperature. The shear viscosity of solution 2 is 150 cP at 4°C, 1920 cP at 25°C and 7922 cP at 32°C. Mixing of a 1:1 ratio of solution 1 and solution 2, resulted in a solution having a viscosity of 2311 cP at 32°C, with little "run off." The thinner the film on the skin, the lower the "run-off."

Mixing of a 1:2 and 1:3 ratio of solution 1 and 2 resulted in viscosities of 3622 cP and 4799 cP with no "run off." All three mixes were gels. A preliminary in-vitro release experiment of the 1:3 ratio gel demonstrated some sustained release of ionized lithium.

9.2 **Determination of the viscosity that can be sprayed from a sprayer.**

This experiment was to generate a series of polyvinyl alcohol solutions formulated in distilled water and measure the viscosities of these solutions, with the purpose of ascertaining whether these solutions could be effectively sprayed from a spraying apparatus.
Materials

1. Polyvinyl Alcohol (PVA); 5, 10, 15, 20, 25, 30% solutions - Lot #D14T017, Alfa Aesar

2. Distilled Water

3. Brookfield Rheometer, DV III

Methods

[001087] a. Six solutions of PVA were prepared by weighing out the appropriate amounts of PVA solid and dissolving it into distilled water.

[001088] b. To measure viscosity, a Brookfield DV-III Ultra programmable rheometer was utilized, and the temperature was regulated by a Neslab RTE-111 water bath. When the temperature was stable at 25° C, mineral oil calibration standards at 9.5, 98, 4800, and 12500 cP were used to calibrate the instrument.

[001089] c. The solutions were then applied and the RPM adjusted until the viscosity reading remained stable over several RPM values. The procedure was then repeated at 30° C.

Data

[001090] Table 49 summarizes the range of viscosities obtained by the PVA solutions, with the lowest viscosity at -150 cP (5% w/v PVA) and the highest viscosity at 9900 cP (30% w/v PVA), measured at 25° C. All solutions had flow characteristics.

Table 49. Viscosity as a function of concentration in % w/v

<table>
<thead>
<tr>
<th>Concentration (% w/v)</th>
<th>Viscosity (25 deg. C)</th>
<th>Viscosity (30 deg. C)</th>
<th>Flow Properties</th>
<th>TESTED WITH SPRAYER</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>154</td>
<td>135</td>
<td>Yes, flows quickly, watery</td>
<td>Can be sprayed</td>
</tr>
<tr>
<td>10</td>
<td>265</td>
<td>164</td>
<td>Yes, flows quickly, watery</td>
<td>Can be sprayed</td>
</tr>
<tr>
<td>15</td>
<td>357</td>
<td>258</td>
<td>Yes, moderately fast, like olive oil</td>
<td>Difficult</td>
</tr>
<tr>
<td>20</td>
<td>773</td>
<td>730</td>
<td>Yes, flows slowly, like syrup</td>
<td>No</td>
</tr>
<tr>
<td>25</td>
<td>952</td>
<td>920</td>
<td>Yes, flows very slowly, like molasses</td>
<td>No</td>
</tr>
<tr>
<td>30</td>
<td>9900</td>
<td>4500</td>
<td>Yes, but flows extremely slowly</td>
<td>No</td>
</tr>
</tbody>
</table>

[001091] Consistent with the properties of Newtonian fluids, PVA lessens in viscosity as temperature increases, as shown in Figure 35. Table 49 includes the actual viscosity values obtained and the flow properties were recorded qualitatively by observing the flow of solution down their vials.

[001092] The purpose of this experiment was to ascertain the viscosity of PVA, which will be used to determine the limits of a spraying apparatus designed to expel a liquid formulation that will eventually solidify into a gel on the target surface. The usage of PVA as a test
mechanism will determine how viscous the formulation can be without jamming or damaging the sprayer. The two temperatures chosen were 25° C and 30° C because the temperature of the apparatus will be approximately room temperature, while the temperature of the body is at 32° C. The results of PVA are consistent with the known properties of Newtonian fluids, as the viscosity decreases as the temperature increases. Therefore, a PVA solution expelled from a sprayer would be less viscous if applied to the surface of the body. As outlined in Table 49, (a) solutions having a concentration of 5% and 10% could be sprayed easily, (b) the solution having a concentration of 15% was difficult to spray, and (c) solutions with concentrations greater than 15% (-337 cP-9900 cP) could not be sprayed.

10. EXAMPLE 17: CO-DELIVERY OF MICRONIZED LITHIUM CARBONATE AND IONIZED LITHIUM FOR CLEANSING AND SUBSEQUENT TREATMENT OF INFECTIONS IN WOUNDS

Lithium carbonate has been shown to have unexpected potential as a broad-spectrum antimicrobial compared to other lithium salts such as lithium gluconate and lithium succinate. Additionally, antimicrobial activity has been shown to be dose-concentration related. That is, formulations with higher concentrations of ionized lithium (formulated as lithium carbonate hydrogels, 0.34 mg Li+/g gel-10.64 mg Li+/g gel) have more of a biocidal effect. However, pharmacokinetics of lithium carbonate hydrogels shows rapid clearance from the site of administration (75% of the drug absorbed is cleared from the wound site within 4 hours). Thus, lithium carbonate hydrogels with both bolus and sustained release of Li+ may offer a sustained antimicrobial effect in addition to potential scarless wound healing.

Additionally, infected wounds need to be debrided and cleansed with a biocompatible cleansing agent prior to application of the antimicrobial therapy. Furthermore, a delivery system that can deliver both a cleansing agent and a sustained release system can be useful in the wound treatment units for both battlefield wounds and burns.

A complete spray delivery system that delivers both a cleansing agent (to clean and debride the wound) and an in-situ "gelling" antimicrobial gel that also accomplishes scarless wound healing is envisioned. The two-chamber spraying system would contain a liquid in each compartment. The spraying mechanism of each of the chambers would be deployed separately, as opposed to simultaneously. In this concept, the spraying mechanism for chamber 1 would deploy the spray stream at high energy, creating a highly effective cleansing method that delivers a sterile liquid to physically debride the infected wound. The sterile liquid delivered as a high energy spray would be comprised of
ionized lithium at a concentration that is microbiocidal, combined with a surfactant molecule like benzalkonium chloride or cetyl Pyridinium chloride. Both molecules are FDA-approved as preservatives and also as potent antimicrobials. It is surmised that a high energy spray of a sterile, cleansing liquid prepares the wound tissue, for delivery of the antimicrobial gel.

[001096] The spraying mechanism for step 2 involves the 2nd chamber. The solution contained in chamber 2 would contain micronized lithium carbonate. Due to the high bond strength of the Li-carbonate linkage (1st Group I alkali in the periodic table), the kinetics of dissolution of this molecule is slow in aqueous solutions. While formulating lithium carbonate into an aqueous formulation, the pH of the solution has to be kept low and the solution heated to allow dissociation of the lithium-carbonate linkage and carbon dioxide to be released. Once the lithium-carbonate linkage is cleaved and carbon dioxide has been released, lithium can exist in a thermodynamically stable, ionized state in solution even when the pH is raised to 7. If lithium carbonate is to exist in a micronized state in an aqueous solution, this can be accomplished by keeping the micronized lithium carbonate at neutral pH. This can be helped further by lowering the temperature of the suspension to refrigeration (2-8 °C). Thus, the spray containers would be stored at refrigerated temperatures. Both chamber 1 and chamber 2 can be sprayed cold, creating a soothing spray to cleanse and treat an infected wound.

[001097] High-energy spraying the contents of chamber 1 (containing a formulation of the surfactant and dissolved lithium) on the wound cleanses and debrides the wound, while eliminating/killing surface bacteria from the wound.

[001098] After spraying the contents of chamber 2 (which contains a formulation of micronized lithium carbonate) on the wound, the dissolution of micronized lithium carbonate is triggered by the higher temperature of the wound releasing a sustained stream of ionized lithium. Sustained release of ionized lithium into the wound maintains an antimicrobial environment, preventing re-establishment of microbial organisms at the site. The micronized lithium carbonate would be formulated in a thermo-reversible polymer system that is liquid when refrigerated and a gel at skin temperatures. This would allow the polymer solution (with micronized lithium) to be held at the site of administration.

[001099] This method provides a complete treatment of the wound at the clinic, by debridement/cleansing followed by administration of a sustained release lithium gel. This step would occur at the clinic, where the spray system would be operated by the medical personnel. The person being treated would be provided with a tube of lithium carbonate hydrogel, which the person will apply twice daily after 2 days as a maintenance regimen.
It is not necessary that chamber 1 contains lithium as the wash. An alcoholic solution (±drug) may be used to first "prepare" the wound by thorough cleansing, followed by spraying the micronized lithium formulation as the sustained release antimicrobial. The spraying mechanism may be at high energy or low energy, depending upon the application.

11. EXAMPLE 18: EVALUATION OF LITHIUM CARBONATE GELS AND EMULSIONS FOR ANTI-MICROBIAL ACTIVITY IN A MOUSE SKIN ABRASION INFECTION MODEL

Lithium Carbonate gels and emulsions were evaluated for anti-microbial activity in a mouse skin abrasion infection model against Methicillin Resistant S. aureus (MRSA). The gels and emulsions contained either 5.66% Lithium Carbonate or 11.5% Lithium Carbonate. The study groups were: (a) vehicle, (b) 2% Mupirocin; (c) 5.66% Lithium Carbonate aqueous gel (d) 11.5% Lithium Carbonate aqueous gel; (e) 5.66% Lithium Carbonate sustained release emulsion; and (f) 11.5% Lithium Carbonate sustained release emulsion.

The pathogen selected was Methicillin-Resistant Staphylococcus aureus (MRSA). The in vivo infective model was a CD-I mouse infection, dermabrasion model. Lithium Carbonate emulsion vehicle was used as one of the test groups. Each group had 10 mice wounded at day 0 using a dermabrader. The wounds were infected with MRSA 24 hours after infection, the mice were dosed twice daily for three days with 0.1 mL of each formulation, using a calibrated wiretrol pipette. The infection control group did not receive treatment. Enumeration of the bio-load on the collected abrasions was assessed five days post infection.

Materials and Methods:

Bacterial strains:
- Methicillin-resistant S. aureus ATCC 33591

Test articles pre-formulated:
- Antibiotic Bactroban Cream (2% Mupirocin) (22 g) Taro Pharmaceuticals, Hawthorne, NY; Exp: 12/2011; Lot# 10055
- Emulsion (Vehicle Control)
- 0.1 mL Lithium Carbonate gel 10.64 mg Li+/g (5.66% w/w Li2C03)
- 0.1 mL Lithium Carbonate gel 20 mg Li+/g (11.5% w/w Li2C03)
- 0.1 mL Lithium Carbonate emulsion 10.64 mg Li+/g (5.66% w/w Li2C03)
- 0.1 mL Lithium Carbonate emulsion 20 mg Li+/g (11.5% w/w Li2C03)
[001106] **Bacterial Growth Media and Vehicles:**
- Trypticase Soy Agar plates - BBL, Franklin Lakes, NJ
- Brain Heart Infusion (BHI) Broth - BBL, Franklin Lakes, NJ
- Sterile Saline - 0.9% prepared from powder as needed at ViviSource Laboratories, Inc
- MacConkey Agar plates - BBL, Franklin Lakes, NJ

[001107] **Animals:** Species: mouse, Strain: CD-l(ICR), Source: Harlan Laboratories, Weight: 20 to 30 grams, Number: N=10 per group

[001108] All procedures in this protocol were in compliance with the ViviSource Laboratories IACUC protocol VVSIO-016. Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

[001109] **Protocol/ Study Design:**

[001110] **Formulations:** The formulations contained 1% carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5% glycerol, 5% citric acid, distilled water and various concentrations of micronized lithium carbonate. The viscosity of the formulations was optimized to have "minimal run-off" from a wound surface. Viscosity was measured using Brookfield Viscometry at 32°C, the temperature of skin. Stability of the formulations were tested at 4°C, 25°C and 40°C, for dose uniformity of Li+, loss in viscosity over time and appearance of turbidity etc. Measurement of Li+ was conducted by Atomic Absorption Spectroscopy. In-vitro release of Li+ was determined in a dissolution chamber containing phosphate buffer saline at 32°C, by sampling at T=0, T=4 hours, T=6 hours and T=24 hours.

[001111] The formulations containing lithium carbonate were transparent and had viscosities in the range 4000-5000 centipoise (cP) at 32°C. When applied topically, the formulations do not exhibit any "run-off. The formulations demonstrated fast release of Li+ (in-vitro release), with 60% released in the first 4 hours and 100% released by 24 hours. Thus, twice-daily application of the topical gel was selected for the in-vivo wound infection study with MRSA. The formulated gels demonstrated dose uniformity, consistent viscosity and appearance for a period of 3 months at temperatures 4°C, 25°C and 40°C.

[001112] **Bacteria preparation:** Methicillin-resistant *S. aureus* ATCC 33591 were grown at 37°C under 5% CO₂ on trypticase soy agar (TSA) plates. The bacterial concentration was prepared by re-suspending colonies from the overnight growth on the TSA plate in brain heart infusion (BHI) broth and adjusting a 1:20 dilution of the suspension to achieve an OD (optimal density) of 0.3 at 625nm. The bacterial suspension was then diluted in saline to achieve an inoculum of approximately in 1x10⁵ CFU/mL (Colony Forming Units).
Bacterial counts were performed to determine actual inoculum concentration; 100 µL of the bacterial suspension was serially diluted (1:10) in saline 7 times. Then 100 µL of the $10^5$, $10^6$, and $10^7$ was pipetted in duplicate on to TSA plates with glass beads, then shaken to spread bacteria on plates, then the beads were removed and plates were placed at 37°C with 5% CO$_2$ overnight, and then counted 24 hours later to determine bacterial concentration of the inoculum the mice received.

**Animal Preparation:** The right rear flank of all mice were clipped to 1.5 cm x 1.5 cm size removing fur and then cleaned with 70% alcohol. Mice were anesthetized with an inhalation mixture of 4% Isoflurane and 1.5% oxygen prior to abrasion.

**Dermabrasion Procedure:** The skin was abraded to a width/length of 13 mm with a sandpapered dowel to abrade the skin without causing any bleeding, by applying pressure and using a constant motion back forth for up to 15 times. This procedure results in a partial thickness wound (Vivisource data).

**Infection establishment:** Mice (ten per group) of weight approximately 25g and age 8-10 weeks were anesthetized with an inhalation of 4% Isoflurane with 1.5% oxygen and fur clipped. The mice were dermabraded using a dermabrader as described in the previous section. The bacterial suspension was pipetted on to the abrasion at a volume of 100µL. The wound was occluded for 24 hours to establish infection, verified by bacterial counting of the tissue at day 0. The wounds were un-occluded for the duration of the study. After the inoculum was pipetted on the skin, a sterile non-absorbent 13mm disc was placed on the wound then taped in place with dermal cloth tape. This maintains the suspension in direct contact with the wound. Animals were observed frequently after the infection procedure to confirm they were awake from the anesthesia and showing no signs of discomfort or pain from the procedure. No analgesic was provided as the abrasion wound will go no deeper than the dermal layer; literature research confirmed that analgesic was unnecessary for this procedure with an anesthetic, however if at any time during the study an animal had shown signs of serious injury or illness that was unlikely to be the result of the experimental procedures, the affected mice would have been immediately euthanized. Animals were monitored daily for any signs of animals exhibiting severe dyspnea (labored breathing), severe depression, weight loss or other neurological signs. Animals with weight loss exceeding 20% from the time 0, body weight and other signs of obvious distress would have been immediately euthanized. Twenty-four hours post infection the tape and disc was removed, and wound area measured. Animals were weighed daily to ensure the animals were not in pain.
[001117] **Treatment:** There were 6 groups in total, with 10 mice per group. At 24 hours post infection with MRSA the tape and disc was removed. Mice were weighed once daily prior to first dose. The abrasion/dose sites were scored twice daily using the Skin Irritation Draize Scoring system prior to dose. Mice were dosed a set volume of 0.100 ml topically (using a wiretrol device) twice daily for three days. The dosing per day was spaced 6 hours apart. If the hydrogel did not spread well on the wound, the tip of the wiretrol device was used to spread the formulation on the wound.

[001118] **Tissue Processing:** Approximately sixteen hours after the last treatment, all mice were euthanized via an inhalation of C0₂. The skin of the animals was aseptically removed, weighed, homogenized in 1mL of sterile saline and serially diluted in sterile saline five times at 1:10. The five diluted samples were plated on TSA medium for bacterial quantification. After 18 hours of incubation the dilution with countable bacterial colonies was recorded for each animal and entered into raw data with weight of excised abrasion for calculation for logioCFU.

[001119] **Data End-Points:** The average logio CFU per gram of skin was calculated for each group and standard deviations determined. The logio CFU per gram of skin versus control and the logio reductions from the untreated controls were calculated. Twenty-four hours after infection was established, erythema and edema in each dose group was assessed twice daily prior to each dose. The wound irritation potential of the formulations was assessed each day, for the duration of the mouse infection study. The erythema scoring scale was: (a) no erythema = 0; (b) very slight erythema =1, (c) well defined erythema = 2, (d) moderate to severe erythema = 3, (e) severe erythema =4. Swelling (edema; Score of 4 =severe, 3=moderate; 2=slight; 1= very slight; 0= none).

[001120] **Results:**

[001121] **Anti-microbial Activity:** As shown in Figure 42, topical administration of 11.5% Lithium Carbonate gel in a mouse skin abrasion infection model against MRSA resulted in significantly increased reduction of bacterial loads as compared to 5.66% Lithium Carbonate gel and 11.5% Lithium Carbonate emulsion (ATCC 33591). Also 11.5% Lithium Carbonate gel was equivalent in effectiveness as compared to 2%> Mupirocin (See Figure 42). At the highest dose tested (11.5% w/w), lithium carbonate reduced the MRSA microbial load by 2.2 log reduction of CFU/g skin, compared to untreated and equivalent to the standard of care, mupirocin (2.17 log reduction of CFU/g skin, compared to untreated). The *in-vitro* MICu-c against 17 strains of MRSA tested (including mupirocin-resistant strains) is 3.5-7 mg/mL.

MICmupirocin against these strains were in the range ≤ 0.06 µg/mL to > 512 µg/mL.
[001122] After three days of twice-daily dosing, both the lithium-treated and Mupirocin groups demonstrated decreased erythema, compared to untreated controls. Li-Carb groups appeared to have lower edema (1-2) than Mupirocin (1-2.5) and untreated (2-3). Thus, the wound irritation potential for the lithium carbonate formulations at all concentrations was deemed minimal.

12. **EXAMPLE 19: EVALUATION OF LITHIUM CARBONATE AND MUPIROCIN COMBINATION FOR ANTI-MICROBIAL ACTIVITY IN A MOUSE SKIN ABRASION INFECTION MODEL**

[001123] Lithium Carbonate formulations in combination with 2% Mupirocin were evaluated for anti-microbial activity in a mouse skin abrasion infection model against *Methicillin Resistant S. aureus* (MRSA). The formulations contained varying concentrations of lithium carbonate. The study groups were: (a) vehicle, (b) 2% Mupirocin; (c) 2% Mupirocin plus placebo; (d) 2% Mupirocin plus 1.46% Lithium Carbonate gel, (e) 2% Mupirocin plus 5.66% Lithium Carbonate gel and (f) 2% Mupirocin plus 11.5% Lithium Carbonate gel.

[001124] The pathogen selected was Methicillin-Resistant *Staphylococcus aureus* (MRSA). The *in vivo* infective model was a CD-I mouse infection, dermabrasion model. Lithium Carbonate vehicle was used as one of the test groups. Each group had 10 mice wounded at day 0 using a dermabrader. The wounds were infected with MRSA 24 hours after infection, the mice were dosed twice daily for three days with 0.1 mL of each formulation, using a calibrated wiretrol pipette. The infection control group did not receive treatment. Enumeration of the bio-load on the collected abrasions was assessed five days post infection.

[001125] **Materials and Methods:**

[001126] **Bacterial strains:**
  - Methicillin-resistant *S. aureus* ATCC 33591

[001127] **Test articles pre-formulated:**
  - Antibiotic Bactroban Cream (2% Mupirocin) (22 g) Taro Pharmaceuticals, Hawthorne, NY; Exp: 12/2011; Lot# 10055
  - Aqueous gel (Vehicle Control)
  - Bactroban + Placebo (0.1 mL aqueous gel)
  - 0.1 mL Bactroban + 0.1 mL Lithium Carbonate 2.74 mg Li+/g (1.46% w/w Li2C03)
o 0.1 mL Bactroban + 0.1 mL Lithium Carbonate 10.64 mg Li+/g (5.66% w/w Li2C03)
o 0.1 mL Bactroban + 0.1 mL Lithium Carbonate 20 mg Li+/g (11.5% w/w Li2C03)

[001128] **Bacterial Growth Media and Vehicles:**
o Trypticase Soy Agar plates - BBL, Franklin Lakes, NJ
o Brain Heart Infusion (BHI) Broth - BBL, Franklin Lakes, NJ
o Sterile Saline - 0.9%> prepared from powder as needed at ViviSource Laboratories, Inc
o MacConkey Agar plates - BBL, Franklin Lakes, NJ

[001129] **Animals:** Species: mouse, Strain: CD-l(ICR), Source: Harlan Laboratories, Weight: 20 to 30 grams, Number: N=10 per group

[001130] All procedures in this protocol were in compliance with the ViviSource Laboratories IACUC protocol VVSIO-016. Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

[001131] **Protocol/Study Design:**

[001132] **Preparation/Characterization of Formulations.** The Li-C formulations contained on a weight-by-weight basis, 1%> carbomethylcellulose, 0.1 % allantoin, 0.1 % alginate, 5%> glycerol, 5%> citric acid, distilled water and various concentrations of lithium carbonate (0, 1.46%, 2.90%, 5.66%, 11.5% w/w). 2% mupirocin (generic, Taro Pharmaceuticals) was used, as is. The formulations were characterized for viscosity (Brookefield Viscometry) and lithium content (AA Spectroscopy). To ensure that the lithium carbonate hydrogels were compatible with mupirocin, 5 mL of each formulation were mixed and placed on stability for 7 days at 40°C to visually assess if precipitation occurred.

[001133] **Bacteria preparation:** *Methicillin-resistant S. aureus* ATCC 33591 were grown at 37°C under 5% C0₂ on trypticasoy agar (TSA) plates. The bacterial concentration was prepared by re-suspending colonies from the overnight growth on the TSA plate in brain heart infusion (BHI) broth and adjusting a 1:20 dilution of the suspension to achieve an OD (optimal density) of 0.3 at 625nm. The bacterial suspension was then diluted in saline to achieve an inoculum of approximately in 1x10⁸CFU/mL (Colony Forming Units).

[001134] Bacterial counts were performed to determine actual inoculum concentration; 100 μL of the bacterial suspension was serially diluted (1:10) in saline 7 times. Then 100 μL of the 10⁵, 10⁶, and 10⁷ was pipetted in duplicate on to TSA pates with glass beads, then shaken to spread bacteria on plates, then the beads were removed and plates were placed at
37°C with 5% CO₂ overnight, and then counted 24 hours later to determine bacterial concentration of the inoculum the mice received.

[001135] Animal Preparation: The right rear flank of all mice were clipped to 1.5 cm x 1.5 cm size removing fur and then cleaned with 70% alcohol. Mice were anesthetized with an inhalation mixture of 4% Isoflurane and 1.5% oxygen prior to abrasion.

[001136] Dermabrasion Procedure: The skin was abraded to a width/length of 13 mm with a sandpapered dowel to abrade the skin without causing any bleeding, by applying pressure and using a constant motion back forth for up to 15 times. This procedure results in a partial thickness wound (Vivisource data).

[001137] Infection establishment: Mice (ten per group) of weight approximately 25g and age 8-10 weeks were anesthetized with an inhalation of 4% Isoflurane with 1.5% oxygen and fur clipped. The mice were dermabraded using a dermabrader as described in the previous section. The bacterial suspension was pipetted on to the abrasion at a volume of 100µL. After the inoculum was pipetted on the skin, a sterile non-absorbent 13mm disc was placed on the wound then taped in place with dermal cloth tape. This maintains the suspension in direct contact with the wound. Animals were observed frequently after the infection procedure to confirm they were awake from the anesthesia and showing no signs of discomfort or pain from the procedure. No analgesic was provided as the abrasion wound will go no deeper than the dermal layer; literature research confirmed that analgesic was unnecessary for this procedure with an anesthetic, however if at any time during the study an animal had shown signs of serious injury or illness that was unlikely to be the result of the experimental procedures, the affected mice would have been immediately euthanized. Animals were monitored daily for any signs of animals exhibiting severe dyspnea (labored breathing), severe depression, weight loss or other neurological signs. Animals with weight loss exceeding 20% from the time 0, body weight and other signs of obvious distress would have been immediately euthanized. Twenty-four hours post infection the tape and disc was removed, and wound area measured. Animals were weighed daily to ensure the animals were not in pain. Biological burden was verified by bacterial enumeration of the tissue at day 0.

[001138] Treatment: There were 7 groups in total, with 10 mice per group. At 24 hours post infection with MRSA the tape and disc was removed. Mice were weighed once daily prior to first dose. The abrasion/dose sites were scored twice daily using the Skin Irritation Draize Scoring system prior to dose. Mice were dosed a set volume of 0.100 ml topically (using a wiretrol device) twice daily for three days. The dosing per day was spaced 6 hours
apart. If the hydrogel did not spread well on the wound, the tip of the wiretrol device was used to spread the formulation on the wound.

[001139] **Tissue Processing:** Approximately sixteen hours after the last treatment, all mice were euthanized via an inhalation of CO₂. The skin of the animals was aseptically removed, weighed, homogenized in 1 mL of sterile saline and serially diluted in sterile saline five times at 1:10. The five diluted samples were plated on TSA medium for bacterial quantification. After 18 hours of incubation the dilution with countable bacterial colonies was recorded for each animal and entered into raw data with weight of excised abrasion for calculation for logioCFU.

[001140] **Data End-Points:** The average logio CFU per gram of skin was calculated for each group and standard deviations determined. The logio CFU per gram of skin versus control and the logio reductions from the untreated controls were calculated.

[001141] Twenty-four hours after infection was established, erythema and edema in each dose group was assessed twice daily prior to each dose.

[001142] **Results:**

**Anti-microbial Activity:** As shown in Figure 43, topical administration of the combination of 2% mupirocin with Lithium Carbonate gel at 1.46%, 5.66%, and 11.5% in a mouse skin abrasion infection model against MRSA (ATCC 33591) resulted in increased reduction of bacterial loads, respectively, compared to 2% mupirocin alone. The combination groups 5.66% Li-Carb/2% MP and 11.5% Li-Carb/2% MP remarkably reduce MRSA microbial load by 4.61 and 6.02 log reduction of CFU/g skin, compared to untreated, with p<0.05.

13. **EXAMPLE 20: EVALUATION OF LITHIUM CARBONATE AND MUPIROCIN COMBINATION AGAINST MRSA STRAINS IN A MOUSE SKIN ABRASION INFECTION MODEL**

[001143] A Lithium Carbonate formulation in combination with 2% Mupirocin was evaluated for anti-microbial activity in a mouse skin abrasion infection model against two strains of *Methicillin Resistant S. aureus* (MRSA). The study groups were: (a) 2% Mupirocin plus 11.5% Lithium Carbonate gel (b) 2% Mupirocin; and (c) vehicle.

[001144] The pathogen tested was two strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA). The *in vivo* infective model was a CD-I mouse infection, dermabraserion model. Lithium Carbonate vehicle was used as one of the test groups. Each group had 10 mice wounded at day 0 using a dermabrader. The wounds were infected with a MRSA strain 24 hours after infection, the mice were dosed twice daily for three days with 0.1 mL of each
formulation, using a calibrated wiretrol pipette. The infection control group did not receive
treatment. Enumeration of the bio-load on the collected abrasions was assessed five days post
infection.

[001145] Materials and Methods:

[001146] Bacterial strains:
  - *Methicillin-resistant S. aureus* ATCC 33591
  - *Methicillin-resistant S. aureus* NEMC 89-4

[001147] Test articles pre-formulated:
  - Antibiotic Bactroban Cream (2% Mupirocin) (22 g) Taro Pharmaceuticals, Hawthorne, NY; Exp: 12/2011; Lot# 10055
  - Aqueous gel (Vehicle Control)
  - 0.1 mL Bactroban + 0.1 mL Lithium Carbonate 20 mg Li+/g (11.5% w/w Li2CO3)

[001148] Bacterial Growth Media and Vehicles:
  - Trypticase Soy Agar plates - BBL, Franklin Lakes, NJ
  - Brain Heart Infusion (BHI) Broth - BBL, Franklin Lakes, NJ
  - Sterile Saline - 0.9% prepared from powder as needed at ViviSource Laboratories, Inc
  - MacConkey Agar plates - BBL, Franklin Lakes, NJ

[001149] Animals: Species: mouse, Strain: CD-l(ICR), Source: Harlan Laboratories, Weight: 20 to 30 grams, Number: N=10 per group

[001150] All procedures in this protocol were in compliance with the ViviSource Laboratories IACUC protocol VVS10-016. Animal Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Office of Laboratory Animal Welfare.

[001151] Protocol/Study Design:

[001152] Preparation/Characterization of Formulations. The Li-C formulations contained on a weight-by-weight basis, 1%> carbomethylcellulose, 0.1% allantoin, 0.1% alginate, 5%> glycerol, 5%> citric acid, distilled water and various concentrations of lithium carbonate (0, 1.46%, 2.90%, 5.66%, 11.5% w/w). 2% mupirocin (generic, Taro Pharmaceuticals) was used, as is. The formulations were characterized for viscosity (Brookefield Viscometry) and lithium content (AA Spectroscopy). To ensure that the lithium carbonate hydrogels were compatible with mupirocin, 5 mL of each formulation were mixed and placed on stability for 7 days at 40 °C to visually assess if precipitation occurred.
[001153] **Bacteria preparation:** Methicillin-resistant *S. aureus* strains ATCC 3359 and NEMC 89-4 were each grown at 37°C under 5% CO₂ on tryptasesoy agar (TSA) plates. The bacterial concentration of each was prepared by re-suspending colonies from the overnight growth on the TSA plate in brain heart infusion (BHI) broth and adjusting a 1:20 dilution of the suspension to achieve an OD (optimal density) of 0.3 at 625nm. The bacterial suspension was then diluted in saline to achieve an inoculum of approximately in 1x10⁶ CFU/mL (Colony Forming Units).

[001154] Bacterial counts were performed to determine actual inoculum concentration; 100 μL of the bacterial suspension was serially diluted (1:10) in saline 7 times. Then 100 μL of the 10⁶, 10⁷, and 10⁸ was pipetted in duplicate on to TSA pates with glass beads, then shaken to spread bacteria on plates, then the beads were removed and plates were placed at 37°C with 5% CO₂ overnight, and then counted 24 hours later to determine bacterial concentration of the inoculum the mice received.

[001155] **Animal Preparation:** The right rear flank of all mice were clipped to 1.5 cm x 1.5 cm size removing fur and then cleaned with 70% alcohol. Mice were anesthetized with an inhalation mixture of 4% Isoflurane and 1.5% oxygen prior to abrasion.

[001156] **Dermabrasion Procedure:** The skin was abraded to a width/length of 13 mm with a sandpapered dowel to abrade the skin without causing any bleeding, by applying pressure and using a constant motion back forth for up to 15 times. This procedure results in a partial thickness wound (Vivisource data).

[001157] **Infection establishment:** Mice (ten per group) of weight approximately 25g and age 8-10 weeks were anesthetized with an inhalation of 4% Isoflurane with 1.5% oxygen and fur clipped. The mice were dermabraded using a dermabrader as described in the previous section. The bacterial suspension was pipetted on to the abrasion at a volume of 100μL. After the inoculum was pipetted on the skin, a sterile non-absorbent 13mm disc was placed on the wound then taped in place with dermal cloth tape. This maintains the suspension in direct contact with the wound. Animals were observed frequently after the infection procedure to confirm they were awake from the anesthesia and showing no signs of discomfort or pain from the procedure. No analgesic was provided as the abrasion wound will go no deeper than the dermal layer; literature research confirmed that analgesic was unnecessary for this procedure with an anesthetic, however if at any time during the study an animal had shown signs of serious injury or illness that was unlikely to be the result of the experimental procedures, the affected mice would have been immediately euthanized. Animals were monitored daily for any signs of animals exhibiting severe dyspnea (labored breathing),
severe depression, weight loss or other neurological signs. Animals with weight loss exceeding 20% from the time 0, body weight and other signs of obvious distress would have been immediately euthanized. Twenty-four hours post infection the tape and disc was removed, and wound area measured. Animals were weighed daily to ensure the animals were not in pain. Biological burden was verified by bacterial enumeration of the tissue at day 0.

[Treatment: There were 9 groups in total, with 10 mice per group. At 24 hours post infection with MRSA the tape and disc was removed. Mice were weighed once daily prior to first dose. The abrasion/dose sites were scored twice daily using the Skin Irritation Draize Scoring system prior to dose. Mice were dosed a set volume of 0.100 ml topically (using a wiretrol device) twice daily for three days. The dosing per day was spaced 6 hours apart. If the hydrogel did not spread well on the wound, the tip of the wiretrol device was used to spread the formulation on the wound.

[Tissue Processing: Approximately sixteen hours after the last treatment, all mice were euthanized via an inhalation of C0₂. The skin of the animals was aseptically removed, weighed, homogenized in 1 mL of sterile saline and serially diluted in sterile saline five times at 1:10. The five diluted samples were plated on TSA medium for bacterial quantification. After 18 hours of incubation the dilution with countable bacterial colonies was recorded for each animal and entered into raw data with weight of excised abrasion for calculation for logioCFU.

[Data End-Points: The average logio CFU per gram of skin was calculated for each group and standard deviations determined. The logio CFU per gram of skin versus control and the logio reductions from the untreated controls were calculated.

[Results: Anti-microbial Activity: As shown in Figure 44, topical administration of the combination of 2% mupirocin with 11.5% Lithium Carbonate gel in a mouse skin abrasion infection model against MRSA strain ATCC 33591 in two duplicate experiments and also MRSA strain NEMC 89-4 resulted in significantly increased reduction of bacterial load as compared to 2% mupirocin alone (4.88 and 4.11 log reduction of CFU/g skin against untreated at p<0.05, for the Li-C/MP group tested).
14. **EXAMPLE 21: DETERMINATION OF MECHANISM OF ACTION OF LITHIUM CARBONATE IN REDUCING BACTERIAL LOAD**

[001163] In order to investigate the mechanism of action of lithium carbonate, the macromolecular synthesis pathways in *Staphylococcus aureus* were tested for inhibition of either DNA, RNA, lipid, protein, or cell wall synthesis.

[001164] **Materials and Methods:**

[001165] The test compound lithium carbonate was stored at 2 - d°C until assayed. The comparator agents were as follows:

[001166] Table 50: List of comparator agents and storage conditions

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Supplier</th>
<th>Catalog No.</th>
<th>Lot No.</th>
<th>Storage Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>USP</td>
<td>1134335</td>
<td>JOH307</td>
<td>-20°C</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Sigma</td>
<td>V-2002</td>
<td>087K0694</td>
<td>4°C</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>Sigma</td>
<td>R-7382</td>
<td>080M1506V</td>
<td>-20°C</td>
</tr>
<tr>
<td>Linezolid</td>
<td>ChemPacific</td>
<td>35710</td>
<td>CHPC091007-01</td>
<td>4°C</td>
</tr>
<tr>
<td>Cefuroxim</td>
<td>Sigma</td>
<td>C-2389</td>
<td>030M4030</td>
<td>-20°C</td>
</tr>
</tbody>
</table>

[001167] **Test Organism:** *S. aureus* ATCC 29213 was originally acquired from the American Type Culture Collection (Manassas, VA).

[001168] **Macromolecular Synthesis Assays:**

[001169] **Bacteria and growth conditions:** The effect of lithium carbonate on whole cell DNA, RNA, cell wall, protein and lipid synthesis was investigated using *S. aureus* ATCC 29213. Cells were grown at 35°C overnight on Trypticase Soy agar, and this culture was used to inoculate 10 ml of Mueller Hinton Broth II (MHB II). The culture was grown to early exponential growth phase (OD<sub>600</sub> = 0.2 to 0.3) while incubating in a shaker at 35°C and 200 rpm.

[001170] **DNA, RNA, and protein synthesis:** Bacterial cells (100 µi/well) in early exponential phase were transferred to triplicate wells of a 96-well microplate containing either MHB II (DNA and RNA synthesis) or M9 (protein synthesis) minimal medium containing various concentrations of lithium carbonate. Bacterial cells (100 µi/well) were also added to wells containing various concentrations of control antibiotics (5 µi) at 20X the final concentration in 100% DMSO. A 5% DMSO treated culture served as the "no drug" control for all experiments. Following a 5 min incubation at room temperature, either [³H] thymidine (DNA synthesis), [³H] uridine (RNA synthesis) or [³H] leucine (protein synthesis) was added at 0.5-1.0 µCi per reaction, depending on the experiment. Reactions were allowed
to proceed at room temperature for 15-30 min and then stopped by adding 12 μl of cold 5% trichloroacetic acid (TCA; DNA and RNA synthesis) or 5% TCA/2% casamino acids (protein synthesis). Reactions were incubated on ice for 30 min and the TCA precipitated material was collected on a 25 mm GF/A filter. After washing three times with 5 ml of cold 5% TCA, the filters were rinsed two times with 5 ml 100% ethanol, allowed to dry, and then counted using a Beckman LS 3801 liquid scintillation counter.

[001171] **Cell wall synthesis:** Bacterial cells in early exponential growth phase were transferred to M9 minimal medium containing various concentrations of test compound and added to 1.5 ml eppendorf tubes in triplicate (100 μl/tube). Bacterial cells were also added to eppendorf tubes containing various concentrations of control antibiotic (5 μl) at 20X the final concentration in 100% DMSO as described above. Following a 5 min incubation at 37°C, [14C] N-acetylglucosamine (0.4 μCi/reaction) was added to each tube and incubated for 45 min in a 37°C heating block. Reactions were stopped by the addition of 100 μl 8% SDS to each tube. Reactions were heated at 95°C for 30 min in a heating block, cooled, briefly centrifuged, and then spotted onto pre-wet HA filters (0.45 μM). After washing three times with 5 ml of 0.1% SDS, the filters were rinsed two times with 5 ml dH20, allowed to dry, and then counted using a Beckman LS 3801 liquid scintillation counter.

[001172] **Lipid synthesis:** Bacterial cells in early exponential growth phase were transferred to MHB II broth containing various concentrations of test compound and added to 1.5 ml eppendorf tubes in triplicate (100 μl/tube). Bacterial cells were also added to eppendorf tubes containing various concentrations of control antibiotic as described above. Following a 5 min incubation at room temp., [3H] glycerol was added at 0.5 μCi per reaction. Reactions were allowed to proceed at room temperature for 15 min and then stopped through the addition of 375 μl chloroform/methanol (1:2) followed by vortexing for 20 sec after each addition. Chloroform (125 μl) was then added to each reaction and vortexed followed by the addition of 125 μl dH20 and vortexing. Reactions were centrifuged at 13,000 rpm for 10 min, 150 μl of the organic phase was transferred to a scintillation vial and allowed to dry in a fume hood for at least 1 hr. Samples were then counted via liquid scintillation counting.

[001173] **Results:**

[001174] It was determined that the Minimal Inhibitory Concentration (MIC) of lithium carbonate for *S. aureus* ATCC 29213 is 7.018 mg/ml. The macromolecular synthesis inhibition studies were performed using concentrations of lithium carbonate that were equivalent to 0.25-, 0.5-, 1-, 2-, 4- or 8-fold the MIC value.
Figure 45 shows the effect of lithium carbonate on DNA synthesis. Dose-dependent DNA synthesis inhibition occurred from 2- to 8-fold the MIC, reaching a maximum of 88% inhibition. The positive control antibiotic ciprofloxacin inhibited DNA synthesis up to approximately 63% at 8-fold the MIC (0.5 µg/ml).

Lithium carbonate also demonstrated dose-dependent inhibition of protein synthesis (see Figure 46) at concentrations ranging from 1- to 8-fold the MIC value. There was more inhibition at 0.5 than at 1-fold the MIC, though it appears that this was an aberrant result since inhibition at 0.25-fold was more in line with what was observed from 1- to 8-fold. The positive control antibiotic linezolid demonstrated 83% inhibition of protein synthesis at 8-fold the MIC (2 µg/ml).

Figure 47 shows that lithium carbonate inhibited lipid synthesis at all concentrations tested. Dose-dependent inhibition occurred at concentrations ranging from 0.5- to 8-fold the MIC value, reaching maximal inhibition of approximately 85% at the highest concentration tested. There was more inhibition at 0.25-fold than at 0.5 - 2-fold the MIC of lithium carbonate, but his appears to be an aberrant value since there was a nice linear dose-dependent effect at 0.5- to 8-fold the MIC. The positive control inhibitor cerulenin demonstrated dose responsive inhibition of lipid synthesis with approximately 76% inhibition at 8-fold the MIC (32 µg/ml).

In Figure 48 lithium carbonate showed dose-dependent inhibition of cell wall synthesis from 2- to 4-fold the MIC. There was a large increase in inhibition observed from 4- to 8-fold the MIC value, reaching >90% inhibition at the highest concentration tested. It should also be noted that precipitation was observed in reactions conducted in the presence of 4- or 8-fold the MIC of lithium carbonate. It is not known whether the precipitation was lithium carbonate, or some component of the medium. In comparison, the positive control vancomycin showed a maximum of 97% inhibition at 8-fold the MIC (2 µg/ml).

Lithium carbonate showed no significant inhibition of RNA synthesis (see Figure 49). In contrast, rifampicin demonstrated good dose responsive inhibition with maximal inhibition of approximately 84% at 8-fold the MIC (0.015 µg/ml).

Figure 50 represents a comparison of the DNA, lipid, protein and cell wall synthesis inhibition observed in the study. Data plotted included inhibition of lithium carbonate for each pathway at 1-, 2-, 4-, and 8-fold the MIC. Lipid synthesis was the most sensitive reaction, showing approximately 40% inhibition at 1-fold the MIC, while the other pathways showed little or no inhibition with this level of drug added. It is possible that repeating the lipid synthesis reaction with expansion of the lithium carbonate dose to 0.05
and 0.1-fold the MIC may provide additional evidence for showing that lipid synthesis is the most sensitive reaction. Both lipid and DNA synthesis were inhibited 50% at 4-fold the MIC, whereas the protein and cell wall pathways would have reached this level of inhibition somewhere between 4- and 8-fold the MIC of drug. Interestingly, each of the pathways shown in Figure 6 was inhibited approximately 80% at 8-fold the MIC.

[001181] Conclusion:

Lithium carbonate demonstrated dose-dependent inhibition of DNA, lipid, protein and cell wall synthesis, whereas RNA synthesis was not affected. It appears that lipid synthesis may be the most sensitive pathway inhibited, followed by DNA synthesis, and expanding the lower range of lithium carbonate added to the lipid synthesis assay may provide more evidence for this hypothesis. It is possible that lithium carbonate targets a variety of pathways in S. aureus by an unknown mechanism.

15. EXAMPLE 22: EVALUATION OF THE NAIL ABSORPTION OF LITHIUM FORMULATIONS INTO AND THROUGH HUMAN EX VIVO TOE NAILS

[001183] The in vitro Franz human nail finite dose model has proven to be a valuable tool for the study of the absorption and associated pharmacokinetics of topically applied compounds. The model uses ex vivo human nails mounted in specially designed diffusion chambers allowing the nail to be maintained at a temperature and hydration that model typical in vivo conditions (Franz, 1975, J Invest Derm 64:190-195). A finite dose of formulation is applied to the outer surface of the nail, and the absorption of compounds of interest is measured by monitoring their rate of appearance in the receptor solution bathing the inner surface of the nail. Data defining total absorption, rate of absorption, as well as nail content can be determined in this model. The Franz finite dose model, when used for assessing skin percutaneous absorption characteristics, has historic precedent for accurately predicting in vivo pharmacokinetics (Franz, 2008, Pharmacopeial Forum 34:1349-1356; Franz, 2009, Skin Pharmacol Physiol 22:276-286).

15.1 STUDY PRODUCTS, RANDOMIZATION, AND DOSING

REFERENCE FORMULATION

[001184] Penlac® (8% Ciclopirox) Topical Solution
TEST FORMULATIONS

[001185] Test Formulation #1: lithium carbonate (11.5%), carboxymethyl cellulose (2%), citric acid (20%), allantoin (0.120%), benzalkonium chloride (0.2%), sodium thioglycolate (0.1%), urea (0.1%), glycerol (10.4%), water (53.15%), pH 8.

[001186] Test Formulation #2: lithium carbonate (11.5%), carboxymethyl cellulose (2%), citric acid (20%), allantoin (0.120%), benzalkonium chloride (0.2%), papain (0.1%), glycerol (10.4%), water (Q.S.), pH 8.

[001187] Test Formulation #3: lithium carbonate (11.5%), carboxymethyl cellulose (2%), citric acid (20%), allantoin (0.120%), benzalkonium chloride (0.2%), hydrogen peroxide (0.1%), glycerol (10.4%), water (Q.S.), pH 8.

RANDOMIZATION SEQUENCE

[001188] Ex vivo nails from different donors were randomized, and the treatments in each test group were performed on randomly assigned nails. Randomization were documented.

DOSING

[001189] For the Penlac® Reference Formulation, a nominal 10 μL formulation/cm²/nail section was applied topically at each dosing event, by pipette.

[001190] For the Test Formulations (TH-003-070a, TH-003-070b, TH-003-070c), a nominal 100 μL formulation/cm² (nail or cellulose membrane) was applied topically at each dosing event, by pipette. The actual dose volume was adjusted depending upon the nominal chamber size (i.e. 38.5 μL on a 7 mm chamber, or 63.6 μL on a 9 mm chamber).

[001191] For treatment groups receiving iontophoresis, 100 μL/cm² of the relevant Test Formulations were deposited upon the (trimmed) iontophoretic patch, and the patch with formulation was then applied to the nail surface for 15 minutes (at a setting of 0.8 mAmps). Following completion of the iontophoresis, the residual formulation on the nail was wiped off using a dry Kimwipe or cotton-tipped swab (as appropriate) and discarded. The nail was then re-dosed with a nominal 100 μL formulation/cm², by pipette. The actual dose volume was adjusted depending upon the nominal chamber size (i.e. 38.5 μL on a 7 mm chamber, or 63.6 μL on a 9 mm chamber).

[001192] Chambers mounted with cellulose membrane received dose applications at 0 and 6 hours on Study Day 1.

[001193] The nails not designated for iontophoresis were dosed twice per day, approximately 6 hours apart each day, for thirteen (13) sequential days. Dosing was
conducted in the morning (approximately at 9 am) and in the afternoon (approximately at 3 pm) each day.

15.2 STUDY PROCEDURES

DIFFUSION CELL AND NAIL/CELLULOSE MEMBRANE PREPARATION

[001194] Human, ex vivo, big toe nails without obvious signs of nail disease or cracks, were used in this study. Following receipt the nails were sealed in a water-impermeable bag and stored at ~ -20°C, until the day of the experiment. Prior to use they were thawed to room temperature, cleared of underlying soft tissue, and then rinsed in water to remove any adherent blood or other material from the surfaces. Following trimming, the nails were soaked at 32°C overnight prior to mounting onto the diffusion cells by immersion in 0.1 × Phosphate Buffered Saline (PBS) with 0.008% Gentamicin (the pH was recorded but not adjusted).

[001195] The nail length and width dimensions, thickness, and weight measurements were documented pre-hydration and post-hydration, before mounting onto the chambers.

[001196] The nails were fitted to nominal 7 or 9 mm diameter modified Franz Diffusion cells and secured in place with silicone sealant. The receptor compartment was filled to capacity with the designated receptor solution. The cells were then placed in a water bath set to achieve a nail surface temperature of 32.0 ± 1.0°C.

[001197] Two (2) of the chambers were mounted with Cellulose dialysis tubing membrane (e.g. MWCO of- 12,000).

SAMPLE COLLECTION

[001198] At each reservoir sampling event, the receptor solution was removed in its entirety, replaced with stock receptor solution, and a pre-determined volume aliquot (e.g. 4 mL) retained. All reservoir samples were dried down using a vacuum centrifugation process.

SAMPLE STUDY SEQUENCE BY DOSING TREATMENT

[001199] Cellulose Membrane Chambers (2 Chambers)

1. Pre-dose receptor sample collected and reservoir re-filled
2. Membranes dosed with nominal 100 µL/cm² (0 hour)
3. Membrane surface wiped with dry Kimwipe or cotton-tipped swab at t = 5.45 hours (wipe discarded)
4. Receptor samples collected and re-filled following wipe
5. Second dose application at t=6 hr with nominal 100 µL/cm²
6. Receptor samples collected at 24 hours
7. Chambers dismounted and membrane discarded

[001200] **Toe Nail Chambers Dosed with the Penlac® Reference Formulation (2 Chambers)**

**Day 1**
1. Pre-dose receptor samples collected and reservoir re-filled
2. Nail dosed with nominal 10 µL/cm² in morning (0 hour)

**Days 2 - 13**
1. Receptor samples collected prior to morning dose and reservoir re-filled
2. Approximately 15 minutes prior to dose application, nail surface to be washed with 1% Sodium Lauryl Sulfate (SLS) solution using cotton-tipped swabs (wash discarded)
3. Nail surface wiped with dry Kimwipe or cotton-tipped swab (wipe discarded)
4. Nail re-dosed with nominal 10 µL/cm² for that morning's dose

**Day 14**
1. Receptor samples collected
2. Nail dismounted
3. Nail surface washed with 1% SLS using cotton-tipped swabs (wash discarded).
4. Membrane surface wiped with dry Kimwipe or cotton-tipped swab (wipe discarded)
5. Nail sectioned

[001201] **Toe Nail Chambers Receiving Test Formulations and No Iontophoresis Treatment (6 Chambers)**

**Day 1**
1. Pre-dose receptor samples collected and reservoir re-filled
2. Nail dosed with nominal 100 µL/cm² in morning (0 hour)
3. Receptor samples collected at 6 hours following morning dose and re-filled
4. Approximately 15 minutes prior to dose application the nail surface is to be washed with 1% SLS using cotton-tipped swabs (wash discarded)
5. Nail surface wiped with dry Kimwipe or cotton-tipped swab (wipe discarded)
6. Nail re-dosed with nominal 100µL/cm² (afternoon dose)

**Day 2 - 13**
1. Pre-dose receptor samples collected and reservoir re-filled
2. Approximately 15 minutes prior to dose application the nail surface is to be washed with 1% SLS using cotton-tipped swabs (wash discarded)
3. Nail surface wiped with dry Kimwipe or cotton-tipped swab (wipe discarded)
4. Nail dosed with nominal 100 µL/cm² in morning (~ 18 hours following prior afternoon dose)
5. Receptor samples collected at 6 hours following morning dose and re-filled
6. Approximately 15 minutes prior to dose application the nail surface is to be washed with 1% SLS using cotton-tipped swabs (wash discarded)
7. Nail surface wiped with dry Kimwipe or cotton-tipped swab (wipe discarded)
8. Nail re-dosed with nominal 100µL/cm² (afternoon dose)

**Day 14**
1. Pre-dose receptor samples collected
2. Nail dismounted.
3. Nail surface washed with 1% SLS using cotton-tipped swabs (wash discarded)
4. Nail surface wiped with Kimwipe or cotton-tipped swab (wipe discarded)
5. Nail sectioned
[001202] Toe Nail Chambers Receiving Test Formulations and Iontophoresis

Treatment (6 Chambers)

Day 1
1. Pre-dose receptor samples collected and re-filled
2. 100 µL of Test Product applied to trimmed iontophoresis pads.
3. Iontophoresis pads with test Product applied nails.
4. Iontophoretic treatment at lmAmp for 15 minutes (0 hour).
5. Nail surface wiped with dry Kimwipe or cotton-tipped swab (wipe discarded)
6. Nail re-dosed with nominal 100 µL/cm²
7. Receptor samples collected at 6 hours following morning dose and re-filled
8. Approximately 15 minutes prior to dose application nail surface is to washed with 1% SLS using cotton-tipped swabs (wash discarded)
9. Nail surface wiped with dry Kimwipe or cotton-tipped swab (wipe discarded)
10. Nail re-dosed as described in steps 2-6 above (afternoon dose)

Day 2 - 13
1. Pre-dose receptor samples collected and reservoir re-filled
2. Approximately 15 minutes prior to dose application nail surface is to washed with 1% SLS using cotton-tipped swabs (wash discarded)
3. Nail surface wiped with dry Kimwipe or cotton-tipped swab (wipe discarded)
4. 100 µL/cm² of Test Product applied to trimmed iontophoresis pads.
5. Iontophoresis pads with test Product applied nails.
6. Iontophoretic treatment at lmAmp for 15 minutes (0 hour).
7. Nail surface wiped with Kimwipe or cotton-tipped swab (wipe discarded)
8. Nail re-dosed with nominal 100 µL/cm²
9. Receptor samples collected at 6 hours following morning dose and re-filled
10. Approximately 15 minutes prior to dose nail surface to be washed with 1% SLS using cotton-tipped swabs (wash discarded)
11. Nail surface wiped with dry Kimwipe or cotton-tipped swab (wipe discarded)
12. Nail re-dosed as described in steps 2-6 above (afternoon dose)

Day 14
1. Receptor samples collected
2. Nail dismounted.
3. Nail surface washed with 1% SLS using cotton-tipped swabs (wash discarded)
4. Nail surface wiped with Kimwipe or cotton-tipped swab (wipe discarded)
5. Nail sectioned

[001203] All samples were stored at ~ -20°C before analysis.

ANALYSIS

[001204] The nails were intact with thicknesses ranging from 0.200 cm-0.400 cm. Drug flux was calculated using the formula: µg/cm²/h, to calculate transport through the nail.

[001205] For data analysis, the variables analyzed were: (a) effect of nail thickness on flux, (b) Effect of formulation on flux, (c) Iontophoresis versus no iontophoresis, (d) drug in receptor fluid, (e) drug remaining in nail.
[001206] Drug Flux was plotted as Li+ Flux (g/cm²/h) versus time in hours.

RESULTS

[001207] Figure 51 shows the Franz Cell set-up for diffusion studies. Figure 52 shows Li+ flux from formulation TH-003-070a, as a function of diffusion time through the nail, as a function of nail thickness, with red bars denoting Li+ flux through a nail of thickness of 0.448 cm and blue bars denoting Li+ flux through a nail of thickness of 0.04775 cm. The nails received no iontophoretic treatment. The samples for analysis were taken as follows:

(a) Day x-1: 6 hours after the 1st dose of that day
(b) Day x-2: 18 h after the 2nd dose of that day.

[001208] Figure 53 shows Li+ flux from formulation TH-003-070b, as a function of diffusion time through the nail, as a function of nail thickness, with red bars denoting Li+ flux through a nail of thickness of 0.0625 cm and blue bars denoting Li+ flux through a nail of thickness of 0.248 cm. The nails received no iontophoretic treatment. The samples for analysis were taken as follows:

(a) Day x-1: 6 hours after the 1st dose of that day
(b) Day x-2: 18 h after the 2nd dose of that day.

[001209] Figure 52 and Figure 53 demonstrate the effect of nail thickness on Li+ flux. For both formulations TH-003-070a and TH-003-070b, the Li+ flux through the thicker nails were slower, indicating a longer diffusion path through the nail. The Li+ fluxes increase over time for both thicknesses and both formulations, indicating saturation of the nail membrane at the later time-points may result in a high Li+ flux.

[001210] Figure 54A and Figure 54B demonstrate the effect of formulation composition on Li+ flux. Figure 54A compares the flux between formulations TH-003-070a, TH-003-070b and TH-003-070c. Sodium thioglycolate and urea, both well-known as keratinolytic agents were ingredients present in TH-003-070a, but were not present in TH-003-070b and TH-003-070c. Papain, a keratin-dissolving agent, was present in TH-003-070b, but was not present in TH-003-070a and TH-003-070c. Formulation TH-003-070c had hydrogen peroxide as the unique ingredient. The flux for the formulations was in the trend: TH-003-070a>TH-003-070b>TH-003-070c.

[001211] Figure 54B shows percent Li+ permeated per time interval, for all three formulations, without iontophoresis. Even for the formulation with the highest flux (TH-003-070a), the percent Li+ permeated was low and did not exceed 10%. One possibility is that the
positively charged lithium ion could be bound to the negatively charged keratin fibers of the nail plate, thus creating a local drug delivery depot.

[001212] Lithium concentrations in the nail plates for formulations TH-003-070a, TH-003-070b and TH-003-070c, with no iontophoretic treatment, were measured. The lithium concentration in the nails for all formulations ranged from 2-4.5 mg Li^+/mL, demonstrating lithium ion build-up in the nail plates, regardless of formulation type.

[001213] Figure 55A and Figure 55B demonstrate the effect of iontophoresis on Li^+ flux. Iontophoresis at 0.8 mA/min for 15 minutes, was applied to the nail, using an lomed Phoresor and Optima gel-pads with Ag/AgCl electrodes. The gel pads were cut down to size to fit the diffusion cells. The formulation was applied on the hydrated nail plate at a volume of 0.1 mL. The gel pad was applied on top of the formulation. One electrode was placed on the top of the gel pad and the other was placed in the receptor chamber. After iontophoresis was completed, the formulation and the gel pad were kept in place for 6 hours, at which time the receptor fluid is retrieved as the analytical sample (Day 1-1) and the formulation is re-applied with iontophoretic treatment. The next time-point was 18 hours after the first dose. At this time-point, the receptor fluid was retrieved as the next analytical sample (Day 1-2) and the formulation was re-applied with iontophoretic treatment. The next time-point was 6 hours later (Day 2-1), with the same regimen of sampling and formulation administration.

[001214] Figure 55A shows a comparison of diffusive flux of Li^+ via formulation TH-003-070a with, and without iontophoresis. The Li^+ flux was enhanced dramatically with iontophoretic treatment. Figure 55B shows the percent Li^+ permeation using via formulation TH-003-070a over each time interval. The percent Li^+ permeation increased with time, indicating membrane saturation at latter time-points. The receptor liquid at each time-point had lithium levels between 0.03-0.40 mg/ml. In contrast, lithium levels present in nails were much higher, suggesting binding of lithium ion to the negatively charged keratin.

[001215] Figure 55C shows a comparison of diffusive flux of Li^+ across the nail plate with iontophoresis, plotted as a function of formulation composition (TH-003-070a, TH-003-070b and TH-003-070c). Formulation TH-003-070a and TH-003-070b showed higher Li^+ flux than formulation TH-003-070c. This trend was identical to that seen with formulations administered without iontophoresis. Briefly, formulation TH-003-070a had sodium thioglycolate and urea as unique ingredients. Formulation TH-003-070b had papain as the unique ingredient and TH-003-070c had hydrogen peroxide as the unique ingredient.

[001216] Lithium levels deposited in the nail plates across all groups, with and without iontophoresis, were measured. The nails were homogenized and extracted with hydrochloric
acid to extract lithium. The homogenates were analyzed by atomic absorption spectroscopy to determine lithium levels. Groups that received iontophoresis had lithium levels between 7-17 mg Li⁺/mL. Formulation TH-003-070a had the highest levels of lithium (15-17 mg Li⁺/mL). Formulation TH-003-070c had the lowest levels of lithium (7-8 mg Li⁺/mL). Formulation TH-003-070b had lithium levels between 11-13 mg Li⁺/mL. Figure 55D shows the Li⁺ concentrations in nail and nail bed using formulation TH-003-070a over each time interval. Based on these data, Formulation TH-003-070a was selected as the formulation to test in an in-vitro infected nail model.

16. **EXAMPLE 23: DETERMINATION OF THE EFFICACY OF LITHIUM FORMULATIONS IN AN IN VITRO NAIL INFECTION MODEL USING FULL THICKNESS HUMAN NAILS**

[001217] An in-vitro infected nail study was conducted using full thickness human nails infected with Trichophyton rubrum. Formulation TH-003-070a was used for this study, administered with and without, iontophoresis and compared to Penlac, the current standard of care for onychomycosis. Placebo vehicle was used as one of the controls of the experiments.

16.1 **MATERIALS:**

[001218] Test item 1: Active Formulation TH-003-070a (lithium carbonate (11.5%), carboxymethyl cellulose (2%), citric acid (20%), allantoin (0.120%), benzalkonium chloride (0.2%), sodium thioglycolate (0.1%), urea (0.1%), glycerol (10.4%), water (53.15%), pH 8)

[001219] Test item 2: Placebo Formulation (TH-03-070a matching vehicle with no lithium carbonate, carboxymethyl cellulose (2%), citric acid (20%), allantoin (0.120%), sodium thioglycolate (0.1%), urea (0.1%), glycerol (10.4%) and distilled water (Q.S.), pH balanced to 8)

[001220] Test item 3: Comparator formulation (PenLac®)

16.2 **TEST SYSTEMS:**

**T. RUBRUM**

[001221] A Sabouraud dextrose agar slope was inoculated with *Trichophyton rubrum* (*T. rubrum*, a clinical isolate) which was originally isolated from an onychomycotic patient. The organism had been sub-cultured on a three monthly basis to maintain viability and the cultures stored at 25°C for seven days after sub-culturing, and then stored 2-8°C until required.
CHUBTUR® TEST SYSTEMS

[001222] The ChubTur® test system was designed and trade marked by MedPharm, and made for MedPharm by an external glass manufacturer. The ChubTur® cells were cleaned, assembled and assembled.

16.3 METHODS:

CHUBTUR® INFECTED NAIL ASSAY

Preparation of Sabouraud dextrose agar (SDA) plate

[001223] Powdered SDA (65 ± 0.1 g) was added to 1 L of MilliQ de-ionised water. The mixture was heated until the agar visibly dissolved. The mixture was then sterilised at 121 ± 2°C for 15 min in an autoclave. After removing the molten agar from the autoclave it was allowed to cool to 50 ± 3°C, before transferring 25 mL into sterile 90 mm Petri dishes. The Petri dishes were left with the lids slightly ajar (ca. 1 cm opening) for 30 min under a laminar flow cabinet and then stored at 2-8°C before use.

Preparation of Ringer's solution

[001224] One Ringer's solution tablet was added to 1 L of MilliQ de-ionised water. The mixture was stirred until the tablet had visibly dissolved. The solution was then sterilised at 121 ± 2°C for 15 min in an autoclave. After removing the solution from the autoclave it was allowed to cool before storage at 2-8°C for no more 7 days before use.

Preparation of a suspension of T. rubrum

[001225] A 90 mm SDA plate (prepared as described above) was seeded with T. rubrum mycelium and spores using a sterile swab removed from a slope culture and transferred onto the surface of the agar. The agar plate was then incubated at 25°C for 7 days. The white spores were then washed from the surface of the plate with Ringers solution (20 mL). The spore suspension was filtered through a sterile gauze (Smith+Nephew, Propax, 7.5 cm x 7.5 cm 8 ply gauze swab, BP Type 13) to remove mycelium and agar debris. A sample of the resulting suspension was removed and diluted 1/10 in Ringer's solution and the absorbance measured on the UV spectrophotometer. The density of the suspension was adjusted to 10^7 CFU/mL according to the 1/10 target absorbance value of 0.1 to 0.15 at 600 nm for T. rubrum. A viable count of the spore suspension was performed to confirm the concentration of 10^7 CFU/mL.
TCCT<sup>TM</sup> INFECTED NAIL SETUP PROCEDURE

Nail preparation

[001226] Prior to cutting the nails into 3 mm x 3 mm segments, the nails were removed from storage at -20°C and placed in a laminar flow cabinet for 30 min to equilibrate at room temperature. Following this the nails were cut into 3 mm x 3 mm segments and washed individually as follows:

[001227] (i) The nails were initially heated to 60°C in sterile Ringer's solution for 15 min to eliminate any background ATP. The nails were then immersed into 10 mL of 70% v/v ethanol in water solution and vortex mixed for 1 min. The ethanol solution was decanted and replaced with fresh 70% v/v ethanol solution and vortex mixed for a further minute.

[001228] (ii) The ethanol solution was decanted and replaced with sterile Ringer's solution, vortex mixed for 1 min and decanted and replaced with fresh sterile Ringer's. This process of washing with Ringer's was carried out three times, replacing the wash solution each time.

[001229] (iii) Once the washing process was complete, the nails were placed into a sterile Petri dish without a lid and air dried under a laminar flow cabinet for 30 min at room temperature. Once the nails were completely dry, nail thickness measurements were recorded using a pair of callipers which had been wiped completely with 70% v/v ethanol in water solution ensuring the callipers were dry before use. Individual thickness is shown in Table 51.

[001230] (iv) Each nail was placed in a sterile 96 well microtitre plate. The thickness of each nail along with its position in the 96 well plate was recorded.

Preparation of ChubTur® cells

[001231] The ChubTur® cells were assembled and used as follows:

[001232] (i) Following the nail preparation described above, the full thickness toe nails were infected on the underside (the side opposite to where the test formulation is applied) using T. rubrum cell suspension (5 μL of approximately 1x10<sup>7</sup> CFU/mL) prepared above.

[001233] (ii) The organism was allowed to dry on the nail for 30 min under a laminar flow cabinet. Once the nail had dried, it was mounted onto the nail gasket within the cell (Figure 56A). The receiver chamber of the cell was then filled with an inert humidity control fluid (sterile Ringers solution) and the top half of the cell was secured with Parafilm® before the cell was incubated at 25°C for 14 days.

[001234] (iii) Following 14 days post infection the ChubTur® cells were removed from incubation at 25°C and the formulations were applied to the surface using the dosing
procedures listed below, in which the active (Test item 1 and Test item 3) and placebo (Test item 2) formulations were applied to the nail as shown in Figure 56A opposite to where the nails were inoculated with the organism suspension (refer to Figure 56A).

**Iontophoresis setup**

[001235] A pre-calibrated iontophoretic device was used. The positive electrode for the iontophoresis device was placed in the formulation onto the surface of the nail and the negative electrode was mounted into the gasket of the ChubTur® cell such that there was contact with the underside of the nail, but ensuring there was no contact with the organism. The negative electrode was mounted into the cell at the start of the investigation and left in position for the duration of the assay. The iontophoresis dosage applied to the nail was 0.8 mA for 15 min (12 mAmin dosage) using the dosing procedures listed below.

**DOSING REGIME**

[001236] The dosing regimen employed for each treatment group has been summarised in Group 1 to Group 5.

**Group 1**

[001237] (i) 0.1 mL of the active formulation (Test item 1) was placed onto the surface of the nail (Figure 56A).

[001238] (ii) The iontophoresis was then applied as described.

[001239] (iii) The iontophoresis positive electrode and formulation were then removed, and 0.1 mL of formulation (Test item 1) was re-applied as described in Step (i).

[001240] (iv) 6 h after applying the first iontophoresis dose, a second dose was applied and step (iii) repeated.

[001241] (v) The iontophoresis dosing was performed once in the morning and then a second dose 6 h later for 14 days.

[001242] (vi) The ChubTur® cells were returned immediately to the incubator following each dosage of formulation.

**Group 2**

[001243] (i) 0.1 mL of the active formulation (Test item 1) was placed onto the nail (Figure 56A).

[001244] (ii) 6 h after applying the first dose, it was removed and a second dose was applied.

[001245] (iii) Dosing was performed once in the morning and then a second dose 6 h later for 14 days.
[001246] (iv) The ChubTur® cells were returned immediately to the incubator following each dosage of formulation.

Group 3

[001247] (i) 5 μL of the comparator formulation (Test item 3) was placed onto the surface of the nail and allowed to dry for 30 s before placing the ChubTur® cell back into the incubator.

[001248] (ii) The comparator formulation (Test item 3) was applied once every day at the same time of day for 14 days, without removing the formulation between each dose, with the exception of day 7, when the previous 7 days of formulation were removed using ethanol cotton buds.

[001249] (iii) Following drying the ChubTur® cells were returned immediately to the incubator after each dosage of formulation.

Group 4

[001250] (i) 0.1 mL of the placebo formulation (Test item 2) was placed onto the surface of the nail (Figure 56A).

[001251] (ii) The iontophoresis was then applied as described.

[001252] (iii) The iontophoresis positive electrode and formulation were then removed, and 0.1 mL of formulation (Test item 2) was re-applied as described in Step (i).

[001253] (iv) 6 h after applying the first iontophoresis dose, a second dose was applied and step (iii) repeated.

[001254] (v) The iontophoresis dosing was performed once in the morning and then a second dose 6 h later for 14 days.

[001255] (vi) The ChubTur® cells were returned immediately to the incubator following each dosage of formulation.

Group 5

[001256] The infected controls were inoculated with the organism, however they were not treated with any test sample over the 14 day treatment period described for Groups 1 to 4. A non infected control was also included which represents total kill of all of the T. mbrum in the nail.

ATP ANALYSIS

[001257] After the dosing period (14 days post application of formulations), the ChubTur® cells were removed from incubation. The nails were dismantled from the gasket of the
ChubTur® cells ensuring the nails have been completely cleaned of all residual formulation by employing the following steps:

[001258] (i) The surfaces of the nails were gently wiped with a cotton swab dampened in ethanol (e.g. all excess solution was removed from swab) as quickly as possible. This procedure was repeated 4 times using four separate swabs with ethanol.

[001259] (ii) Following step (i), the surface of the nail was wiped with sterile water twice using two separate pre-soaked cotton swabs and then a final dry swab to wipe the surface of the nail to remove any moisture. The nails were then stored at -20°C until required.

[001260] (iii) All the nails were analysed for the presence of ATP from the viable fungi.

16.4 RESULTS AND CONCLUSION

[001261] Figure 56B compares the percentages of ATP recovered (mean ± range) following the dosing regimen summarised above for 14 days against T. rubrum. Data has been normalised for nail thickness (Table 51) and presented as a percentage of the infected control, as presented in Table 54. Table 52 represents the ATP recovered after treatment with the test formulations (data has not been normalised for nail thickness). Table 53 represents the ATP recovered after treatment with test formulations in which data has been normalised for nail thickness (Table 51).

[001262] Figure 56B indicates without iontophoresis the active formulation achieves a 93.34% decrease in viable organisms (6.64 % recovery of ATP) while the results for Penlac® are consistent with previous investigations with a ca. 71 % decrease in viable organisms.

[001263] Figure 56B also indicates that there was complete kill of the organisms as the result of the iontophoresis, with the active (Test item 1) and placebo (Test item 2) formulations. On completion of the dosing prior to performing the ATP assay, it was observed while dismantling the nails from the cells that there was a distinct colour change in all of the nails treated with iontophoresis which was not observed upon initiation of the dosing procedure or the nails without iontophoresetic treatment. It is hypothesised that due to the high electrical resistance across the nail, the cumulative heat generated which may have inadvertently lead to the killing of the organisms and nail discolouration.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nail thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=1</td>
</tr>
<tr>
<td>Group 1</td>
<td>0.5</td>
</tr>
<tr>
<td>Group</td>
<td>ATP recovered (AU)</td>
</tr>
<tr>
<td>-------</td>
<td>------------------</td>
</tr>
<tr>
<td>Group 1 (Active with iontophoresis)</td>
<td>6</td>
</tr>
<tr>
<td>Group 2 (Active without iontophoresis)</td>
<td>864.6</td>
</tr>
<tr>
<td>Group 3 (Placebo with iontophoresis)</td>
<td>4.8</td>
</tr>
<tr>
<td>Group 4 (Penlac® without iontophoresis)</td>
<td>802.2</td>
</tr>
<tr>
<td>Infected control</td>
<td>4275.2</td>
</tr>
<tr>
<td>Non-infected control</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Table 52. ATP recovered after treatment with the Test items.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Luminescence (AU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=1</td>
</tr>
<tr>
<td>Group 1 (Active with iontophoresis)</td>
<td>7.2</td>
</tr>
<tr>
<td>Group 2 (Active without iontophoresis)</td>
<td>864.6</td>
</tr>
<tr>
<td>Group 3 (Placebo with iontophoresis)</td>
<td>5.76</td>
</tr>
<tr>
<td>Group 4 (Penlac® without iontophoresis)</td>
<td>962.64</td>
</tr>
<tr>
<td>Infected control</td>
<td>5130.24</td>
</tr>
<tr>
<td>Non-infected control</td>
<td>6.24</td>
</tr>
</tbody>
</table>

Table 53. ATP recovered after treatment with the Test items normalised for nail thickness (Table 6).
Table 54. % ATP recovered compared to infected control after treatment with the Test items, following data normalised for nail thickness (Table 8)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% recovered compared to infected control</th>
<th>Means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=1</td>
<td>N=2</td>
</tr>
<tr>
<td>Group 1 (Active with iontophoresis)</td>
<td>0.1658</td>
<td>0.4468</td>
</tr>
<tr>
<td>Group 2 (Active without iontophoresis)</td>
<td>19.9110</td>
<td>0.0055</td>
</tr>
<tr>
<td>Group 3 (Placebo with iontophoresis)</td>
<td>0.1326</td>
<td>0.1050</td>
</tr>
<tr>
<td>Group 4 (Penlac® without iontophoresis)</td>
<td>22.1688</td>
<td>48.3999</td>
</tr>
<tr>
<td>Infected control</td>
<td>118.1451</td>
<td>91.9361</td>
</tr>
<tr>
<td>Non-infected control</td>
<td>0.1437</td>
<td>0.1050</td>
</tr>
</tbody>
</table>

17. **EXAMPLE 24: WOUND DATA**

[001264] As shown in Figures 75 and 76, formulations of topical lithium 8% and 16% decrease the area of healed wounds in the FTE experiments. Figure 76 shows that treatment with LiCl 8% leads to an increased number of NHF; treatment with LiCl 8% or Lithium gluconate 16% result in the same, reduced, total wound area; treatment with LiCl 8% results in increased % of wound coverage by new hair follicles compared to Lithium gluconate 16%. Together, these data show that an increased number of neogenic hair follicles is correlated to the decrease in wound area in 8% lithium treated animals, but while decreased wound area was observed in the 16% lithium treated animals, this was not correlated to an increase in hair follicles.

[001265] Topical lithium treatment also appeared to reduce scarring, as shown by histology results showing an increased regeneration zone (reduced scar zone) with topical 8% lithium gluconate treatment (Figure 77).

2005, "Multiple roles for elastic fibers in the skin," *J.Histochem.Cytochem.* 53:431-443) and elastogenic cells have been detected in close apposition to hair follicles (Starcher et al, 1999, "UVB irradiation stimulates deposition of new elastic fibers by modified epithelial cells surrounding the hair follicles and sebaceous glands in mice," *J.Invest.Dermatol.* 112:450-455). The FTE mouse model of wounding and hair follicle neogenesis is useful for studying factors that modulate normal skin regeneration versus scar formation because the healed wound has a central zone of skin regeneration surrounded by a peripheral scar zone. Using elastin immunohistochemistry of FTE wounded mouse skin, it was found that the scar zone lacks both elastin and neogenic hair follicles, unlike the regeneration zone which has both, suggesting that hair follicle neogenesis may support wound healing by reduced scarring by enhancing the deposition of elastin in the healing wound. (Figure 78).

[001267] In summary, this example shows that in acute full thickness wounds in mice, lithium gluconate to promotes wound healing with reduced scarring. Lithium treatment increases hair follicle neogenesis and may concomitantly generate important extracellular matrix components, such as elastin, both of which are missing in scars.
CLAIMS

1. A method for treating and/or preventing a microbial infection and/or preventing microbial colonization, comprising administering a lithium compound in combination with mupirocin.

2. A method for treating and/or preventing acne comprising administering a lithium compound.

3. The method of claim 1 or 2, wherein the lithium compound is lithium carbonate.

4. The method of claim 1 or 2, wherein the lithium compound is administered at a concentration of 1.46% w/w lithium carbonate.

5. The method of claim 1 or 2, wherein the lithium compound is administered at a concentration of 5.66% w/w lithium carbonate.

6. The method of claim 1 or 2, wherein the lithium compound is administered at a concentration of 11.5% w/w lithium carbonate.

7. The method of any one of the preceding claims wherein 2% mupirocin is administered.

8. The method of claim 1 wherein the microbial infection is a bacterial infection.

9. The method of claim 8, wherein the bacterial infection is caused by *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Propionibacterium acnes*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Streptococcus pyogenes*, *corynebacterium species*, enterococci, *Proteus mirabilis*, group D streptococci, *other gram-positive aerobes*, or *Bacteroides fragilis*.

10. The method of claim 8, wherein the bacterial infection is caused by Methicillin-resistant *Staphylococcus aureus*.

11. The method of any one of claims 8-10, wherein the bacteria is present in a biofilm.
12. The method of any one of claims 1 and 3-7, where in the microbial infection is a fungal infection.

13. The method of claim 12, wherein the fungal infection is caused by *Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans, Microsporum gypseum, Microsporum gypseum, Microsporum canis, Epidermophyton floccosum, Candida albicans, and Candida parapsilosis, Malassezia furfur, or Aspergillus fumigatus.*

14. The method of claim 12 or 13, wherein the fungi is present in a biofilm.

15. The method of any one of claims 1 and 3-7, wherein the colonization is bacterial colonization.

16. The method of claim 15, wherein the colonization is caused by *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Propionibacterium acnes, Staphylococcus epidermidis, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae, Streptococcus pyogenes, corynebacterium species, enterococci, Proteus mirabilis, group D streptococci, other gram-positive aerobes, or Bacteroides fragilis.*

17. The method of claim 15, wherein the colonization is caused by Methicillin-resistant *Staphylococcus aureus.*

18. The method of any one of claims 1 and 3-7, wherein colonization is fungal colonization.

19. The method of claim 18, wherein the colonization is caused by *Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton tonsurans, Microsporum gypseum, Microsporum gypseum, Microsporum canis, Epidermophyton floccosum, Candida albicans, and Candida parapsilosis, Malassezia furfur, and/or Aspergillus fumigatus.*

20. A composition comprising a lithium compound and mupirocin.

21. The composition of claim 20, wherein the lithium compound is lithium carbonate.

22. The composition of claim 21, wherein the lithium compound is present at a concentration of 1.46% w/w lithium carbonate.
23. The composition of claim 21, wherein the lithium compound is present at a concentration of 5.66% w/w lithium carbonate.

24. The composition of claim 21, wherein the lithium compound is present at a concentration of 11.5% w/w lithium carbonate.

25. The composition of any one of claims 20-24, wherein 2% mupirocin is present.

26. The composition of any one of claims 20-24, suitable for use as a hand sanitizer.

27. The composition of any one of claims 20-24, suitable for use as a skin sanitizer.

28. The composition of any one of claims 20-24, suitable to disinfect surfaces.

29. The composition of any one of claims 20-28 further comprising chlorhexidine, iodine, or povidone-iodine.

30. The method of claim 2 further comprising administering sulfonamides, trimethoprim/sulfamethoxazole, dapsone, sulfapyridine, sulfasalazine, tetracycline (tetracycline natural products, tetracycline semisynthetic compounds, or chemically modified tetracylines), minocycline, macrolides, erythromycin, ampicillin, ciprofloxacin, cephalosporin, clindamycin, doxycycline, erythromycin stearate, erythromycin base, doxycycline hyclate, oxytetracycline, lymecycline, trimethorprim, cotrimoxazole, oxytetracycline, quinolones, aminoglycosides, chloramphenicol; adapalene (Differin), tazarotene (Tazorac), tretinoin (Retin-A), tretinoin microsphere (Retin-A micro), isotretinoin (Accutane), arotinoid, MDI-301, retinaldehyde, retinol, bexarotene, motretinide, retinoyl-b-glucuronide; norethidrone, norethidrone acetate, ethynodiol diacetate, norgestimate, cyproterone acetate/ethinyl estradiol, spironolactone, flutamide, levonorgestrel, drospirenone, chlormadinone; benzoyl peroxide, azelaic acid (Azelex), sulfacetamide (Klaron), sulfacetamide-sulfur (Sulfacet-R), salicylic acid, alpha hydroxy acids, botanicals, resorcinol, aluminum chloride, zinc, nicotinamide, complementary and alternative medications, corticosteroids (such as, e.g., Dermatop®), NTHEs, COX-2 inhibitors, adrenocorticoids, beclomethasone, budesonide, flunisolide, fluticasone, triamcinolone, methylprednisolone, prednisolone, prednisone, hydrocortisone, or non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, diclofenac, and COX-2 inhibitors).
31. A pharmaceutical composition formulated for topical administration, comprising lithium carbonate and a pharmaceutically acceptable carrier or excipient.

32. The pharmaceutical composition of claim 31, formulated as a hydrogel comprising 1.46% w/w lithium carbonate.

33. The pharmaceutical composition of claim 31, formulated as a hydrogel comprising 5.66% w/w lithium carbonate.

34. The pharmaceutical composition of claim 31, formulated as a hydrogel comprising 11.5% w/w lithium carbonate.

35. The pharmaceutical composition of any one of claims 31 to 34 for treating and/or preventing a microbial infection and/or preventing microbial colonization.

36. The pharmaceutical composition of claim 35 for administration in combination with mupirocin.

37. The pharmaceutical composition of claim 36, wherein 2% mupirocin is present.

38. Use of a lithium compound in the manufacture of a medicament for treatment and/or prevention of a microbial infection and/or prevention of microbial colonization.

39. The use of claim 38, wherein the lithium compound is for administration in combination with mupirocin.

40. Use of a lithium compound in the manufacture of a medicament for treatment and/or prevention of acne.

41. The use of any one of claims 38 to 40, wherein the lithium compound is lithium carbonate.
Fig. 1
**Fig. 2A – 2B**
Fig. 2C – 2D
**Fig. 3**

Time Kill Log reduction of CFUs/mL of MRSA

**Fig. 4**

Time Kill Log reduction of CFUs/mL of *E. coli*
Time Kill Log reduction of CFUs/mL of

*P. aeruginosa*

Fig. 5
Fig. 6

\[ y = 0.401x + 0.013 \]

\[ R^2 = 0.998 \]
Calibration Curve of Li (0-0.2 ppm)

\[ y = 0.333x + 7E-05 \]

\[ R^2 = 0.999 \]

Absorbance

Conc/ppm

Fig. 10
Calibration Curve of Li+ (0-2 ppm)

\[ y = 0.303x + 0.002 \]

\[ R^2 = 0.999 \]

Fig. 11
Calibration Curve of Li⁺ (0-10 ppm)

\[ y = 0.148x + 0.169 \]

\[ R^2 = 0.9 \]

Conc/ppm

0 2 4 6 8 10

Absorbance

0 0.5 1 1.5 2
<table>
<thead>
<tr>
<th>Group</th>
<th>Test article</th>
<th>Number of Animals/Sex</th>
<th>Dose Volume (mL)</th>
<th>Dose Frequency</th>
<th>Formulation Concentration (mg Li/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Lithium carbonate</td>
<td>16 F</td>
<td>0.1 mL</td>
<td>Twice per day, at least 4 hours apart</td>
<td>0.34</td>
</tr>
<tr>
<td>Low-dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>Lithium carbonate</td>
<td>16 F</td>
<td>0.1 mL</td>
<td>Twice per day, at least 4 hours apart</td>
<td>2.74</td>
</tr>
<tr>
<td>Mid-dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>Lithium carbonate</td>
<td>16 F</td>
<td>0.1 mL</td>
<td>Twice per day, at least 4 hours apart</td>
<td>5.48</td>
</tr>
<tr>
<td>High-dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 4</td>
<td>Lithioderm</td>
<td>16 F</td>
<td>0.1 mL</td>
<td>Twice per day, at least 4 hours apart</td>
<td>2.74</td>
</tr>
<tr>
<td>Comparator</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

**Pharmacokinetic Group Assignment: Dosing and Tissue Sampling**

```
0-1h  4-5h
0-1h  4-5h
0-1h  4-5h
0-1h  4-5h
0-1h  4-5h
0-1h  4-5h
0-1h  4-5h
```

- Dosing: Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7

= dermabrasion

**Fig. 14**
Fig. 17
A.  

![Graph showing lithium levels in blood after different doses of Li+ and Li-Glu.]

- **0.34 mg Li+/G Li-Carb**
  - Day 1: 0.06
  - Day 2: 0.12
  - Day 3: 0.08
- **2.74 mg Li+/G, Li-Carb**
  - Day 1: 0.6
  - Day 2: 0.55
  - Day 3: 0.39
- **5.48 mg Li+/G, Li-Carb**
  - Day 1: 0.85
  - Day 2: 0.89
  - Day 3: 0.85
- **2.74 mg Li+/G, Li-Glu**
  - Day 1: 0.51
  - Day 2: 0.40
  - Day 3: 0.32

B.  

![Graph showing lithium levels in blood after different doses of Li+ and Li-Glu over 18 hours.]

- **0.34 mg Li+/G Li-Carb**
  - ~18h after dose, day 1: 0.01
  - ~18h after dose, day 2: 0.02
  - ~18h after dose, day 6: 0.01
- **2.74 mg Li+/G, Li-Carb**
  - ~18h after dose, day 1: 0.11
  - ~18h after dose, day 2: 0.07
  - ~18h after dose, day 6: 0.03
- **5.48 mg Li+/G, Li-Carb**
  - ~18h after dose, day 1: 0.07
  - ~18h after dose, day 2: 0.1
  - ~18h after dose, day 6: 0.06
- **2.74 mg Li+/G, Li-Glu**
  - ~18h after dose, day 1: 0.09
  - ~18h after dose, day 2: 0.03
  - ~18h after dose, day 6: 0.04

---

**Fig. 18**
A. Skin Levels of Li+

B. Blood Levels of Li+

Fig. 19
Polyethylene oxide-amine

Glutarate

$pH$ 7.4-8

Hydrolytically Labile Ester Linkages

Crosslinked Network

Crosslink
Fig. 26
Fig. 27
Fig. 28

\[ y = 1.8715x^2 - 21.876x + 68.444 \]
Fig. 30

Fig. 31
Fig. 32

Cumulative Release of Li+ PLG Microspheres

% Lithium Released

Hours

Fig. 33

Gelation Time (minutes)

Percent Gels

$y = 1.8715x^2 - 21.876x + 68.444$
Fig. 34
Fig. 35

Viscosity of PVA Solutions as a Function of Temperature
Fig. 36

A

B

C

PLA/PLG: 0/100
(Prepared by cotton candy machine)
Fig. 51
Fig. 52
Fig. 53

Li+ Flux (μg/h/cm²)

TH-003-070B
21.8 MG Li+/G

No IP/70B CHAMBER 29; N=1
no IP/70B CHAMBER 167; N=2

NO IONTOPHORESIS

TIME (hrs)
NO IONTOPHORESIS

**Comparison of Formulations**

- No IP/70A CHAMBER 162
- No IP/70B CHAMBER 167
- No IP/70C CHAMBER 184

**Fig. 54A**

**NO IONTOPHORESIS**

% Li+ Permeated in Receptor/Time Interval

**Fig. 54B**
Fig. 55A

Fig. 55B
Iontophoresis 0.8 mA/min for 15 minutes

- Chamber 78 TH-003-070c
- Chamber 137 TH-003-070b
- Chamber 179 TH-003-070a

Li+ Flux (µg/h/cm²)

Day 1-1, Day 2-1, Day 3-1, Day 4-1, Day 5-1, Day 6-1, Day 7-1, Day 8-1, Day 9-1, Day 10-1, Day 11-1, Day 12-1, Day 13-1

Chamber 78: nail thickness: 0.13625 cm
Chamber 137: nail thickness: 0.06225 cm
Chamber 179: nail thickness: 0.04475 cm

Time Intervals

Fig. 55C
Iontophoresis 0.8 mA/min for 15 minutes

- No IP Chamber 162 TH-003-070a
- IP Chamber 179 TH-003-070a
- Linear (No IP Chamber 162 TH-003-070a)
- Linear (IP Chamber 179 TH-003-070a)

Fig. 55D
Lithium 8%

regeneration zone
neogenic hair follicles

scar zone
no hair follicles

adjacent normal skin
pre-existing hair follicles

placebo

Fig. 77
A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 59/22 (2012.01)
USPC - 424/677

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC-424/677

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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</thead>
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<tr>
<td>X</td>
<td>US 2009/0214628 A1 (de Rijk) 27 August 2009 (27.08.2009) entire document esp. para[0002], [0024],[0026], [0042], [0058], [0085], [0145], [0244], [0246], [0358], [0407]-[0408]</td>
<td>1-3, 8-9, 11(8-9), 20-21, 30, 38-41</td>
</tr>
<tr>
<td>Y</td>
<td>US 5,223,271 A (Horrobin) 29 June 1993 (29.06.1993) entire document esp. col. 1, 4-8, col. 2, ln 26, 55; col.3, ln 5-11, col. 4, ln 14</td>
<td>4-6, 10, 11/10, 22-28, 36-37</td>
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<td>US 2009/0196945 A1 (Walsh et al.) 06 August 2009 (06.08.2009) entire document esp. para[0035]-[0034]</td>
<td>31-33, 35/31-33, 34, 35/34</td>
</tr>
</tbody>
</table>

* Special categories of cited documents:
**A** document defining the general state of the art which is not considered to be of particular relevance
**E** earlier application or patent but published on or after the international filing date
**L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
**O** document referring to an oral disclosure, use, exhibition or other means
**P** document published prior to the international filing date but later than the priority date claimed

**T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
**X** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
**Y** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Date of the actual completion of the international search
11 June 2012 (11.06.2012)

Date of mailing of the international search report
25 JUN 2012

Name and mailing address of the ISA/US
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Authorized officer: Lee W. Young
PCT Helpdesk: 571-272-4300
PCT QSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)
# INTERNATIONAL SEARCH REPORT

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. □ Claims Nos.:  
   because they relate to subject matter not required to be searched by this Authority, namely:

2. □ Claims Nos.:  
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ✗ Claims Nos.: 7, 12-19, 29  
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. □ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. □ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. □ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. □ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- □ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.
- □ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- □ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)