(54) Title: METHODS AND COMPOSITIONS FOR MODULATING HAIR GROWTH, WOUND HEALING AND SCAR REVISION

(57) Abstract: The invention relates to methods for (i) modulating hair growth and (ii) improving wound healing. e.g., scarless wound healing, and scar revision in human subjects involving the administration of one or more compounds provided herein. Compositions and uses of compositions containing the compounds provided herein are also described. The compound, composition thereof, or treatment(s) comprising the compound may be administered alone or in combination with (e.g., concurrently with, before, and/or after) other treatments for the enhancement or inhibition of hair growth or for the improvement of wound healing or scar revision. Such combination treatments may involve mechanical or physical treatments that cause integumental perturbation (e.g., laser, dermabrasion, etc); immune stimulation; chemical treatments; and/or surgical treatments for the enhancement or inhibition of hair growth or for the improvement of wound healing or scar revision.

FIG. 1
before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))
Fig. 7C

**DD** In Vitro Migration of ARCaP Cells

- **AA**

<table>
<thead>
<tr>
<th>Condition</th>
<th>OD 590nm</th>
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</thead>
<tbody>
<tr>
<td>ARCaPM</td>
<td></td>
</tr>
<tr>
<td>1ug/ml OPG</td>
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<tr>
<td>ARCaPE</td>
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</tr>
<tr>
<td>200ng/ml RANKL</td>
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<tr>
<td>200ng/ml RANKL+ OPG</td>
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**DD** In Vitro Invasion of ARCaP Cells

- **BB**

<table>
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<tbody>
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<td>ARCaPM</td>
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<tr>
<td>1ug/ml OPG</td>
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<td>200ng/ml RANKL+ OPG</td>
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**DD** In Vitro Migration and Invasion of LNCaP Cells

- **CC**

<table>
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<td></td>
</tr>
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<td>200ng/ml RANKL+ OPG</td>
<td></td>
</tr>
</tbody>
</table>

- **AA** Migration *in vitro* de cellules ARCaP
- **BB** Invasion *in vitro* de cellules ARCaP
- **CC** Cells Migration et invasion *in vitro* de cellules LNCaP
- **DD** DO 590 nm
follicles that produce "vellus hair" while certain other hair follicles may contain or produce no hair (see Figure 1). Essentially, only the glabrous skin on palmar and plantar aspects of hands and feet, respectively, and the lips and labia lack hair follicles. Only a minority of human hair follicles produce a hair fiber that can be appreciated visibly (a "terminal hair") and these specialized follicles are localized on specific regions of skin. Accordingly, both the presence and absence of visible hair on human non-glaborous skin is mediated by regulation of activity of specialized follicles.

[0004] Hair follicles, and particularly human hair follicles, are crypt structures comprised of distinct components, each comprised of several different specialized cells (see Figures 2 and 3). In addition to the cells and structures associated with making and anchoring the hair shaft, the vast majority of hair follicles contain units called sebaceous glands (which produce sebum). Some hair follicles have apocrine glands attached to them, and are located in the axilla and other specific areas of the body.

[0005] In addition to the hair shaft, the structures of the hair follicle include the follicular papilla (FP) and the germinative epithelium (GE) (together, the bulb). The FP is comprised of mesenchymal cells (and connective tissue). The other cells of the follicle are epithelial and include at least 8 cellular lineages including the outer root sheath (ORS), the companion layer (CL), the internal root sheath Henle's layer (He), internal root sheath Huxley's layer (Hu), the cuticle of the internal root sheath (Csth), the cuticle of the hair shaft (Csf), the cortex of the hair shaft, and the medulla of the shaft (Med). (Stenn & Paus, 2001, Physiol. Revs. 81: 449-494.) (See also Figures 2-4.)

[0006] Scalp and certain other hair in humans tend to grow in follicular units. A follicular unit of scalp hair is typically composed of one to four terminal hair follicles; one to two vellus hair follicles; their associated sebaceous glands, neurovascular plexus, an erector pilorum muscle and a circumferential band of adventitial collagen, termed the "perifolliculum" (Headington JT, 1984, Arch. Dermatol. 120:449-456; Bernstein RM, 2005, "Follicular Unit Hair Transplantation," Ch. 34 in Surgery of the Skin, Robinson et al., eds., St. Louis: Mosby, pp. 549-574).

[0007] Hair follicles are believed to produce approximately 20 individual hair shafts over the life of the follicle as the follicle progresses through cycles of hair production, shedding (ejection), involution and new growth. The regulation of hair growth and follicle regeneration have been investigated in murine systems. However, the biology of hair follicles in the mouse is different from those of the human in several important aspects. In the mouse, a thick fur coating is essential to healthy life (because hair plays roles in
thermoregulation and other functions.) Mouse skin is covered with hair follicles that produce terminal hair (fur), whereas significant regions of human skin are covered with hair follicles that produce vellus hair, which is invisible. Mouse and other non-primate mammals have synchronous Follicle Cycles, whereas human follicles progress through the Follicle Cycle in an asynchronous fashion. While the mouse has certain specialized follicles (e.g., whiskers, guard, awl, auchene, and zigzag hair), mouse follicles are generally not subject to developmental and gender-specific hair patterning. In contrast, a significant number of human follicles are individual participants in choreographed hair patterning that affects the type, length and color of shaft produced at different times in development and aging and in a gender specific manner.

2.1 THE HAIR FOLLICLE CYCLE

[0008] Hair growth in each follicle occurs in a cycle that includes the following principal phases: anagen (growth phase), catagen (involuting/regressing stage), telogen (the quiescent phase), exogen (shedding phase), and re-entry into anagen (sometimes referred to herein as the "Follicle Cycle"). In addition there is a latent phase, termed kenogen, which is a persistent suspension of growth after a hair follicle has been shed. The Follicle Cycle has been relied on to explain many phenomena associated with hair growth and hair loss. However, it is an incomplete explanation for the regulation of human hair growth.

2.1.1 ASYNCHRONOUS CYCLING IS UNIQUE TO HUMANS

[0009] In fur-covered terrestrial, non-primate mammals the hair cycle is typically synchronized across large areas of the skin, sometimes in "waves" across the skin and sometimes the entire fur coat sheds in response to environmental or other triggers. See, e.g., Stenn & Paus, 2001, Physiol Rev 81:449-494.

[0010] Human hair follicles are relatively unique among mammals (and particularly non-primates) since hair follicles in a region of skin are not synchronized. On an adult human scalp, at any particular time approximately 90% are in anagen; 10-14% in telogen and 1-2% in catagen.

2.1.2 SPECIALIZED FOLLICLES IN HUMANS

[0011] While certain other mammals do have specialized hair follicles (e.g., whiskers and eyelashes on canines), humans are unique in the number and variety of their specialized
Hair follicles. Hair patterning in humans is mediated by distinct specialized hair follicles that play out their predetermined programs over the life of the human.

It is believed that humans are born with their complement of hair follicles and that each hair follicle belongs to a class of follicles that accounts for the distinctive length, temporal appearance, regulation by sex hormones, etc., of the hair shaft it produces. The duration of the Follicle Cycle length is believed to be a characteristic of each distinct specialized human hair follicle that accounts for the length of hair produced and other aspects of the follicle function. For example, the anagen stage for eyebrow hair follicles is approximately 4 months and for scalp hair follicles is approximately 3-4 years. It has been proposed that eyebrow hairs have a shorter length compared to scalp hairs because the former have a shorter anagen phase.

It also has been proposed that in humans, sex hormones regulate the length of anagen in the Follicle Cycle of certain specialized hair follicles that participate in hair patterning: decreasing anagen in programmed hair loss and increasing anagen in programmed hair growth.

However, while current descriptions of the hair Follicle Cycle are powerful for explaining features of fur covered terrestrial mammals, certain features of human hair follicle biology remain unexplained.

2.1.3 FOLLICLE CYCLE AND PATHOLOGICAL HAIR LOSS

The Follicle Cycle has also been used to distinguish two different types of pathologic hair loss (effluvium): Anagen effluvium and Telogen effluvium.

Anagen is associated with intense mitotic activity; therefore, follicles in anagen are sensitive to cancer chemotherapeutic agents. Anagen effluvium is believed to be the process in which certain hair follicles undergo hair loss and involvation during chemotherapy because these agents typically target cells with high metabolic or mitotic activity (e.g., 5-fluorouracil, methotrexate, cyclophosphamide, vincristine). In addition to chemotherapy drugs, Anagen effluvium can be caused by other toxins, radiation exposure, radiation overdose, endocrine diseases, trauma, pressure, and certain diseases like alopecia areata (an autoimmune disease that attacks anagen follicles.)

Telogen effluvium is a premature interruption of anagen and early entry of anagen follicles into the Telogen (or resting) phase. The proportion of telogen hairs on the scalp increases to 25-50% and telogen follicles remain in telogen for more than the usual 3-6 months. Telogen effluvium is caused frequently by drugs like lithium and other drugs like
valproic acid and carbamazepine and numerous other drugs including the commonly used beta blockers and oral contraceptives. In addition to psychiatric drugs, telogen effluvium can be induced by childbirth, traction, febrile illnesses, surgery, stress, or poor nutrition (see Mercke et al, 2000, Ann. Clin. Psych. 12:35-42).

2.2 HUMAN HAIR PATTERNING IS MEDIATED BY DISTINCT HAIR FOLLICLE TYPES WITH SPECIFIC FEATURES

[0018] At a microscopic level, human skin is essentially covered with hair follicles. The portions of human skin that lack visible hair contain, for the most part, hair follicles that produce "vellus hair" which is thin and short (i.e., less than 2 cm in length) and often colorless. Certain other hair follicles may contain or produce no hair. Only a minority of human hair follicles produce a hair fiber that can be appreciated visibly (a "terminal hair") and these specialized follicles are localized on specific regions of skin. Another follicle type is the "sebaceous" follicle, which has a large sebaceous gland and a vellus-like hair shaft localized in the acne-prone areas. Accordingly, both the presence and absence of visible hair on human skin is mediated by regulation of the activity of specialized hair follicles.

[0019] The spatial and temporal aspects of human hair patterning are believed to depend on the localization of specialized hair follicles with unique features during embryogenesis. It is further believed that this complement of hair follicles is maintained throughout life without renewal or replacement. Human fetus follicles may produce lanugo hair during gestation, which is intermediate between vellus and terminal hair in thickness and length and is typically shed by the time of normal birth. By the time of birth, distinct specialized follicle types are positioned in specific areas of the skin where they will each play a programmed role in hair patterning over the life of the human individual, producing various hair types (lanugo, vellus or terminal hair) either constitutively or depending on certain signals, such as sex hormones or other factors (e.g., lanugo hair can reappear in starvation or in eating disorders such as anorexia nervosa and bulimia).

[0020] Gender is associated with specific patterning of human hair. The growth and loss of visible hair in specific areas of the skin, in stereotypical gender dimorphic patterns, are regarded as "Secondary Sexual Characteristics." This terminology relates "secondary" features such as hair patterning to the genitals and reproductive organs, which are termed "Primary Sexual Characteristics." The distinctive genitals and reproductive organs of males and females acquired during embryonic development undergo further changes in puberty and
menopause/andropause. In addition to hair growth and loss, breasts in females are also considered Secondary Sexual Characteristics.

[0021] Certain human hair follicles are targeted to specific skin areas and develop specialized characteristics during embryogenesis under the influence of sex hormones such as testosterone and dihydrotestosterone ("androgens") and/or estrogens. Further, certain human hair follicles are driven to change activity by sex hormones during puberty and in menopause/andropause.

[0022] The appearance and intensity of secondary sex characteristics can be described as being regulated by ratios of androgens and estrogens, since to a certain extent either of these groups of hormones (androgens and estrogens) can act to induce certain activities or to inhibit the effect of the other group (i.e., androgens inhibit estrogen effects and estrogens inhibit androgen effects). For example, androgens induce male characteristics and suppress female characteristics while estrogens induce female characteristics and suppress male characteristics. Male and female, as used herein, refer to the extremes of genetic gender dimorphism and include by reference the various conditions and states that represent a spectrum of male and female features (such as XO syndromes or conditions that result from exogenous sex steroid administration).

[0023] Specialized human hair follicles have quantitative variation in activity as well as qualitative variation. For example, sex steroids have qualitative effects on hair patterning either in embryogenesis or in adult life or both (e.g., males have beard hair follicles that produce terminal hair after puberty whereas females do not). Males and females also vary in the amount of gender-specific hair patterning (e.g., a higher density of leg hair follicles produce terminal hair on male rather than female legs). Also, individuals of the same gender exhibit quantitative variation. For example, male chest and back hair presents in different individuals as a spectrum from almost hairless to dense hair and from small regions of follicles producing terminal hair to large regions.

[0024] Gender specific human hair patterning highlights the distinct biological programming of specific hair follicles. Distinct hair follicles in relative proximity on the male scalp and face respond to high androgen/estrogen ratios in diametrically opposite ways: high androgen/estrogen ratios induce vellus to terminal hair transformation in male moustache/beard skin (particularly during puberty), but induce terminal to vellus follicle transformation change in male frontal/temporal scalp (progressively post puberty) in male pattern hair loss (MPHL).
[0025] The effects of androgen/estrogen levels on other regions evidences further variations in the biological programming of specific hair follicles. Hair follicles on the occipital scalp are relatively insensitive to high androgen/estrogen ratios (but later, after more prolonged androgen exposure, undergo age-related thinning). Hair follicles in the axillary and pubic regions (anogenital region) appear to be more sensitive to androgen than moustache/beard follicles; since terminal hair in axillae/pubis grows: (a) in females with relatively low levels of androgen; (b) early in male puberty before beard/moustache; and (c) in patients with genetic 5-alpha-Reductase Type II deficiency.

2.2.1 MALE PATTERN HAIR LOSS

[0026] Male pattern hair loss (MPHL) is a type of "androgenetic alopecia." Androgenetic alopecia is a genetically-mediated disorder that occurs in approximately 50% of men by the age of 50 years (see review, Stough et al, 2005). In women, the histological features of the condition are the same as in men, but susceptibility, age at onset, rate of progression and pattern of hair loss differ between genders (Dinh and Sinclair, 2007).

[0027] After puberty, males begin to lose the scalp hair over the vertex, crown and frontal/parietal areas in a relatively characteristic pattern that is a continuum (described by the Hamilton Norwood scale; see Figure 5). The process of hair loss occurs at the level of the hair follicles by "miniaturization" through which the hair follicle becomes progressively smaller both in depth and circumference, and the hair shaft produced becomes shorter and thinner. The ratio of terminal-to-vellus-like hairs may be reduced from approximately 7:1 to less than 2:1. Miniaturization results in club hair shafts or vellus hair shafts. The loss of scalp hair in men is known to be a process driven by the androgen, DHT, which can be inhibited and to some extent reversed by finasteride, which inhibits 5-alpha-reductase II (which converts testosterone to DHT). In advanced stages of MPHL, the affected hair follicles on the bald vertex or temples are considered to be atrophied, or perhaps involuted irreparably ("senescent"). The process by which this occurs is not completely understood. One theory holds that androgens change the length of anagen and telogen phases, so that a normal ratio of anagen to telogen ratio of approximately 12:1 becomes approximately 5:1 in MPHL. Telogen hairs are more loosely anchored and prone to shedding or being pulled out (for example, by combing or brushing hair). At the end of telogen, a club hair is produced that is a fully keratinized hair. The hair follicles on MPHL affected areas also undergo follicular miniaturization in which a growing proportion of terminal follicles become vellus follicles. Additionally, androgenetic alopecia is thought to involve the progressive...
conversion of hair follicle units with 3 or more terminal hairs to follicular units having fewer terminal hairs (e.g., units with 2 terminal hairs progress to units with 1 terminal hair).

MPHL is associated with specific polymorphisms of the androgen receptor, the EDA2R gene. Men who are genetically deficient in Type II 5-alpha-reductase do not experience MPHL (see Jenkins et al., 1992, J Clin Invest 89:293-300).

Several lines of investigation have elucidated mechanistic aspects of the sensitivity to androgen of male frontal parietal and coronal hair follicles. Androgen activity may be mediated by a co-factor to the androgen receptor Hic-5/ARA55 (Inui, 2007, J Invest Dermatol 127:2302-2306). Hic-5/ARA55 mRNA expression was high in dermal papilla cells from the beard and bald frontal scalp but low in cells from the occipital scalp. Another androgen receptor coactivator ARA70/ELE1 had decreased expression of a splice variant form (ARA70beta/ELE1beta) in the dermal papilla of balding recipient areas than non-balding areas (Lee et al., 2005, J Cutan Pathol 32:567-571).

2.2.2 FEMALE PATTERN HAIR LOSS

In addition to the progression of MPHL, both males and females develop diffuse hair loss in the frontal/parietal scalp called "thinning," which begins between 12 and 40 years of age. Collectively, MPHL and diffuse thinning in males and females is termed "androgenetic alopecia." Perhaps more than males, females notice (and complain of) diffuse hair thinning progressively in middle age more than males, perhaps because diffuse alopecia is more noticeable and problematic for females because they do not suffer from MPHL and retain the frontal hairline. In females, thinning is known as "Female Pattern Hair Loss (FPHL)" and may be caused or exacerbated by androgens (Price, 2003, J. Investig. Dermatol. Symp. Proc. 8:24-27).

Mechanistically, FPHL is thought to share some features with MPHL in terms of progressive reduction in the duration of anagen and progressive follicular miniaturization, although recent studies have found a prolongation of kenogen.

2.2.3 DONOR DOMINANCE

The unique features of specialized human hair follicles continue to show the characteristics of the donor site when skin, hair follicles, or hair follicle units are transplanted, which has been referred to as "donor dominance" (Orentreich N, 1959, Ann NY Acad Sci. 83:463-479). This principle is evidenced by the results of the commonly performed procedure of transplanting scalp hair (skin, follicles or follicle units) in males from
areas that are not subject to androgen-triggered, MPHL (e.g. occipital scalp) to areas in which specialized follicles have begun producing vellus hair or have stopped producing hair under the influence of androgens (e.g. frontal/temporal; crown or vertex scalp. The transplanted follicles retain the programmed terminal hair producing features from their original location. However, more recent studies suggest that the recipient site may affect some characteristics of transplanted hairs. See Hwang et al, 2002, Dermatol. Surg. 28:795-799.

2.3 HAIR FOLLICLE MORPHOGENESIS AND REGENERATION AND ROLE IN SKIN BIOLOGY

[0033] 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.

[0034] In non-human mammals, when the depth of a wound in mammalian skin approaches approximately one-half of the total skin thickness, the resultant repair process leads to a scar (Dunkin et al., 2007). This is in contrast to similar fetal wounds which heal by regeneration, a process that results in complete restoration of normal skin function (Buchanan et al., 2009). In several species (Buchanan et al., 2009), including primates (Lorenz et al., 1993), a prominent feature of complete skin regeneration in fetuses is the formation of hair follicles, a property that is lost in the perinatal period. The mechanism for the switch from regeneration to repair with age in mammals is not fully known, but involves cytokines and an immune response that promotes rapid epidermal closure, dermal fibroplasia and increased collagen deposition. During this reparative process, hair follicles do not form, thus contributing to the lack of adnexal structures in a scar. The abnormal structure of scars contributes to the their associated morbidity. Due to lack of eccrine glands, there is poor thermoregulation. Increased fibroplasia and collagen can lead to contracture and loss of mobility in affected areas of the body.

[0035] Several lines of evidence indicate that formation of hair follicles in a healing wound can reduce or even prevent scarring. In adult rodents, burn wounds heal faster if hair follicles are actively growing at the time of wounding (Zawacki & Jones, 1967). Direct evidence for the anti-scarring properties of hair follicles comes from full thickness excisional wounds in adult mice (such wounds normally form a scar) that heal with near-complete skin
regeneration when dissociated hair follicle cells are implanted at the time of wounding (Prouty et al., 1996; Prouty et al., 1997). The regenerated skin contains hair follicles and normalized collagen and extracellular matrix. When hair follicles of mouse dorsal and tail skin (areas rich in hair follicles) are experimentally removed, such seen in EDAR mutant mice (Langton et al., 2008) and in photoepilated mouse skin (Huh et al., 2006), alterations in wound healing such as delayed re-epithelialization and dermal changes are observed.

[0036] It is also well known from surgical and medical experience that after comparable injury in humans, areas rich in hair follicles heal more rapidly and with less scarring compared to those relatively devoid of adnexal structures (Martinot et al., 1994; Jahoda & Reynolds, 2001). This is consistent with the hair follicle (both in mouse and human) being rich in Keratin 1-15 (K1-15; K15)-positive stem cells and being a known central repository of skin regenerative capacity (Morris et al., 2004; Roh & Lyle, 2006). There is also evidence that connective tissue cells of the follicle play a role in dermal repair. For example, it has been observed that hair follicle keratinocytes contribute to epidermal closure, and hair follicle dermal sheath fibroblasts play central roles in dermal healing. See, Ito et al., 2005; Jahoda & Reynolds, 2001; Stenn & Paus, 2001, Physiol. Revs. 81:449-494.

[0037] 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.

[0038] The only skin tissue (aside from scar tissue) that normally lacks hair follicles is the glabrous skin on palmar and plantar aspects of hands and feet, respectively, and the lips and labia. Although human glabrous skin lacks hair follicles, it is rich in eccrine sweat glands. Wound healing studies in pig (Miller et al., 1998) have shown that sweat glands, by themselves, are capable of regenerating epidermis, which likely accounts for lack of scarring in glabrous skin wounds that spare the base of sweat glands. However, as with deep wounds to hairy skin, palmar (Barret at al., 2000) and plantar (Barret & Herndon, 2004) wounds can result in scarring, a sequelae related to increased depth of wound and delayed wound healing.

[0039] Until recently, it was believed that the only hair follicles involved in wound healing migrated into the wound from the surrounding epithelium, as the general belief is that hair follicle formation occurs but once in a lifetime (in utero), so that a mammal, and particularly a human, is born with a fixed number of follicles, which does not normally increase thereafter. Despite early suggestions of the regenerative capacity of the adult mammalian skin to recreate the embryonic follicle, 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 of 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); for example, hair follicles were not understood to be capable of neogenesis and bulge cells were not proven to be a source of stem cells for hair follicle neogenesis. Well-designed studies in the 1950s using full thickness skin excisions generated support both for and against hair follicle neogenesis. Following a 2.5 cm diameter full thickness skin excision on the rabbit dorsum, and placement of a barrier device to retard contracture, small neogenic hair follicles were observed histologically in the healed wound (Breedis, 1954). However, another experiment, using a very similar design in rabbits and a histological endpoint, was carried out specifically to address the issue of contracture and concluded that, despite the barrier device, the wound margins contracted and "dragged" pre-existing hair follicles into the center of the wound (Straile, 1959). In human volunteers, following dermabrasion of the cheek to remove of 2 mm of skin (to depth of mid-derm is), neogenic hair follicles were identified histologically (Kligman & Strauss, 1956). However, without molecular markers to define neogenic hair follicles, these studies were only speculative of hair follicle neogenesis.

[0040] More recently, based on studies primarily in rodents, it has been proposed that wound healing in animals is associated with hair follicle neogenesis or reformation of hair follicles from dissociated parts. See, Stenn & Paus, 2001, Physiol. Revs. 81:449-494. Prouty and colleagues showed that a full thickness excisional wound in mice, which normally forms a scar, heals with near-complete skin regeneration when dissociated hair follicles are implanted at the time of wounding. The regenerated skin contains hair follicles and normalized collagen and extracellular matrix. See Prouty et al, 1997, Lab. Invest. 76: 179-189. Nielsen & Sun (U.S. Patent No. 5,767,152) showed that application of the hair growth enhancer n-butyl cyanoacrylate to incisional and excisional wounds in mice stimulated hair follicle growth in the wound site in what would normally be a scar devoid of skin appendages, suggesting that hair follicle growth in wounds accelerates the process of wound remodeling. 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 22:4 11-17; Ito et al., 2004, Differentiation 72:548-57; and Ito et al., 2005, Nature Medicine 11:135 1-1354).

[0041] Some of the molecular signals involved in the regulation of hair follicle regeneration have been investigated in murine systems. Based on experiments performed on
murine cells in culture, Morgan implicated a role for Wnt signaling to the dermal papillae (DP) (e.g., resulting from β-catenin accumulation in the epidermis) in the coordination of hair follicle development (Kishimoto et al., 2000, Genes & Dev. 14: 1181-1 185; see also U.S. Patent Application Publication No. US 2006/0134074, which postulates a role for Wnt produced in three-dimensional tissue cultures in vitro promoting hair growth). Previous and subsequent reports were conflicting. One group, consistent with Morgan's theory, showed that continuous expression of stabilized β-catenin in the epidermis of transgenic mice resulted in hair follicle morphogenesis, but unfortunately led to the development of hair follicle tumors (Gat et al., 1998, Cell 95:605-614). Another group found that continuous activation of a hyperstabilized and highly amplified (12-21 copies) form of β-catenin in mice also resulted in hair follicle tumors, but that transient activation of this hyperstabilized and highly amplified form of β-catenin led to normal hair follicle patterning, although no hair growth was observed in mice harboring a single copy of the hyperstabilized form of β-catenin (Lo Celso et al., 2004, Development 131:1787-1799). These results are in contrast to the results of another study, in which a reduction of β-catenin signaling was found to cause hair follicles to develop into cysts in postnatal mice (Niemann et al., 2002, Development. 129:95-109). Moreover, there are examples in which increased β-catenin was found to decrease hair growth and hair follicle formation. For example, one group showed that forced expression of β-catenin dependent Wnt 3a in murine skin decreased hair growth (Millar et al., 1999, Dev. Biol. 207:133-149). In another study, continuous expression of β-catenin during embryogenesis was found to induce placodes, but they became aborted and did not produce a hair shaft (Narhi et al., 2008, Development. 135:1019-1028). In yet another study, increased β-catenin expression during embryogenesis was found to alter cell fate toward formation of hair follicles, but continued presence of β-catenin prevented further development of the hair follicles (Zhang et al., 2008, Development. 135:2161-2172). Finally, overexpression of Lef-1 (a transcription factor in Wnt pathway) was found to lead to abnormal hair follicle growth, as reported in Zhou et al., 1995, Genes Dev. 9:700-713.

Despite this confusion about a role of β-catenin in hair growth, Morgan speculated that exogenous Wnt could extend the hair cycle and promote hair growth, and further, compounds that inhibit the activity of glycogen synthase kinase^ (GSK-3β) may induce Wnt signal transduction (through the accumulation of β-catenin), and thus, may be used to promote hair growth (US Patent 6,924,141; US Patent 7,175,842; and US Patent Application Publication 2008/0286261).
Fathke's experiments tested Morgan's theory. In animal studies designed to explore the role of Wnt in tissue patterning following wounding, Fathke showed that prolonged activation of β-catenin dependent Wnt signaling (by continuous topical administration of lithium chloride for two weeks until wound closure) during wound healing in mice resulted in generation of rudiments of hair follicles but did not result in the formation of hair follicles or growth of more hair (Fathke et al., 2006, BMC Cell Biol. 7:4). Fathke also noted that the epithelium that typically forms over the wound during wound healing was covered with cysts. Id. Fathke then turned the focus of their investigation to the β-catenin-independent Wnts expressed in the skin - which are not activated by lithium chloride. Unfortunately, the prolonged expression of the β-catenin independent Wnt yielded the same results - mature hair follicles were not generated in the mice and large epithelial cysts formed in the wounds. Although Fathke interpreted their data as evidence for the restoration of tissue patterning in the adult mammalian wound epithelium - a feature not normally seen in adult cutaneous wound healing - they provided neither evidence of hair follicle neogenesis, nor the concomitant hair growth and enhanced wound healing that would be expected in connection with it.

The most compelling evidence for hair follicle neogenesis to date comes from studies in mice by Cotsarelis and coworkers, in which hair follicle neogenesis following wound healing was investigated. As noted by Fathke, cutaneous repair in adult mammals following a full thickness wound is understood to result in scar tissue and the loss of the regenerative capability of the hair follicle. However, 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 447:3 16-321). Physically disrupting the skin and existing follicles was found to lead to follicle neogenesis. Here again the role of Wnt was examined. In these experiments, inhibition of Wnt signaling at the time of wounding decreased the number of new hairs formed in the healed wound. While increasing Wnt expression increased the number of new hairs formed in the healed wound, Cotsarelis noted that "the hair follicle dermal papilla of these mice contain -25-38% more cells compared with normal. It is possible that the larger number of dermal papilla cells contributes to the greater number of hair follicles that form after wounding" (Ito et al. 2007, Nature 447:316-321, full Methods, available at www.nature.com/nature). This suggests that these Wnt-overexpressing mice were already predisposed to growing more hair. Thus, Cotsarelis' recognition that "... to date there has been no evidence that extracellular Wnt ligands can promote actual hair follicle neogenesis in
adult skin" (Ito et al. 2007, Nature 447:316-321, at p. 319) remains correct. Moreover, in both of Cotsarelis' experiments - the blockade of Wnt or Wnt overexpression - wound closure was normal (Ito et al. 2007, Nature 447:316-321, at p. 319).

Thus, while Wnt signaling in hair follicle neogenesis and, in turn, wound healing has generated interest, its role in these processes is, at best, unclear. Moreover, the evidence that β-catenin activation causes cyst formation, is abnormally activated in the epidermis of chronic wounds, and that its overactivation shifts the wound healing process from the re-epithelialization stage to the proliferative stage, suggests that activating Wnt in the epidermis could also have a negative effect on wound healing. Continuous activation of Wnt, it seems, may actually be bad for wound healing.

It is also important to note that although the above-described studies implicate hair follicles, and perhaps hair follicle neogenesis, in wound healing, mice are an imperfect model for human wound healing and scar formation. Mice tend to heal most wounds rapidly, with little or no scarring. In humans, however, severe wounds and burns are usually associated with cutaneous repair that results in scar tissue, no hair follicles, and the loss of regenerative capability that hair follicles may provide (see, Fathke et al., 2006, BMC Cell Biol. 7:4). One reason for the difference in wound healing capability between humans and mouse may be that the biology of hair follicles in humans and mice differs in several significant respects. In the mouse, a thick fur coating is essential to healthy life (because hair plays roles in thermoregulation and other functions). Mouse skin is covered with hair follicles that produce terminal hair (fur), whereas significant regions of human skin are covered with hair follicles that produce vellus hair, which is invisible. Finally, as explained in Section 2.1.1, supra, mouse and other non-primate mammals have synchronous Follicle Cycles, whereas human follicles progress through the Follicle Cycle in an asynchronous fashion.

2.4 BACKGROUND ON WOUND HEALING AND SCAR FORMATION

2.4.1 PHASES OF WOUND HEALING

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 (involving angiogenesis, granulation tissue formation and fibroplasia, re-epithelialization, and contraction, the latter phase reducing the size of the wound); 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.

[0048] 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 discussed in Section 2.4.2.

2.4.1 PRIMARY INTENTION

[0049] 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." These wounds are usually surgically closed in layers along tissue planes by a physician. In primary intention, a linear scar results at the intersection of the approximated tissues. Scarring is often minimal, but can be variable depending on the size and location of the wound, the tension on tissue and other factors. Most surgical wounds are sutured closed, so they heal by primary intention. In primary intention, wound closure is usually performed with sutures, staples, or an adhesive. Other examples of wounds that heal by primary intention are well repaired lacerations, well reduced bone fractures, and wounds that heal after flap surgery (the edges of which tend to appear scarred).

2.4.1.2 SECONDARY INTENTION

[0050] 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." For example, wounds formed by blast injury, shrapnel (e.g., from improvised explosive devices ("IEDs")), blunt trauma, dental wounds (e.g., gingivectomy, gingivoplasty, tooth extraction sockets), poorly reduced fractures, and third degree burns heal by secondary intention. Healing by secondary intention follows the same basic steps as wounds that heal by primary intention, i.e., inflammation, proliferation, and remodeling, but each sequence may take much longer, especially the proliferative phase. Since the wound edges are not approximated, epithelial cell migration much occur over a longer distance, and before epiboly can occur, a provisional matrix must be present. Thus, in healing by secondary intention, there is much more
granulation tissue formation and contraction, which carries a greater risk of scarring. The secondary intention healing process can be slow due to the presence of drainage from infection, and the surgeon may pack the wound with a gauze or use a drainage system. Wound care must be performed daily to encourage wound debris removal to allow for granulation tissue formation. Depending on the size and location of the wound, placement of a partial or full-thickness skin graft may be considered if no infection is present and the area is of sufficient size that healing may not be complete for at least 2 or 3 weeks. Infection and inflammation of the wound can dysregulate repair and transform the wound into a clinically non-healing wound.

2.4.1.3 TERTIARY INTENTION

[0051] 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.

2.4.2 SCAR FORMATION

[0052] 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.

[0053] Adult mammalian skin repairs wounds in a process that results in scars, whereas fetal skin heals by a regenerative process that results in complete restoration of normal skin. Scarring is the consequence of the adult wound repair mechanism that focuses on hemostasis and rapid epidermal closure (to avoid infection) in lieu of cosmetic or functional outcomes - regeneration of the original tissue architecture is sacrificed. For example, during wound repair, adult skin undergoes epithelialization without adnexal structures such as hair follicles and sweat glands. This leads to alopecia, desiccation, and thermal dysregulation of the affected tissue. See, e.g., Fathke et al., 2006, BMC Cell Biol. 7:4. Scar tissue is also usually of inferior functional quality. A scar is a collagen-rich, elastin-poor dermal matrix with a simple stratified epithelial covering. Deposition of such a collagen-rich matrix in the neo-dermis is prone to contracture, loss in elasticity, and reduced tensile strength. Scars in the skin are also less resistant to ultraviolet radiation. For example, scars from skin transplants
are typically dysfunctional, discolored, etc. Skin flaps and grafts are common methods of achieving rapid closure of large defect wounds. Not only do these methods tend to result in scarring at the donor site, but the sites of apposition of flap or graft edges to the wound edges can also result in linear scars.

[0054] Scars form differently based on the location of the injury on the body and the age of the person who was injured.

[0055] Recent research has implicated osteopontin in scarring. http://jem.rupress.org/cgi/content/abstract/205/l/43 "Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring"; BBC NEWS - Health - Gel "to speed up wound healing." Transforming Growth Factors (TGFs) are also believed to play a critical role in scar development, and current research is investigating the manipulation of these TGFs for drug development to prevent scarring from the emergency adult wound healing process. Another recent study implicated the protein Ribosomal S6 kinase (RSK) in the formation of scar tissue.

[0056] Scarring is likely to be extensive when wounds heal by secondary intention. In secondary intention, the wound heals by granulation, wherein epithelial cells grow over the wound from all sides of the normal skin, which results in a shiny layer of epithelial cells and fibrous tissue that is rich in collagen but does not contain underlying structures ("adnexal structures," including hair follicles). In addition to lacking adnexal structures, the scar also lacks the suppleness of normal skin. This type of scar can result in contractures when it occurs over the mouth or eyes or on the skin around joints, and can be disfiguring.

[0057] In addition to scars that form by secondary intention, there are numerous other types of scars that we distinguish, including atrophic scars, hypertrophic scars, keloid scars, hypopigmented scars, hyperpigmented scars, depressed scars (which, compared with atrophic scars, also have contour abnormality, while "atrophic" scars are implied to have only thinning), ice-pick scars (a type of depressed scar), spread scars (scars that widen due to tension over a time period, and which may become somewhat atrophic in the center), fineiine scars, widespread (or stretched) scars, scar contractures, and other "intermediate" types of scars that are difficult to categorize. For more information on types of scars, see Bayat et al., 2003, BMJ. 326:88-92, the contents of which are incorporated herein by reference in its entirety.
2.5 CURRENT TREATMENTS FOR HAIR LOSS IN HUMAN SUBJECTS

Human hair loss can be categorized as (1) gender specific hair patterning, (2) pathological hair loss, or (3) hair loss after wounding, all which can be associated with effects on self-esteem and self-image, and many individuals explore whether their hair loss process can be treated. Current treatments offered involve a limited selection of agents and regimens, such as chemical and surgical approaches that either stimulate or transplant pre-existing hair - none are associated with true follicular neogenesis.

Chemical treatments involve the use of drugs for the treatment of certain MPHL. These include, for example, minoxidil (an antihypertensive drug that opens the K+ channel); and antiandrogens such as finasteride, dutasteride or ketoconazole. While these types of treatments are reasonably effective in preventing or delaying MPHL, they are less effective in stimulating the growth of significant terminal hair in scalp of MPHL after baldness has been present for 6 months or more. Moreover, minoxidil and finasteride require continuous treatment for lasting effects. Consequently, patients with advanced MPHL may express dissatisfaction with even statistically significant, but cosmetically insignificant increase in hair counts and such frustration may contribute to poor compliance and further unsatisfactory outcomes.

Recently, bimatoprost (a prostaglandin analog used to control the progression of glaucoma in the management of ocular hypertension) has been FDA approved to lengthen eyelashes and is marketed under the name Latisse®, with the claim of growing eyelashes, making them longer, thicker and darker.

A device that uses low level light energy directly on the scalp (the HairMax Lasercomb) has received FDA clearance as a 510K device. Although the device is advertised as a "Laser," it operates by applying low level monochromatic light energy directly to the scalp, which is thought to stimulate hair growth through "photo-biostimulation" of hair follicles. Various types of devices operating on similar principles were referenced as the predicate for HairMax (see, Lolis et al., 2006, J. Cosmetic Dermatol. 5:274-276).

Finally, more drastic measures for treating hair loss involve hair transplantation — in which scalp strips, hair follicles or follicular units from the occipital scalp (which are resistant to the effects of androgens in inducing MPHL type alopecia) are excised and transplanted to a person's balding or thinning areas. Another surgical method that has been used is scalp reduction; in this procedure, the skin in the balding area of the scalp is surgically excised and the surrounding skin (with hair) is pulled together and sutured. Surgical methods
are best for focal hair loss, and are less effective for diffuse hair loss, are less effective for 
women and younger patients are not ideal candidates because the pattern and extent of hair 
loss is variable, and may be inconvenient because of the expense of the surgery, duration of 
time to show a cosmetic effect, creation of scarring. Despite surgical advances in hair 
transplantation, cosmetic coverage is constrained by the area of and the number of hairs in a 
patient's donor sites.

[0063] Because of limited effective treatment options, there is substantial interest among 
individuals for novel, safe and effective treatments for hair loss, including those that lead to 
true follicular neogenesis.

2.6 CURRENT TREATMENTS FOR REMOVAL OF UNWANTED HAIR 
IN HUMAN SUBJECTS

[0064] Current methods for removing unwanted hair involve depilation and epilation with 
or without the use of hair growth retardants.

[0065] Depilation affects the part of the hair above the surface of the skin. The most 
common form of depilation is shaving. Another popular option is the use of chemical 
depilatories (e.g., Nair®), which work by breaking the disulfide bonds that link the protein 
chains that give hair its strength, making the hair disintegrate.

[0066] Epilation is removal of the entire hair, including the part below the skin, and is 
believed to be longer-lasting. Methods include plucking with tweezers, waxing, sugaring, 
epilation devices, threading, home pulsed light, and can include the use of hair growth 
retardants (e.g., Vaniqa® (eflornithine)).

[0067] Electrology (electrolysis), laser and intense pulsed light are used for permanent 
hair removal. However, permanent hair removal is an imperfect process. For example, laser 
hair removal does not work well on light hair and/or on dark skin. Moreover, multiple 
sessions with trained medical personnel are required.

2.7 CURRENT TREATMENTS FOR WOUNDS AND SCARS IN HUMAN 
SUBJECTS

2.7.1 WOUND TREATMENT.

[0068] Acute treatment of wounds is generally focused on hemostasis, cleaning and 
closing the wound, and preventing and treating infection. The treatment depends on the type, 
cause, and depth of the wound as well as whether other structures beyond the skin are 
involved. If a laceration occurred some time ago, it may be allowed to heal by secondary
intention due to the high rate of infection associated with immediate closure. Minor wounds like bruises tend to heal on their own with skin discoloration that usually disappears within 1-2 weeks. Abrasions usually require no active treatment except keeping the area clean with soap and water, although scarring may occur. Puncture wounds may be prone to infection depending on the depth of penetration. The entry of a puncture wound is usually left open to allow for bacteria or debris to be removed from the inside.

If closure of a wound is decided upon a number of techniques can be used, such as bandages, a Cyanoacrylate glue, staples, and sutures. Absorbable sutures have the benefit over non-absorbable sutures of not requiring removal. They are often preferred in children. Most clean open wounds do not require any antibiotics unless the wound is contaminated or the bacterial cultures are positive. Excess use of antibiotics may lead to resistance and side effects.

Appropriate treatment of chronic wounds seeks to address the problems at the root of chronic wounds, including ischemia and hypoxia, bacterial load, and imbalance of proteases. Various methods exist to ameliorate the problems associated with wounds, including antibiotic and antibacterial use, debridement, irrigation, vacuum-assisted closure, warming, oxygenation, moist wound healing, removing mechanical stress, and adding cells or other materials to secrete or enhance levels of healing factors. However, many of the foregoing treatments are good temporary measures but are not concerned with, and thus not designed for, optimal wound recovery. The addition of exogenous hair follicle cells to acute wounds can result in regenerated skin, after healing, as long as the hair follicles formed. See, e.g., Prouty et ai, 1997, Lab. Invest. 76:179-189; and Prouty et ai, 1996, Am. J. Pathol. 148:1871-1885.

2.7.1.1 **TREATMENT WITH GROWTH FACTORS, PROTEASE INHIBITORS AND HORMONES**

Since some wounds, particularly chronic wounds, underexpress growth factors necessary for healing tissue, chronic wound healing may be speeded by replacing or stimulating those factors and by preventing the excessive formation of proteases like elastase that break them down.

One way to increase growth factor concentrations in wounds is to apply the growth factors directly, though this takes many repetitions and requires large amounts of the factors. Another way is to spread onto the wound a gel of the patient's own blood platelets, which then secrete growth factors such as vascular endothelial growth factor (VEGF),
insulin-like growth factor 1-2 (IGF), PDGF, transforming growth factor-β (TGF-β), and epidermal growth factor (EGF). Other treatments include implanting cultured keratinocytes into the wound to re-epithelialize it and culturing and implanting fibroblasts into wounds.

Since levels of protease inhibitors are lowered in chronic wounds, some researchers are seeking ways to heal tissues by replacing these inhibitors in them. Secretory leukocyte protease inhibitor (SLPI), which inhibits not only proteases but also inflammation and microorganisms like viruses, bacteria, and fungi, may prove to be an effective treatment.

Research into hormones and wound healing has shown estrogen to speed wound healing in elderly humans and in animals that have had their ovaries removed, possibly by preventing excess neutrophils from entering the wound and releasing elastase. Thus the use of estrogen is a possibility for treating acute and chronic wounds. For information on the hormonal regulation of wound healing see, e.g., Gilliver et al., 2007, Clin. Dermatol. 25:56-62, which is incorporated herein by reference in its entirety.

### 2.7.1.2 GRAFTING AND DRESSINGS

Some patients are treated with artificial skin substitutes that have fibroblasts and keratinocytes in a matrix of collagen to replicate skin and release growth factors. In other cases, skin from cadavers is grafted onto wounds, providing a cover to keep out bacteria and preventing the buildup of too much granulation tissue, which can lead to excessive scarring. Though the allograft (skin transplanted from a member of the same species) is replaced by granulation tissue and is not actually incorporated into the healing wound, it encourages cellular proliferation and provides a structure for epithelial cells to crawl across. On the most difficult chronic wounds, allografts may not work, requiring skin grafts from elsewhere on the patient, which can cause pain and further stress on the patient’s system.

Collagen dressings are another way to provide the matrix for cellular proliferation and migration, while also keeping the wound moist and absorbing exudate.

### 2.7.2 SCAR REVISION

Although healing in embryos and some animals even after extended injuries can occur without scarring, no adult human skin scars can be completely removed. Current treatments involve methods that remove the scar by surgical intervention (scar revision) and non-surgical methods that reduce the appearance of the scar. However, no scar treatment to date has been completely effective. As stated by Lalwani A K (2008, Chapter 71, "Scar Revision," in *Current Diagnosis & Treatment in Otolaryngology—Head & Neck Surgery*, 2d
ed., McGraw-Hill Companies, Inc.), "no technique has been devised to allow total and permanent removal or effacement of scars. Patients should be counseled to understand that the goal of scar revision is to replace one scar for another to improve the appearance and the acceptability of the scar." Clearly, there is a large unmet need for techniques to revise scars, particularly those that form from wounds and burns.

The only current commonly used surgical scar revision methods are skin grafts or serial expansion of surrounding skin. Other surgical approaches are primary excision/straight line closure, W plasty, Z plasty, and geometric broken line closure. See, Lalwani AK, 2008, Chapter 71, "Scar Revision," IN Current Diagnosis & Treatment in Otolaryngology—Head & Neck Surgery, 2d ed., McGraw-Hill Companies, Inc.

Skin grafts (either full thickness or split thickness) do not fully address the need for effective scar revision because (1) there is a limited supply of donor tissue (typically buttocks, abdomen, legs, in-front or behind the ear, etc.); (2) scarring occurs at donor sites and contractures (i.e., around the eyes and mouth); and (3) skin grafts typically (but not always) (with the possible exception of scalp) lack qualities of the donor site (porosity, vascularity, color, pigmentation, thickness, texture and overall cosmetic appearance, etc.). In serial expansion, the scar is serially excised, and a balloon is implanted at the wound site, which pushes the tissue around the scar to expand. Surgical excision of hypertrophic or keloid scars is often used with other methods such as pressotherapy or silicone gel sheeting (see below). Lone excision of keloid scars shows a high recurrence rate, close to 45%. Surgical excision in combination with the immunomodulator imiquimod 5% cream (Aldara) may also have a benefit on scar reduction.


Injection of corticosteroids into scars was introduced in the 1960s. A long term course of steroid injections into the scar under medical supervision may help flatten and soften the appearance of keloid or hypertrophic scars. The steroid is injected into the scar itself; since very little is absorbed into the blood stream, side effects of this treatment are minor. However, it does cause thinning of the scar tissue. This treatment is repeated at 4-6 week intervals. Topical steroids are ineffective as scar treatments.

Other intralesional injections (such as anti-mitotics and collagen) can also be used. For example, collagen injections or other soft tissue fillers can be used to raise sunken scars.
to the level of surrounding skin. Its effects are temporary, however, and it needs to be regularly repeated. There is also a risk in some people of an allergic reaction.

Silicone scar treatments improve scar appearance and are often used to prevent and treat hypertrophic scarring. The exact mechanism of action is unknown, though some studies suggest a manipulation of local ionic charges or a decrease in production of "pro-inflammatory" substances like TGFp2. See, e.g., Kuhn et al., 2001, "Silicone sheeting decreases fibroblast activity and downregulates TGFp2 in hypertrophic scar model," Int J Surg Invest 2:467. Dimethicone silicone gel appears to be as effective as silicone sheeting in improving scar appearance. See Mustoe TA, 2008, "Evolution of silicone therapy and mechanism of action in scar management," Aesth Plast Surg 32:82-92. Polyurethane bandages are also used.

Pressure garments are used under supervision by a medical professional. They are most often used for burn scars that cover a large area, and is only effective on recent scars. Pressure garments are usually custom-made from elastic materials, and fit tightly around the scarring. They work best when they are worn 24 hours a day for six to twelve months. It is believed that they work by applying constant pressure to surface blood vessels and eventually causing scars to flatten and become softer.

Needling is an inexpensive process where the scarred area is continuously needled to promote collagen formation. Once needled the area is allowed to fully heal, and needled again if required depending on the intensity of the scar. Scarring needles and needling rollers are available for home use; however, needling should not be done on parts of the face or areas where major nerves are located without professional medical supervision. Needling at home must also be done in line with hygienic and sterilization requirements.

Dermabrasion involves the removal of the surface of the skin with specialist equipment and usually involves a general anesthetic. It is useful with raised scars, but is less effective when the scar is sunken below the surrounding skin.

The use of lasers on scars is a new form of treatment. Several cosmetic lasers have been approved for the treatment of acne scars by using laser resurfacing techniques. Vascular lasers have been proven to greatly reduce the redness of most scars 6-10 weeks after the initial treatment. Fractional lasers and 1064 Nd:YAG lasers have also been shown in clinical studies to benefit scars. Pulsed dye laser has been reported to reduce scar erythema.

Low-dose, superficial radiotherapy is used to prevent re-occurrence of severe keloid and hypertrophic scarring. It is usually effective, but only used in extreme cases due to the risk of long-term side effects.
Although several natural remedies to treat scars have been proposed, such as vitamin E and A, research shows the use of vitamin E and onion extract as a treatment for scars is ineffective. Vitamin E causes contact dermatitis in up to 33% of users and in some cases it may worsen scar appearance. See, Baumann & Spencer, 1999, "The effects of topical vitamin E on the cosmetic appearance of scars," Dermatol Surg. 25:31 1-315; and Jenkins et al, 1986, "Failure of topical steroids and vitamin E to reduce postoperative scar formation following reconstructive surgery," J Burn Care Rehabil 7: 309-312. However, there is evidence that vitamin C normalizes collagen production and encourages the production of an organized, healthy collagen framework, which improves scar appearance. Vitamin C and some of its esters also fade the dark pigment associated with some scars. See, Fitzpatrick & Rostan, 2002, "Double-blind, half-face study comparing topical vitamin C and vehicle for rejuvenation of photodamage," Dermatol Surg 28:231-236; and Farris PK, 2005, "Topical vitamin C: a useful agent for treating photoaging and other dermatologic conditions," Dermatol Surg 31:814-818.

Thus, scar revision and wound management are limited by the limited regenerative capacity of adult human skin. Extensive skin damage (e.g., from blast trauma, penetrating munitions, flying debris, or burns) results in severe scars that can limit mobility or function. 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.

3. SUMMARY OF THE INVENTION

Methods for modulating hair growth in human subjects are described, involving the administration of compounds described herein ("Compound") to a human subject in need of such treatment. Methods for inducing or enhancing hair growth in human subjects are described. Methods for hair removal or inhibiting hair growth in human subjects are also described. Methods for promoting or improving wound healing, promoting wound healing with reduced scarring, scar prevention, and scar revision are also described, involving the administration of the Compound to a human subject in need of such treatment.

Uses of the Compound for modulating hair growth in human subjects are described. Uses of the Compound for inducing or enhancing hair growth in human subjects are described. Uses of the Compound for hair removal or inhibiting hair growth in human subjects are described. Uses of the Compound for promoting or improving wound healing,
promoting wound healing with reduced scarring, scar prevention, and scar revision are also described.

[0093] Pharmaceutical compositions comprising the Compound for modulating hair growth in human subjects are described. Pharmaceutical compositions comprising the Compound for inducing or enhancing hair growth in human subjects are described. Pharmaceutical compositions comprising the Compound for hair removal or inhibiting hair growth in human subjects are described. Pharmaceutical compositions comprising the Compound for promoting or improving wound healing, promoting wound healing with reduced scarring, scar prevention, and scar revision are also described.

[0094] In certain embodiments, the Compound inhibits glycogen synthase kinase 3 (GSK-3). In certain embodiments, the Compound inhibits GSK-3beta (GSK-3P). In one embodiment, the Compound is a compound having formula I described hereinbelow. In certain embodiments, the Compound is a compound having formula II or formula III described hereinbelow. In a particular embodiment, the Compound is a compound having formula IV described hereinbelow. In other particular embodiments, the Compound is one of compounds 1-14 described hereinbelow. In another embodiment, the Compound is a compound having formula V described hereinbelow. In particular embodiments, the Compound is one of compounds 15-24 described hereinbelow. In another embodiment, the Compound is a compound having formula VI described hereinbelow. In particular embodiments, the Compound is one of compounds 25-32 described hereinbelow. In another embodiment, the Compound is a compound having formula VII described hereinbelow. In another embodiment, the Compound is a compound having formula VIII described hereinbelow. In another embodiment, the Compound is a compound having formula IX described hereinbelow. In another embodiment, the Compound is a compound having formula X described hereinbelow. In particular embodiments, the Compound is one of compounds 33-45. In certain more particular embodiments, the Compound is compound 41.

[0095] In some embodiments, the Compound inhibits the activity of GSK-3P in an in vitro assay, as described in Section 5.7.2 infra. In some embodiments, the Compound inhibits the activity of GSK-3b in a cell-based assay, as described in Section 5.7.2 infra. In some embodiments, the Compound modulates the growth of hair in an animal model described herein (see, e.g., Section 5.7.3 and Sections 10, 16, 24, and 25). In some embodiments, the Compound, either alone or in combination with a therapy described in Section 5.3 infra, promotes hair growth in an animal model described herein. In alternative embodiments, the Compound, either alone or in combination with a therapy described in
Section 5.3 *infra*, reduces hair growth in an animal model described herein. In some embodiments, the Compound, either alone or in combination with a therapy described in Section 5.3 *infra*, promotes wound healing, *e.g.*, wound healing with reduced scarring, or is used to aid scar revision in an animal model described herein.

[0096] The Compound is preferably administered topically, but other routes can be employed as well, as such the transdermal, intradermal, cutaneous, subcutaneous, intramuscular, intravenous, oral, sublingual, or buccal routes. 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. Topical formulations and topical treatment methods comprising the Compound are preferred embodiments because high local concentrations can be achieved while minimizing systemic exposure. The Compound may be formulated to be released in a sustained fashion, or in a "pulsed" fashion. In order to achieve a pulsed mode of administration, the Compound may be administered multiple times. Another way to achieve pulsed delivery is to formulate the Compound in beads with multiple coatings, with the Compound contained in alternate coating layers. Other Compound formulations, combinations, and delivery methods for use in the pharmaceutical compositions and treatment methods described herein are described in Sections 5.2 and 5.3 *infra*. For example, the Compound formulations, including various modified release forms, may be delivered topically as additives to shampoos and other hair products, as a lotion, cream, or ointment, or may be delivered using devices such as iontophoresis, micro-needle injection arrays, or auto-injector devices.

[0097] The Compound may be administered as a single-agent or in combination with other treatments for the enhancement or inhibition of hair growth. The Compound may be administered in combination with other treatments that promote scar revision or wound healing, including wound healing with reduced scarring. Embodiments of the invention include combination therapies involving the addition of other treatment(s) concurrently with, or before and/or after treatment with the Compound. 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 low voltage electric current, electrology, laser, intense pulsed light, dermabrasion, or surgical treatments (*e.g.*, hair transplant, strip harvesting, follicular unit extraction (FUE), scalp reduction, *etc.*) that either promote or inhibit the growth of hair and/or which promote scar revision or wound healing.
In certain embodiments, the Compound is formulated for administration together with the other treatment(s).

In one embodiment, the Compound is administered concurrently or in sequential/alternating combination with other agents or treatments that stimulate hair growth. For example, treatment with the Compound can be concurrently or in sequential/alternating combination with other agents or treatments that increase overall hair density in a human subject. For example, the Compound can be administered concurrently or alternating sequentially with one or more of the following agents to promote or induce hair growth: a prostaglandin F2a analog, bimatoprost (Latisse), latanoprost, travoprost, tafluprost, a 5a-reductase inhibitor, minoxidil, kopexil (for example, the product Keranique™), finasteride, CaCb, or adenosine. In another embodiment, the Compound is administered concurrently or alternating sequentially with one or more agents that prevent hair follicle cells from senescing to promote hair growth and hair follicle formation (which may, in turn, promote wound healing with reduced scarring), for example, anti-oxidants such as glutathione, ascorbic acid, tocopherol, uric acid, or polyphenol antioxidants; activators of Wnt signaling; inhibitors of EGF signaling; inhibitors of BMP signaling; 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.

Moreover, the Compound, with or without an additional agent for promoting hair growth, can be used in combination with integumental perturbation to promote hair growth and hair follicle formation (which, in turn, may promote wound healing), such as by, e.g., 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 CO₂-based, or Erbium-YAG-based, etc.), 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.). For example, a low-level laser therapy treatment (e.g., HairMax) can be applied concurrently with administration of the Compound to stimulate hair growth.

In one embodiment, treatment with the Compound can be in combination with perturbation (e.g., debriding, peeling, or wounding) of the skin and/or other tissues of the integumentary system by methods such as dermabrasion, microneedles, laser treatment, 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 the Compound. For example, the integumental perturbation procedure can be any "wounding" procedure used for scar revision, or the integumental perturbation has already occurred by virtue of wound formation. 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.

[00101] Success of treatment with the Compound can be measured by:

- increased terminal hair formation
- increased maturation of hair follicles
- increased hair density
- increased hair count
- increased hair weight
- increased hair shaft thickness (diameter)
- increased hair length
- follicle synchronization so that the overall hair density appears to be greater compared to previous asynchronous hair growth
- increased proportion of follicles in anagen or decreased proportion of follicles in telogen
- follicle regeneration
- increased numbers of follicular units with 3 or more hair follicles
- hair cuttings
- subjective patient measures of increased hair.

[00102] Human subjects who are candidates for such treatments include any subject for whom increased hair growth is desired including, but not limited to, subjects with nonscarring (noncicatricial) alopecia, such as androgenetic alopecia (AGA), including MPHL or FPHL, or any other form of hair loss caused by androgens, toxic alopecia, alopecia areata (including alopecia universalis), scarring (cicatricial) alopecia, pathologic alopecia (caused by, e.g., medication, chemotherapy, trauma, wounds, burns, stress, autoimmune diseases), senescence (age-related hair loss), malnutrition, or endocrine dysfunction), or hypotrichosis, or any other disease, disorder, or form of hair loss as discussed infra and/or known in the art.

[00103] In particular embodiments, the invention provides a method for inducing hair growth on the scalp of a male or a female subject with androgenetic alopecia wherein the
method comprises in the following order: (a) applying integumental perturbation; (b) administering an inhibitor of GSK-3, such as, in particular embodiments, a GSK-3beta inhibitor, topically; (c) a period without topical treatment with an inhibitor of GSK-3 and without topical treatment with minoxidil; and (d) administering minoxidil topically. In more specific embodiments, integumental perturbation is performed using dermabrasion with an estimated depth of 100 microns, CHIR99021 is administered topically, and minoxidil is administered in the form of 5% minoxidil foam. In specific embodiments, an inhibitor of GSK-3, such as, in particular embodiments, a GSK-3beta inhibitor, is administered topically immediately following dermabrasion twice daily for about 1 week, followed by a 3 week period without topical treatment, which in turn is followed by a period of at least 5 months of minoxidil treatment.

[00104] In particular embodiments, the invention provides a method for inducing hair growth on the scalp of a male or female subject with androgenetic alopecia, wherein the method comprises:

[00105] (a) Dermabrasion (estimated depth 100 microns) at Day 0;
[00106] (b) Commencing at Day 0, topical administration of CHIR99021 gel twice daily for about 7 days;
[00107] (c) Period without topical treatment for about 21 days;
[00108] (d) Immediately following step (c), topical administration of 5% minoxidil foam for at least 5 months.

[00109] In a particular embodiment, a human subject who is a candidate for treatment with a Compound described herein is a human subject with scarring (cicatricial) alopecia. Forms of cicatricial alopecia that may be treated in accordance with the methods described herein include primary cicatricial alopecia (PCA) and secondary cicatricial alopecia. Primary cicatricial alopecias that may be treated in accordance with the methods described herein include lymphocyte-mediated PCAs, such as lichen planopilaris (LPP), frontal fibrosing alopecia (FFA), central centrifugal cicatricial alopecia (CCCA), and pseudopelade (Brocq); neutrophil-mediated PCAs, such as folliculitis decalvans and tufted folliculitis; and PCAs involving a mixed inflammatory infiltrate, such as occurs in dissecting cellulitis and folliculitis keloidalis.

[00110] In a particular embodiment, provided herein is a method for enhancing hair growth in a patient with scarring alopecia comprising controlled integumental perturbation using a fractional ablative laser, followed by twice daily topical administration of a Compound. In certain embodiments, treatment with a Compound is begun on the same day
as the laser treatment. In one embodiment, the patient has primary scarring alopecia. In one embodiment, the patient has lichen planopilaris. In another embodiment, the patient has frontal fibrosing alopecia.

[0011] Success of treatment, such as an intermittent or pulse treatment, with a Compound for scarring alopecia may be measured using the methods described above. In some embodiments, successful treatment is determined as an increase in the number of photographically detected hairs. In other embodiments, successful treatment is evaluated by a skin biopsy for hair follicle structures and scar attributes. Success may also be measured as a reduction or elimination of itching, burning, pain, and tenderness associated with the condition, or a reduction of scalp redness, scaling, and/or pustules. Success may also be measured as a reduction or elimination of inflammation of the scalp.

[0012] In alternative embodiments, the Compound is administered concurrently or in sequential combination with a cytotoxic drug, a hair growth retardant, epilation or depilation methods to reduce unwanted hair growth. For example, the Compound is administered alternating sequentially with one or more of the following agents or treatments can be used to inhibit unwanted hair growth: efomithine HCl (Vaniqa), 5-fluorouracil (5-FU) (e.g., Efudex 5% cream), and/or epilation. Success of treatment can be measured by:

- decreased terminal hair formation
- follicle synchronization so that synergies are achieved when the hair growth retardant is sequentially applied
- decreased proportion of follicles in anagen or increased proportion of follicles in telogen
- inhibition of follicle regeneration
- decreased hair length
- decreased hair thickness
- decreased pigmentation, which will make thinner hairs less visible
- decreased hair weight
- subjective patient measures of decreased hair.

[0013] Human subjects who are candidates for such treatment include any subject for whom elimination of unwanted visible hair is desired including, but not limited to, those afflicted with hypertrichosis, excess hair in androgen-dependent areas of the skin, idiopathic hirsutism, female post-menopausal facial hair, axillary hair, leg hair, back hair, ear hair, nares.
or nose hair, or any other disease, disorder, or form of unwanted hair or excessive hair as discussed infra and/or known in the art.

[00114] The invention is based in part on the realization that human hair follicles and follicular units are relatively unique (among other mammals, particularly non-primates) in that they enter and progress through different stages of the hair Follicle Cycle relatively independently of each other, even independently of neighboring follicles or follicular units. Consequently, the normal biology of human hair patterning is based on a probability distribution of the hair cycle stage that follicles will be in, generated by a stochastic (random) process by which follicles cycle independently.

[00115] Thus, an object of the invention is to synchronize hair follicle growth in the treated area to more effectively promote the growth of terminal hair (in preference to vellus hair); and/or promote the branching of pre-existing hair follicles (seen as an increased number of hair shafts per pore); and/or increase the width of hair follicles (thereby promoting growth of an increased shaft width); and/or promote regeneration of hair follicles or generation of new hair follicles ("follicle neogenesis"); and/or delay or prevent follicle senescence. In the alternative, the object is to inhibit the growth of unwanted hair (as measured by, e.g., decreased terminal hair formation or inhibition of follicle regeneration or generation of new follicles). Without being bound to any theory, treatment with the Compound may achieve these results by:

- regulating the unique human processes that regulate visible hair growth.
- regulating the activity of specialized human hair follicles.
- regulating specific activities of specialized human hair follicles.
- regulating gender-specific specialized human hair follicles, including those under the influence of sex-steroid regulation.
- altering the activity of specialized human hair follicles, sometimes in conjunction with transplantation.
- regulating the differentiation of stem cells into gender-specific specialized human hair follicles, that may result 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).
- altering, delaying or preventing programmed senescence of hair follicles.

[00116] In some embodiments, treatments that promote hair growth, or, alternatively, treatments that prevent hair growth, may also be used in combination with the Compound in
order to promote the establishment of desired hair patterning in a 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 a Compound 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, Compound treatment, such as intermittent or pulse Compound 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 efornithine HCl (Vaniqa), 5-fluorouracil (5-FU) (e.g., Efudex 5% cream), or other epilation or depilation methods to prevent or reduce hair growth, as described supra.

[00117] In some embodiments, a Compound described herein may improve wound healing (including promoting wound healing with reduced scarring) or scar revision, as 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 Compound
• 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.

[00118] In some embodiments, human subjects who are candidates for Compound treatments described herein include any subject in need of improved wound healing, particularly wound healing with reduced 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.

[00119] Without being bound by any theory, the invention is based in part on the recognition that the timing of the administration of the Compound is important for it to function as an effective modulator of hair follicle formation and wound healing (and thus, scar revision) in human subjects. In some embodiments, the Compounds provided herein are potent and specific inhibitors of protein kinase glycogen synthase kinase 3 (GSK-3) (see Ring et al, 2003, Diabetes 52: 588-595; Bain et al, 2007, Biochem. J. 408:297-215; Meijer et al, 2004, Trends in Pharmacological Sciences 25(9): 471-480). GSK-3P inhibitors are known to increase 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 even lead to decreased hair growth (Millar et al, 1999, Dev. Biol. 207:133-149).
Thus, in some embodiments, by using Compounds in formulations for \textit{intermittent or pulse} treatments described herein, for example, before, concurrently with, or after integumental perturbation or another treatment that modulates hair growth or wound healing, it functions as an effective treatment for modulation of hair growth and wound and scar revision in humans.

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 Compound is administered in combination with methods that mobilize tissue stem cells (\textit{e.g.}, using integumental perturbation); and/or methods that mobilize bone marrow-derived stem cells (\textit{e.g.}, growth factors such as G-CSF and/or chemical agents such as plerixafor (Mozobil®)); and/or methods that regulate the differentiation of these stem cells into gender-specific specialized human hair follicles (\textit{e.g.}, using agents such as finasteride, fluconazole, spironolactone, flutamide, diazoxide, 17-alpha-hydroxyprogesterone, 11-alpha-hydroxyprogesterone, ketoconazole, RU58841, dutasteride, fluridil, or QLT-7704, an antiandrogen oligonucleotide, cyoctol, topical progesterone, topical estrogen, cyproterone acetate, 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; \textit{e.g.}, such as valproate), \textit{etc.}, known in the art), that can result in the appearance of specialized follicles having features that are different from natural follicles in the target location of skin. Such combination treatments can further include the use of agents that enhance the growth of hair (\textit{e.g.}, minoxidil, kopexil (\textit{e.g.}, Keranique), finasteride, prostaglandin F2a analogs, bimatoprost (Latisse), latanoprost, travoprost, tafluprost, CaCb, adenosine, a 5a-reductase inhibitor, and others described herein) or aid in the removal of hair (\textit{e.g.}, 5-fluorouracil, eflornithine (Vaniqa), or others described herein). The Follicle Stem Cells involved can be derived from (1) other Follicle Stem Cells, (2) from other tissue stem cells, termed "pre-Follicle Stem Cells" (from the interfollicular skin), (3) from bone marrow-derived stem cells ("BMST"), and/or (4) from mesenchymal stem cells such as adipocyte stem cells. In the case of bone marrow derived stem cells (BMST), their differentiation into Follicle Stem Cells requires intact follicles, whose cells can play the role of "nurse cells" and provide appropriate signals to guide the differentiation of bone marrow derived stem cells into Follicle Stem Cells. Integumental perturbation (for example, by the induction of inflammation, wounding, or laser treatment) (1) provides signals for Follicle Stem Cells to divide symmetrically to begin the process of forming new follicles; (2) mobilizes tissue stem cells ("pre-Follicle Stem Cells") from interfollicular skin to
differentiate into stem cells and (3) increases the trafficking of bone marrow derived stem
cells to affected areas of skin and promotes their differentiation into Follicle Stem Cells by
nurse cells in existing follicles. When used in combination with such procedures, treatment
with one or more Compounds provided herein organizes the normally asynchronous state of
human hair follicle cells in Cell Cycle and human hair follicles in Follicle Cycle into
relatively more synchronous states of human hair follicle cells in Cell Cycle and human hair
follicles in Follicle Cycle.

[00121] The methods, uses, and pharmaceutical compositions of the invention are
illustrated by the examples described in Sections 6 to 25.

[00122] 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
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.

[00123] 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.

[00124] 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.

[00125] In certain embodiments, the drug spraying device disclosed herein enables the sustained release of a GSK-3 inhibitor, such as a Compound as defined herein, without the use of highly hydrophobic, occlusive matrices. In particular, the drug spraying device enables the delivery of a GSK-3 inhibitor 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.

[00126] In certain embodiments, the drug spraying device disclosed herein enables the sustained release of a GSK-3 inhibitor and uptake by the skin through a scab. In particular, the drug spraying device enables the delivery of a GSK-3 inhibitor such that the delivery system is incorporated into the scab. This can be accomplished by placing a GSK-3 inhibitor 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.

[00127] 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).

[00128] 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 a GSK-3 inhibitor. Any wound-cleansing solution known to the skilled artisan can be used with these embodiments.
3.1 GLOSSARY OF TERMS FOR HAIR AND DISORDERS OF HAIR GROWTH AND DEFINITIONS

[00129] The following terms are used herein consistently with their art-accepted meanings summarized below.


[00131] Alopecia areata: Hair loss in patches, thought to be caused by an autoimmune response to hair follicles in the anagen stage; extensive forms of the disorder are called alopecia areata totalis (hair loss over the entire scalp) and alopecia areata universalis (hair loss over the entire body).

[00132] Anagen: Growth stage of the hair-Follicle Cycle.

[00133] Anagen effluvium: Abrupt shedding of hair caused by interruption of active hair-follicle growth (e.g., in patients undergoing chemotherapy).

[00134] Androgenetic alopecia (AGA): Baldness caused by miniaturization of genetically predisposed follicles in the MPHL pattern (frontal recession and thinning at the vertex) or the FPHL pattern (loss of hair primarily over the crown, with sparing of frontal hair).

[00135] Bulb: Lowermost portion of the hair follicle, containing rapidly proliferating matrix cells that produce the hair.

[00136] Bulge: Portion of the outer-root sheath of the hair follicle, located at the region of the insertion of the arrector pili muscle; thought to contain epithelial stem cells responsible for regenerating follicles in the anagen stage.

[00137] Catagen: Stage of the hair cycle characterized by regression and involution of the follicle.

[00138] Cicatricial (scarring) Alopecia: Abnormal hair loss with scarring. Caused by destruction of hair follicles and replacement with scar tissue as a result of inflammation, trauma, fibrosis, or unknown causes; examples include lichen planopilaris and discoid lupus erythematosus.

[00139] Club hair: Fully keratinized, dead hair — the final product of a follicle in the telogen stage; 50 to 150 club hairs are shed daily from a normal scalp.

[00140] Exogen: Phase of the hair Follicle Cycle where hair shaft is shed from the follicle.

[00141] Female Pattern Hair Loss (FPHL): form of gender specific hair patterning in females (also sometimes referred to as female pattern alopecia).
[00142] Follicle cycle: Hair growth in each follicle occurs in a cycle that includes the following phases: anagen (growth phase), catagen (involuting/regressing stage), telogen (the quiescent phase), exogen (shedding phase), and re-entry into anagen.


[00144] Hypertrichosis: Excessive hair growth (usually diffuse) beyond that considered normal according to age, race, sex, and skin region.

[00145] Integumental: Pertaining to the integumentary system, which comprises the skin (epidermis, dermis, hypodermis (or subcutanea)) and all cells contained therein regardless of origin, and its appendages (including, e.g., hair and nails).

[00146] Kenogen: Latent phase of hair cycle after hair shaft has been shed and growth is suspended in follicle.

[00147] Lanugo hair: Fine hair on the body of the fetus, usually shed in utero or within weeks after birth.

[00148] Male Pattern Hair Loss (MPHL): form of gender specific hair patterning in men (also sometimes referred to as male pattern alopecia).

[00149] Miniaturization: Primary pathological process in androgenetic alopecia, resulting in conversion of large (terminal) hairs into small (vellus) hairs.

[00150] Permanent alopecia: Caused by destruction of hair follicles as a result of inflammation, trauma, fibrosis, or unknown causes; examples include lichen planopilaris and discoid lupus erythematosus. Include diseases referred to as scarring alopecia.

[00151] Telogen: Resting stage of the hair cycle; club hair is the final product and is eventually shed.

[00152] Telogen effluvium: Excessive shedding of hair caused by an increased proportion of follicles entering the telogen stage; common causes include drugs and fever.

[00153] Terminal hair: Large, usually pigmented hairs on scalp and body.

[00154] Vellus hair: Very short, nonpigmented hairs (e.g., those found diffusely over nonbeard area of face and bald scalp as a result of miniaturization of terminal hairs).

[00155] The term "alkyl" refers to a linear or branched saturated monovalent hydrocarbon radical, wherein the alkylene may optionally be substituted as described herein. The term "alkyl" also encompasses both linear and branched alkyl, unless otherwise specified. In certain embodiments, the alkyl is a linear saturated monovalent hydrocarbon radical that has 1 to 20 (C1-20), 1 to 15 (C1-15), 1 to 10 (C1-10), or 1 to 6 (C1-6) carbon atoms, or branched saturated monovalent hydrocarbon radical of 3 to 20 (C3-20), 3 to 15 (C3-15), 3 to 10 (C3-10), or 3 to 6 (C3-6) carbon atoms. As used herein, linear C1-6 and branched C3-6 alkyl groups are also
referred as "lower alkyl." Examples of alkyl groups include, but are not limited to, methyl, ethyl, propyl (including all isomeric forms), n-propyl, isopropyl, butyl (including all isomeric forms), n-butyl, isobutyl, sec-butyl, t-butyl, pentyl (including all isomeric forms), and hexyl (including all isomeric forms). For example, C\textsubscript{1-6} alkyl refers to a linear saturated monovalent hydrocarbon radical of 1 to 6 carbon atoms or a branched saturated monovalent hydrocarbon radical of 3 to 6 carbon atoms.

[00156] The term "alkenyl" refers to a linear or branched monovalent hydrocarbon radical, which contains one or more, in one embodiment, one to five, carbon-carbon double bonds. The alkenyl may be optionally substituted as described herein. The term "alkenyl" also embraces radicals having "cis" and "trans" configurations, or alternatively, "Z" and "E" configurations, as appreciated by those of ordinary skill in the art. As used herein, the term "alkenyl" encompasses both linear and branched alkenyl, unless otherwise specified. For example, C\textsubscript{2-6} alkenyl refers to a linear unsaturated monovalent hydrocarbon radical of 2 to 6 carbon atoms or a branched unsaturated monovalent hydrocarbon radical of 3 to 6 carbon atoms. In certain embodiments, the alkenyl is a linear monovalent hydrocarbon radical of 2 to 20 (C\textsubscript{2-20}), 2 to 15 (C\textsubscript{2-15}), 2 to 10 (C\textsubscript{2-10}), or 2 to 6 (C\textsubscript{2-6}) carbon atoms, or a branched monovalent hydrocarbon radical of 3 to 20 (C\textsubscript{3-20}), 3 to 15 (C\textsubscript{3-15}), 3 to 10 (C\textsubscript{3-10}), or 3 to 6 (C\textsubscript{3-6}) carbon atoms. Examples of alkenyl groups include, but are not limited to, ethenyl, propen-1-yl, propen-2-yl, allyl, butenyl, and 4-methylbutenyl.

[00157] The term "alkynyl" refers to a linear or branched monovalent hydrocarbon radical, which contains one or more, in one embodiment, one to five, carbon-carbon triple bonds. The alkynyl may be optionally substituted as described herein. The term "alkynyl" also encompasses both linear and branched alkynyl, unless otherwise specified. In certain embodiments, the alkynyl is a linear monovalent hydrocarbon radical of 2 to 20 (C\textsubscript{2-20}), 2 to 15 (C\textsubscript{2-15}), 2 to 10 (C\textsubscript{2-10}), or 2 to 6 (C\textsubscript{2-6}) carbon atoms, or a branched monovalent hydrocarbon radical of 3 to 20 (C\textsubscript{3-20}), 3 to 15 (C\textsubscript{3-15}), 3 to 10 (C\textsubscript{3-10}), or 3 to 6 (C\textsubscript{3-6}) carbon atoms. Examples of alkynyl groups include, but are not limited to, ethynyl (-\text{C}≡\text{CH}) and propargyl (-\text{CH}=\text{C}≡\text{CH}). For example, C\textsubscript{2-6} alkynyl refers to a linear unsaturated monovalent hydrocarbon radical of 2 to 6 carbon atoms or a branched unsaturated monovalent hydrocarbon radical of 3 to 6 carbon atoms.

[00158] The term "cycloalkyl" refers to a cyclic saturated bridged and/or non-bridged monovalent hydrocarbon radical, which may be optionally substituted as described herein. In certain embodiments, the cycloalkyl has from 3 to 20 (C\textsubscript{3-20}), from 3 to 15 (C\textsubscript{3-15}), from 3 to 10 (C\textsubscript{3-10}), or from 3 to 7 (C\textsubscript{3-7}) carbon atoms. Examples of cycloalkyl groups include, but are
not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, decalinyl, and adamantyl.

[00159] The term "aryl" refers to a monocyclic aromatic group and/or multicyclic monovalent aromatic group that contain at least one aromatic hydrocarbon ring. In certain embodiments, the aryl has from 6 to 20 (C6-20), from 6 to 15 (C6-15), or from 6 to 10 (C6-10) ring atoms. Examples of aryl groups include, but are not limited to, phenyl, naphthyl, fluorenyl, azulenyl, anthryl, phenanthryl, pyrenyl, biphenyl, and terphenyl. Aryl also refers to bicyclic or tricyclic carbon rings, where one of the rings is aromatic and the others of which may be saturated, partially unsaturated, or aromatic, for example, dihydronaphthyl, indenyl, indanyl, or tetrahydronaphthyl (tetralinyl). In certain embodiments, aryl may be optionally substituted as described herein.

[00160] The term "heteroaryl" refers to a monocyclic aromatic group and/or multicyclic aromatic group that contain at least one aromatic ring, wherein at least one aromatic ring contains one or more heteroatoms independently selected from O, S, and N. Each ring of a heteroaryl group can contain one or two O atoms, one or two S atoms, and/or one to four N atoms, provided that the total number of heteroatoms in each ring is four or less and each ring contains at least one carbon atom. In certain embodiments, the heteroaryl has from 5 to 20, from 5 to 15, or from 5 to 10 ring atoms. Examples of monocyclic heteroaryl groups include, but are not limited to, pyrrolyl, pyrazolyl, pyrazolinyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, thiadiazolyl, isothiazolyl, furanyl, thienyl, oxadiazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, and triazinyl. Examples of bicyclic heteroaryl groups include, but are not limited to, indolyl, benzothiazolyl, benzoazolyl, benzothienyl, quinolinyl, tetrahydroisoquinolinyl, isoquinolinyl, benzimidazolyl, benzopyryl, indolizinyl, benzofuranyl, isobenzofuranyl, chromonyl, coumarinyl, cinnolinyl, quinoxalinyln, indazolyl, purinyl, pyrrolopyridinyl, furopyridinyl, thienopyridinyl, dihydroquinolinyl, and tetrahydroquinolinyl. Examples of tricyclic heteroaryl groups include, but are not limited to, carbazolyl, benzindolyl, phenanthroquininyl, acridinyl, phenanthridinyl, and xanthenyl. In certain embodiments, heteroaryl may also be optionally substituted as described herein.

[00161] The term "heterocyclyl" or "heterocyclic" refers to a monocyclic non-aromatic ring system and/or multicyclic ring system that contains at least one non-aromatic ring, wherein one or more of the non-aromatic ring atoms are heteroatoms independently selected from O, S, or N; and the remaining ring atoms are carbon atoms. In certain embodiments, the heterocyclyl or heterocyclic group has from 3 to 20, from 3 to 15, from 3 to 10, from 3 to 8, from 4 to 7, or from 5 to 6 ring atoms. In certain embodiments, the heterocyclyl is a
monocyclic, bicyclic, tricyclic, or tetracyclic ring system, which may include a fused or
bridged ring system, and in which the nitrogen or sulfur atoms may be optionally oxidized,
the nitrogen atoms may be optionally quaternized, and some rings may be partially or fully
saturated, or aromatic. The heterocyclol may be attached to the main structure at any
heteroatom or carbon atom which results in the creation of a stable compound. Examples of
such heterocyclic radicals include, but are not limited to, acridinyl, azepinyl, benzimidazolyl,
benzindolyl, benzoisoxazolyl, benzisoxazinyl, benzodioxanyl, benzoxazolyl,
benzofuranonyl, benzofuranyl, benzonaphthofuranyl, benzopyranonyl, benzopyranyl,
benzotetrahydrofuranyl, benzotetrahydrothienyl, benzothiazolyl, benzothiazolyl,
benzothiophenyl, benzotriazolyl, benzothiopyranyl, benzoxazinyl, benzoxazolyl,
benzothiazolyl, β-carbolinyl, carbazolyl, chromanyl, chromonyl, cinnolinyl, coumarinyl,
decahydroisoquinolinyl, dibenzofuranyl, dihydrobenzisothiazinyl, dihydrobenzisoxazinyl,
dihydrofuranyl, dihydropyranyl, dioxyolanyl, dihydropyrazinyl, dihydropyridinyl,
dihydropyrazolyl, dihydropyrimidinyl, dihydropyrrolyl, dihydrofuranyl, dioxolanyl, dioxolanylid,
1,4-dithianyl, furanonyl; furanyl, imidazolidinyl, imidazolinyl, imidazolyl, imidazopyridinyl, imidazothiazolyl,
indazolyl, indoliny, indolizinyl, indolyl, isobenzotetrahydrofuranyl,
isobenzotetrahydrothienyl, isobenzothenyl, isochromanyl, isocoumarinyl, isoindoliny,
isooindolyl, isoquinolinyl, isothiazolidinyl, isothiazolyl, isoxazolidinyl, isoxazolyl,
morpholinyl, naphthyridinyl, octahydroindolyl, octahydrosoindolyl, oxadiazolyl,
oxazolidinonyl, oxazolinyl, oxazolopyridinyl, oxazolyl, oxiranylid, perimidinyl,
phenanthridinyl, phenanthrolinyl, phenarsazinyl, phenazinyl, phenothiazinyl, phenoxazinyl,
phthalazinyl, piperezinyl, piperidinyl, 4-piperidonyl, pteridinyl, purinyl, pyrazinyl,
pyrazolidinyl, pyrazolyl, pyridazinyl, pyridinyl, pyridopyridinyl, pyrimidinyl, pyrroldinyl,
pyrrolidinyl, pyrrolyl, quinazolinyl, quinolinyl, quinoxalinyl, quinclidinyl, tetrahydrofuranyl,
tetrahydrofuranyl, tetrahydroisoquinolinyl, tetrahydropranyl, tetrahydrothienyl, tetrathiazolyl,
thiadiazolopyrimidinyl, thiazolyl, thiamorpholinyl, thiazolidinyl, thiazolyl, thienyl,
triazinyl, triazolyl, and 1,3,5-trithianyl. In certain embodiments, heterocyclic may also be
optionally substituted as described herein.

[00162] The term "alkoxy" refers to an -OR radical, wherein R is, for example, alkyl,
alkenyl, alkynyl, cycloalkyl, aryl, heteroaromatic, or heterocyclol, each as defined herein.
Examples of alkoxy groups include, but are not limited to, methoxy, ethoxy, propoxy, n-
propoxy, 2-propoxy, n-butoxy, isobutoxy, /erf-butoxy, cyclohexyloxy, phenoxy, benzoxy,
and 2-naphthyloxy. In certain embodiments, alkoxy may be optionally substituted as described herein. In certain embodiments, alkoxy is C_{1-6} alkyl-ox.

[00163] The term "halogen", "halide" or "halo" refers to fluorine, chlorine, bromine, and/or iodine.

4. DESCRIPTION OF THE FIGURES

[00164] Figure 1. Types of human hair follicles.

[00165] Figure 2. Architecture of the skin.

[00166] Figure 3. Diagram of human hair follicle.

[00167] Figure 4. Cellular structure of the human hair bulb.

[00168] Figure 5. Hamilton Norwood classification of male pattern hair loss (MPHL).

[00169] Figure 6. Franz cell set-up for in vitro CHIR99021 release experiments.

[00170] Figure 7. In vitro release of CHIR99021 from a hydrophilic hydrogel matrix comprised of chitosan gluconate and PEG300. In vitro cumulative release of CHIR99021 from a hydrophilic gel matrix using a Franz Cell-type set-up, with a cellulose membrane. The formulation contained 0.1 mg/ml CHIR99021, 100 mg/ml PEG300, 30 mg/ml chitosan gluconate and water.

[00171] Figure 8. In vitro release of CHIR99021 from a lecithin/PEO-PPO-PEO organogel matrix. In vitro cumulative release of CHIR99021 from a liquid-crystalline lecithin/PEO-PPO-PEO/Lanolin Alcohol organogel using a Franz Cell-type set-up, with a cellulose membrane. Due to low saturated solubility of CHIR99021 in water, the receptor chamber contained 50% PEG300. Analysis was performed by RP-HPLC.

[00172] Figure 9. Study Design, Dose Administration and Sampling Schedule For CHIR99021 Pharmacokinetics (DA Model). Skin and Blood were sampled and analyzed for CHIR99021 by LC/MS/MS.

[00173] Figure 10. Study Design, Dose Administration and Sampling Schedule For CHIR99021 Pharmacokinetics (FTE Model).

[00174] Figure 11. Dose response was achieved at all time-points CHIR99021 Pharmacokinetics (FTE Model).

[00175] Figure 12. Blood concentrations of CHIR99021 are many-fold lower than in skin at all doses (FTE Model).

[00176] Figure 13. CHIR99021 has a sustaining presence in skin, but does have clearance from site (FTE Model).
Figure 14. A and B. Dose response achieved at all time-points at all dose levels in the DA Model, for (A) skin and (B) whole blood. Dose response is also maintained 48 h after last dose administration. Blood levels are many-fold lower than skin levels, and blood levels at dose levels 0.01 and 0.001 µg are below the level of detection by LC/MS/MS. C and D. Dose response to CHIR99021 in (C) skin and (D) blood at 96 hours in mouse DA and FTE models.

Figure 15. PK and Neogenesis Experiments (Skin and Blood Levels Compared). In the DA experiments only, a small amount of CHIR99021 contaminated the placebo, which explains the values in the "0" dose categories. The "0" group experiments were subsequently repeated, and the amount of CHIR99021 measured in the "0" dose categories was confirmed to be below the level of detection.

Figure 16. Trend toward increased number of germs in 10 µg CHIR99021 treatment group in mouse FTE study. Graph represents a scatterplot with mean ± SD.

Figure 17. Trend toward increased area of germ forming region in 10 µg CHIR99021 treatment group in mouse FTE study. Graph represents a scatterplot with mean ± SD.

Figure 18. Germ density in the germ forming area, FTE and CHIR99021 experiment. Graph represents a scatterplot with mean ± SD.

Figure 19. Total wound area unchanged in CHIR99021 treatment group in mouse FTE study. Graph represents a scatterplot with mean ± SD.

Figure 20. Germ density in the total wound area has a statistically significant increase in the 10 µg CHIR99021 treatment group in the mouse FTE study. Graph represents a scatterplot with mean ± SD. By parametric 1-way ANOVA (P<0.05): 10 vs. 0.01; 10 vs. 0.1.

Figure 21. Coverage of the wound (mean percent coverage represented on y-axis) has a statistically significant increase in the 10 µg CHIR99021 treatment group in the mouse FTE study. Graph represents a scatterplot with mean ± SD. By parametric 1-way ANOVA (P<0.05): 10 vs. placebo; 10 vs. 0.01; 10 vs. 0.1; by non-parametric 1-way ANOVA (P<0.05): 10 vs. 0.01; 10 vs. 0.1

Figure 22. Representative confocal images of coverage of wound with germs in the FTE CHIR99021 study.

Figure 23. Coverage for the three highest responding mice from the 10 µg CHIR99021 FTE group. Representative images were selected as follows: For each group, the four mice closed to the mean for the %GFR/TWA (germ forming region/total wound
area) statistic were ranked by their closeness to the two density measurements (germs per GFR and germs per TWA). If the confocal image for the highest ranking mouse was of poor quality, the next images in the rankings were interrogated until one was found to be suitable.

[00187] **Figure 24.** Shaft diameter in CH1R99021 DA experiment. No statistically significant differences were found in this experiment. A small amount of CHIR99021 contaminated the "Placebo." The Placebo group experiments were subsequently repeated (shown as "Placebo B"), and the amount of CHIR99021 measured in the Placebo B dose categories was confirmed to be below the level of detection.

[00188] **Figure 25.** Hair shafts per pore in CHIR99021 DA experiment. No branching observed, no statistical significance detected for parametric 1-way ANOVA or non-parametric 1-way ANOVA. A small amount of CHIR99021 contaminated the "Placebo." The Placebo group experiments were subsequently repeated (shown as "Placebo B"), and the amount of CHIR99021 measured in the Placebo B dose categories was confirmed to be below the level of detection.

[00189] **Figure 26.** Shaft density in CHIR99021 DA experiment. No statistically significant differences were found in this experiment. A small amount of CHIR99021 contaminated the "Placebo." The Placebo group experiments were subsequently repeated (shown as "Placebo B"), and the amount of CHIR99021 measured in the Placebo B dose categories was confirmed to be below the level of detection.

[00190] **Figure 27.** Hair pore density in CHIR99021 DA experiment. No statistically significant differences were found in this experiment. A small amount of CHIR99021 contaminated the "Placebo." The Placebo group experiments were subsequently repeated (shown as "Placebo B"), and the amount of CHIR99021 measured in the Placebo B dose categories was confirmed to be below the level of detection.

[00191] **Figure 28.** Schematic representation of active treatment and control treatment.

[00192] **Figure 29** is a front perspective view of a spraying device, according to an embodiment of the present invention.

[00193] **Figure 30** is a rear perspective view of a spraying device, according to an embodiment of the present invention.

[00194] **Figure 31** is a front perspective view of a spraying device, according to an embodiment of the present invention.

[00195] **Figure 32** is a plan view of showing the components of a hand piece depicted in Figure 29 and Figure 30.
Figure 33 is a rear perspective view of a hand piece, according to an embodiment of the present invention;

Figure 34 is a plan view of a drug cartridge, according to an embodiment of the present invention.

Figure 35 is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

Figure 36 is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

Figure 37 is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

Figure 38 is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

Figure 39 is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

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

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

Figure 42 is a plan view of a drug cartridge and the front end of a hand piece, according to an embodiment of the present invention.

Figure 43 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.

Figure 44 is a cross-linking reaction between PEG-AM and PEG-NHS.

Figure 45 is a graph depicting the gel time as a function of pH for PEG-NHS/PEG-AM hydrogels.

Figure 46 is a graph depicting the gel time as a function of PEG concentration for PEG-NHS/PEG-AM hydrogels.

Figure 47 is a graph depicting the gel time as a function of total PEG concentration for PEG-NHS/PEG-AM hydrogels.

Figure 48 is graph depicting gel time as a function of PEG concentration in Phosphate buffer (0.1M, 0.2M and 0.5M) for PEG-NHS/PEL hydrogels.
Figure 49 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 50 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 51 shows a standard calibration curve for Li+ determination in film.

Figure 52. Figure 52A shows a method for identification of the regenerating zone, based on changes in hair follicle orientation, thickening of epidermis, morphology of adjacent normal - scar - regenerating zone - scar - adjacent normal, lack of pigmented hair shafts in regenerating zone, immature hair follicles within regenerating zone, and loss of subcutaneous fat. Figure 52B shows an example of pigmented hair follicles in the regenerating zone, which was observed in some samples taken from mice treated with full thickness excision wounding followed by administration of topical CHIR99021. Samples with pigmented hair follicles in regenerating zone were not scored; samples with pigmented hair follicles were 23x5 (placebo), 23x8 (placebo), 23x29 (0.01 µg CHIR99021), and 23x44 (0.1 µg CffIR99021).

Figure 53 shows examples of the staging of hair follicles by histology. Stage 1 (Figure 53A): Germ visible as epidermal thickening and increase in number and change in orientation of cells; Stage 2 (Figure 53B): Elongated hair germ with convex proximal end and condensation of dermal fibroblasts; Stage 3 (Figure 53C): Hair peg with concave basal border; Stage 4 (Figure 53D): Bulb-like thickening of hair peg, formation of inner root sheath (IRS), and dermal papilla (DP) is longer than wide and enclosed by more than 50%; Stage 5 (Figure 53E): Elongation of IRS halfway up to the follicle, Bulge visible, and DP now almost completely enclosed; Stage 6 (Figure 53F): Sebocytes form sebaceous glands (SG), hair canal visible, and hair shaft grows up to level of canal; Stage 7 (Figure 53G): Tip of hair shaft leaves IRS and enters canal and SG localized to posterior wall of hair follicle (HF); Stage 8 (Figure 53H): Hair shaft emerges from epidermis, HF reaches maximal length and reaches subcutaneous muscle layer.

Figure 54 shows representative images from histology samples taken from mice treated with full thickness excision wounding followed by administration of topical CHIR99021, as follows: placebo (Figure 54A), 0.01 µg CHIR99021 (Figure 54B); 0.1 µg CHIR99021 (Figure 54C); 1 µg CHIR99021 (Figure 54D); 10 µg CHIR99021 (Figure 54E); and 100 µg CHIR99021 (Figure 54F).
Figure 55 shows results of histology of samples taken from mice treated with full thickness excision wounding followed by administration of topical CHIR99021. Figure 55A shows proportion of stageable hair follicles at or above stage 5. The numbers above the columns represent the number of follicles at or above stage 5 / total number of stageable follicles. The numbers in the columns represent the following: upper number = number of tissue sections with stageable follicles; middle number = number of tissue sections with no stageable follicles; lower number = number of tissue sections that were not readable. One tissue section per biological replicate analyzed. Figure 55B shows numbers of hair follicles at stage 4 and below. The numbers above the columns represent the number of follicles at stage 4 and below / total number of stageable follicles. The numbers in the columns represent the following: upper number = number of tissue sections with stageable follicles; middle number = number of tissue sections with no stageable follicles; lower number = number of tissue sections that were not readable. One tissue section per biological replicate analyzed. Figure 55C shows average follicle maturity (average germ stage). The numbers above the columns represent the following: (number of tissue sections that had no germs), (number of tissue sections that were not readable). One tissue section per biological replicate analyzed.

5. DESCRIPTION OF THE INVENTION

5.1 COMPOUNDS

In some embodiments, the Compounds described herein are potent and specific inhibitors of GSK-3, and in particular in some embodiments, GSK-3beta.

In a first embodiment, provided herein are Compounds having Formula 1:

\[
\begin{array}{c}
\text{Formula 1} \\
\end{array}
\]

or pharmaceutically salt, solvate, or prodrug thereof, wherein, Y is N or CH;
Ri, R2, R3, and R4 are independently hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, or alkoxy;
R5 and R7 are independently hydrogen or halo or optionally substituted alkyl, cycloalkyl,
aryl, aminoalkyl, aminoaralkyl, aminocycloalkylaryl, arylearboxamidocycloalkylaralkyl, arylearboxamidocycloalkylaryl, arylearboxarnidoalkylcycloalkyl, arylearboxamidoaryl, arylearboxamidoalky1, arylearboxamidoaralky1, arylearboxamidoalkoxyalky1, aminoalkoxyalkyl, arsulfonamidoaralkyl; 

Ri is hydrogen, carboxyl, nitro, amino, cyano, or an optionally substituted alkyl, aryl, aralkyl, aminoalkyl, aminoaryl, aminoaralkyl, arylaminoalkyl, arylaminoaryl, arylaminoaralkyl, arylalkylamino, arylalkylaminoalkyl, arylalkylaminoaryl, arylalkylaminoaralkyl, arylalkylaminoalkoxyalkyl, sulfonylalkyl, carbamylalkyl, carbamylaryl, carbamylaralkyl, carbamylalkylamino, carbamylalkylaminoalkyl, carbamylalkylaminoaryl, or carbamylalkylaminoaralkyl; and 

A is pyridyi, pyrimidinyl, thiazolyl, indolyl, imidazolyl, oxadiazolyl, tetrazolyl, pyrazinyl, triazolyl, thiophenyl, furanyl, quinolinyl, purinyl, naphthyl, benzothiazolyl, benzopyridyl, or benzimidazolyl, which can be optionally substituted with from 0 to 3 of the following substitution groups: nitro, amino, cyano, halo, thioamido, amidino, oxamidino, alkoxyamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, loweralkyl, haloloweralkyl, loweralkoxyalkyl, haloloweralkoxy, haloloweralkoxyalkyl, loweralkylamino, loweralkoxy, loweralkylcarbonyl, loweralkylcarbonyl, lowerheteroaralkylcarbonyl, alkylthio, aminoalkyl or cyanoalkyl.

[00221] In certain embodiments, provided herein are Compounds having Formula I, or a pharmaceutically salt, solvate, or prodrug thereof, wherein Ri, R2, R3, and R4 are hydrogen. In certain embodiments, provided herein are Compounds having Formula I, or a pharmaceutically salt, solvate, or prodrug thereof, wherein Y is N.

[00222] In another embodiment, provided herein are Compounds having Formula II:

\[
\text{Formula II}
\]

or pharmaceutically salt, solvate, or prodrug thereof, wherein, R5, R6, and R7 are as defined above; and

R8 and R9 are independently hydrogen, nitro, amino, cyano, halo, thioamido, sulfonamido, carboxyl, formyl, loweralkyl, haloloweralkyl, aminoloweralkyl, cyanoloweralkyl,
loweralkoxy, haloloweralkoxy, loweralkoxyalkyl, loweralkylaminoloweralkoxy, alkylthio, aryl, and aralkyl.

[00223] In certain embodiments, provided herein are Compounds having Formula II, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_7 \) is H. In certain embodiments, provided herein are Compounds having Formula II, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_7 \) is H and \( R_8 \) is heterocycl. In certain embodiments, provided herein are Compounds having Formula II, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_8 \) and \( R_9 \) are independently hydrogen, nitro, cyano, or halo. In certain embodiments, provided herein are Compounds having Formula II or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_8 \) and \( R_9 \) are independently hydrogen or cyano.

In another embodiment, provided herein are Compounds having Formula III:

![Formula III](image)

or pharmaceutically salt, solvate, or prodrug thereof, wherein, \( R_i \), \( R_7 \), \( R_8 \), and \( R_9 \) are as defined above; and \( R_i \), \( R_{11} \), \( R_{12} \), \( R_{13} \), and \( R_{14} \) are independently hydrogen, nitro, amino, cyano, halo, thioamido, carboxyl, hydroxy, or optionally substituted loweralkyl, loweralkoxy, loweralkoxyalkyl, haloloweralkyl, haloloweralkoxy, aminoalkyl, alkylamino, alkylthio, alkylcarbonylamino, aralkylcarbonylamino, heteroaralkylcarbonylamino, arylcarbonylamino, heteroarylcarbonylamino, aminocarbonyl, loweralkylaminocarbonyl, aminoaralkyl, loweralkylaminocarbonyl, aryl, heteroaryl, cycloaralkyl, aralkyl, alkyloxycarbonyloxy, aralkyloxycarbonyloxy, arylcarbonyloxyalkyl; alkylcarbonyloxyalkyl, heteroarylcarbonyloxyalkyl, aralkylcarbonyloxyalkyl, or heteroaralkylcarbonyloxyalkyl.

[00225] In certain embodiments, provided herein are Compounds having Formula IV, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_9 \), \( R_{11} \), \( R_{12} \), \( R_{13} \), and \( R_{14} \) are independently hydrogen, hydroxy, nitro, amino, cyano, or halo. In certain embodiments, provided herein are Compounds having Formula III, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_9 \), \( R_{11} \), \( R_{12} \), \( R_{13} \), and \( R_{14} \) are independently hydrogen, hydroxy,
nitro, amino, cyano, or halo, and wherein \( R_8 \) and \( R_9 \) are independently hydrogen, nitro, cyano, or halo. In certain embodiments, provided herein are Compounds having Formula III, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_7 \), \( R_{11} \), \( R_{12} \), \( R_{13} \), and \( R_{14} \) are independently hydrogen or halo. In certain embodiments, provided herein are Compounds having Formula III, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_7 \) is H. In certain embodiments, provided herein are Compounds having Formula III, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_7 \) is H and \( R_8 \) is heterocyclyl. In certain embodiments, provided herein are Compounds having Formula III, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R_6 \) is heterocyclyl, \( R_7 \) is hydrogen, \( R_8 \) is hydrogen, \( R_9 \) is cyano, \( R_{10} \) is chloro, \( R_{11} \) is hydrogen, \( R_{12} \) is chloro, \( R_{13} \) is hydrogen, and \( R_{14} \) is hydrogen.

In another embodiment, provided herein are Compounds having Formula IV:

![Formula IV](image)

or pharmaceutically salt, solvate, or prodrug thereof.

In other embodiments, provided herein are Compounds having the following formulae:

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In a second embodiment, provided herein are Compounds having Formula V:

![Formula V](image)

or a pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

- **Z** is H or Br;
- **X** is H, F, Cl, Br, I, or CH=CH₂;
- **R** is H or CH₃;
- **W** is H, CH₃, Cl, NH₂, or NO₂;
- **L** is H or Cl; and
- **Y** is O, NOH, NOCH₃, or NOC(=O)CH₃.

In certain embodiments, provided herein are Compounds having Formula V, or a pharmaceutically salt, solvate, or prodrug thereof, wherein **Z** is H or Br; **X** is H or Br; **R** is H or CH₃; **W** is H; **L** is H; and **Y** is O or NOH.

In a particular embodiment, **Z** is H; **X** is H; **R** is H; **W** is H; **L** is H; and **Y** is NOH.

In a particular embodiment, **Z** is H; **X** is Br; **R** is H; **W** is H; **L** is H; and **Y** is NOH.

In other embodiments, provided herein are Compounds having the following formulae:
In one embodiment, provided herein are Compounds having Formula V, wherein R is H.
In one embodiment, the Compound for use in the methods described herein is not a compound having Formula V. In one embodiment, the Compound for use in the methods described herein is not a compound having Formula V-18.

In a further embodiment, additional examples of the Compounds provided herein are disclosed in International Patent Application Publication No. WO 05/41 954, which is incorporated by reference herein in their entirety and for all purposes.

In a third embodiment, provided herein are Compounds having Formula VI:

\[
\begin{align*}
\text{Formula VI} & \\
\end{align*}
\]

or a pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

- X and Y are independently O, S, NR; and \( \text{CR} \);
- \( R^1 \) and \( R^2 \) are independently H, alkyl, cycloalkyl, haloalkyl, aryl, \( (Z)_n \)-aryl, heteroaryl, OR, C(=O)R, (Z)_n—C(=O)OR, or together with \( NR \) can form a fused aryl group;
- Z is independently CR^3R^4, C(=O)—, C(=NR)—, SO,—, or NR;
- \( n \) is 0, 1, or 2;
- t is 0, 1, or 2;
- \( R^3 \) and \( R^4 \) are independently H, alkyl, aryl, or heterocyclyl; and
- \( R^a \) and \( R^b \) are independently H, alkyl, cycloalkyl, haloalkyl, aryl, \( (Z)_n \)-aryl, heteroaryl, OR, C(=O)R, (Z)_n—C(=O)OR, or \( (Z)_n—C(=O)OR \).

In certain embodiments, provided herein are Compounds having Formula VI, or a pharmaceutically salt, solvate, or prodrug thereof, wherein X and Y are independently O, S, or NR where \( R^3 \) is heterocyclyl. In a particular embodiment, \( R^3 \) is 6-membered heterocyclyl with one heteroatom that is nitrogen, optionally being heteroaryl and optionally being oxidized or quaternized. In a particular embodiment, X and Y are both O.

In certain embodiments, provided herein are Compounds having Formula VI, or a pharmaceutically salt, solvate, or prodrug thereof, wherein \( R^a \) and \( R^b \) are independently H, alkyl, cycloalkyl, aryl (optionally substituted with an alkyl, halo, or alkoxy), CR^aR^b—aryl (the aryl being optionally substituted with an alkyl, halo, or alkoxy), OR, C(=O)R, or CR^aR^b—C(=O)OR, wherein \( R^3 \) and \( R^4 \) are independently H, alkyl, or heterocyclyl. In certain embodiments, \( R^a \) and \( R^b \) are independently alkyl, aryl (optionally substituted with an alkyl, halo, or alkoxy), CH—aryl (the aryl being optionally substituted with an alkyl, halo,
or alkoxy), or CH₂C(=0)OR or CH₂C(=0)OR₂, wherein R³ is H or alkyl. In certain embodiments, Rᵃ and Rᵇ are independently methyl, ethyl, propyl, benzyl, phenyl (optionally substituted with methyl, fluoro, chloro, bromo, or methoxy), or CH₂C(=0)OCH₃CH₃.

[00240] In particular embodiments, X and Y are both O, Rᵇ is alkyl, and Rᵃ is CH₂—aryl (where the aryl is optionally substitute with an alkyl, halo, or alkoxy). In a specific embodiment, X and Y are both O, Rᵇ is methyl, and Rᵃ is benzyl.

[00241] In other embodiments, provided herein are Compounds having the following formulae:

![Chemical structures](image)

[00242] In a further embodiment, additional examples of the Compounds provided herein are disclosed in International Patent Application Publication Nos. WO 01/385685 and WO
05/971 17, and which are incorporated by reference herein in their entirety and for all purposes.

[00243] In one embodiment, the Compound for use in the methods described herein is not a compound having Formula VI. In one embodiment, the Compound for use in the methods described herein is not a compound having the Formula VI, wherein X and Y are both O, \( R^b \) is methyl, and \( R^a \) is benzyl. In one embodiment, the Compound for use in the methods described herein is not a compound having the Formula VI-25.

[00244] In a fourth embodiment, provided herein is a Compound having Formula VII

\[
\begin{align*}
A & \quad Z \\
\quad Y \\
\quad N \\
\quad X
\end{align*}
\]

or a pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein

- X is O, S, or NH (optionally substituted with a lower alkyl);
- Y is O or NH (optionally substituted with a lower alkyl);
- Z is N or CH (optionally substituted with a lower alkyl);
- A is H, F, Cl, Br, CN, N\( \text{O}_2 \), or lower alkyl; and
- B is H, lower alkyl, lower alkoxy, or NH\( _2 \) (optionally substituted with one or two lower alkyl).

[00245] In a particular embodiment, \( X = S, Y = O, Z = N, A = N\( \text{O}_2 \), and \( B = \text{OCH}_3 \). 

[00246] In a specific embodiment, \( A = \text{Cl} \). In another specific embodiment, \( A = \text{OH} \). In another specific embodiment, \( A = \text{C}0\text{2} \).

[00247] A compound of Formula VII is described in International Patent Application Publication No. WO 03/04478, incorporated by reference herein in its entirety and for all purposes. In one embodiment, a Compound for use in the methods described herein is not a compound having Formula VII. In one embodiment, the Compound for use in the methods described herein is not a compound having Formula VII, wherein \( X = S, Y = O, Z = N, A = N\( \text{O}_2 \), and \( B = \text{OCH}_3 \).
In a fifth embodiment, provided herein is a Compound having Formula VIII

\[
\begin{array}{c}
\text{Formula VIII} \\
\end{array}
\]

wherein \( A \) and \( E \) are independently selected from the group consisting of a hydrogen substituted carbon atom and a nitrogen atom; \( Z \) is selected from \( O \); alternatively, \( Z \) is selected from dihydro; wherein each hydrogen atom is attached by a single bond;

\( R_4 \) and \( R_5 \) are independently selected from C1-8 alkyl, C2-8 alkenyl and C2-8 alkylnyl optionally substituted with oxo;

\( R_2 \) is selected from the group consisting of --Cl-8 alkyl-, --C2-8 alkenyl-, --C2-8 alkylnyl-, --0-(C1-8)alkyl-0~, --0-(C2-8)alkenyl-0~, --0-(C2-8)alkylnyl-0~, --C(0)–(Cl-8)alkyl-C(0)~ (wherein any of the foregoing alkyl, alkenyl and alkylnyl linking groups are straight carbon chains optionally substituted with one to four substituents independently selected from the group consisting of hydrogen and C1-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C1-4 alkyl), halogen, (halo)1-3 (Cl-8)alkyl, (halo)1-3 (Cl-8)alkoxy, hydroxy, hydroxy(Cl-8)alkyl and oxo; and, wherein any of the foregoing alkyl, alkenyl and alkylnyl linking groups are optionally substituted with one to two substituents independently selected from the group consisting of heterocyclyl, aryl, heteroaryl, heterocyclyl(Cl-8)alkyl, aryl(Cl-8)alkyl, heteroaryl(Cl-8)alkyl, spirocycloalkyl and spiroheterocyclyl (wherein any of the foregoing cycloalkyl, heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, Cl-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and C1-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and C1-4 alkyl), halogen, (halo)1-3 (Cl-8)alkyl, (halo)1-3 (Cl-8)alkoxy, hydroxy, hydroxy(Cl-8)alkyl and oxo; and, wherein any of the foregoing heterocyclyl
substituents are optionally substituted with oxo)), cycloalkyl, heterocyclyl, aryl, heteroaryl
(wherein cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted with one to
four substituents independently selected from the group consisting of C I -8 alkyl, C I -8

alkoxy, Cl-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a
substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl),
amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected
from the group consisting of hydrogen and Cl-4 alkyl), halogen, (halo) 1-3 (Cl-8)alkyl,

(halo) 1-3 (Cl-8)alkoxy, hydroxy and hydroxy(Cl-8)alkyl; and, wherein heterocyclyl is

optionally substituted with oxo), ~(0-(CH
-, ~0--( CH2)l-6

CH2)l-6 -6)0-5 -S-, -0-(

- ,- N

--0-, -0-(CH

)l-6 -0-(CH

2)l-6

-O-

0 --( CH )l-6 -0--( CH2)l-6 --0--, --(0--( CH2)l-6)0-5 -- N R --, -0-(

- ( CH2)l-6 --0-, ~0--( CH2)l-6 -0-(
CH2)l-6 -S--( CH2)l-6 -0-,

-0-(

CH2)l-6 -- N¾ - , --(0--( CH2)1CH2)l-6 -0-(

CH )l-6 --S--, - N R

~NR 7 - , -NRe - ( CH2)l-6 ~NR 7 - , - NR6 ~ ( CH2)l-6 -NR 7 - ( CH2)l-6 -NR ~

, - NR6 -C(O)-, -C(O)- NR - , -C(0)-(
CH2)0-6 ~C(0)~( CH2)l-6 -C(0)-(
N

2) 1-6)0-5

CH2)0-6 - NR6 - ( CH2)0-6 -C(O)-,

CH2)0-6 - NR7~ , - NR« ~C(0)-,

- NR - (

-C(O)- N¾ --, -

--C(NR 7)-NR 8 --, --0--( CH2)l-6 - NRe --( CH2)l-6 --S-, --S--( CH2)l-6 - NR --(

CH2)l-6 --0-, -S-(

CH )l-6

R --( CH2)l-6 --S-, -

R -(.CH )l-6 -S--( CH2)l-6 --

NR7 and —S0 2 ~ (wherein R6, R7 and R are independently selected from the group
consisting of hydrogen, Cl-8 alkyl, Cl-8 alkoxy(Cl-8)alkyl, carboxyl(Cl-8)alkyl, amino(Cl8)alkyl (wherein amino is substituted with a substituent independently selected from the

group consisting of hydrogen and Cl-4 alkyl), hydroxy(Cl-8)alkyl, heterocyclyl(Cl-8)alkyl,

aryl(Cl-8)alkyl and heteroaryl(Cl-8)alkyl (wherein the foregoing heterocyclyl, aryl and
heteroaryl substituents are optionally substituted with one to four substituents independently

selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, Cl-8 alkoxy(Cl-8)alkyl,
carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected
from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is

substituted with a substituent independently selected from the group consisting of hydrogen
and Cl-4 alkyl), halogen, (halo)l-3 (Cl-8)alkyl, (halo)l-3 (Cl-8)alkoxy, hydroxy and

hydroxy(Cl-8)alkyl; and, wherein heterocyclyl is optionally substituted with oxo));
with the proviso that, if A and E are selected from a hydrogen substituted carbon atom,
then R2 is selected from the group consisting of --C2-8 alkynyl-, —0~(Cl-8)alkyl-0~,

(C2-8)alkenyl-0~, -0-(C2-8)alkynyl-0-,

-C(0)-(Cl-8)alkyl-C(0)-

~0~

(wherein any of the

foregoing alkyl, alkenyl and alkynyl linking groups are straight carbon chains optionally
substituted with one to four substituents independently selected from the group consisting of


Cl-8 alkyl, Cl-8 alkoxy, Cl-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, -C(O)0--(Cl-8)alkyl, --C-8 alkyl-C(O)0--(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), halogen, (halo)1-3 (Cl-8)alkyl, (halo)1-3 (Cl-8)alkoxy, hydroxy, hydroxy(Cl-8)alkyl and oxo; and, wherein any of the foregoing alkyl, alkenyl and alkoxy linking groups are optionally substituted with one to two substituents independently selected from the group consisting of heterocyclyl, aryl, heteroaryl, heterocyclyl(Cl-8)alkyl, aryl(Cl-8)alkyl, heteroaryl(Cl-8)alkyl, spirocycloalkyl and spiroheterocyclyl (wherein any of the foregoing cycloalkyl, heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), halogen, (halo)1-3 (Cl-8)alkyl, (halo)1-3 (Cl-8)alkoxy, hydroxy and hydroxy(Cl-8)alkyl; and, wherein any of the foregoing heterocyclyl substituents are optionally substituted with oxo)), cycloalkyl (wherein cycloalkyl is optionally substituted with one to four substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), halogen, (halo)1-3 (Cl-8)alkoxy, hydroxy and hydroxy(Cl-8)alkyl).
(wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), hydroxy(Cl-8)alkyl, heterocyclyl(Cl-8)alkyl, aryl(Cl-8)alkyl and heteroaryl(Cl-8)alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, Cl-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), halogen, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alcohol, hydroxy and hydroxy(Cl-8)alkyl; and, wherein heterocyclyl is optionally substituted with oxo);

and, wherein R₈ is selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy(Cl-8)alkyl, carboxyl(Cl-8)alkyl, amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), hydroxy(Cl-8)alkyl, heterocyclyl(Cl-8)alkyl, aryl(Cl-8)alkyl and heteroaryl(Cl-8)alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, Cl-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), halogen, (halo)-3 (Cl-8)alkyl, (halo)-3 (Cl-8)alcohol, hydroxy and hydroxy(Cl-8)alkyl; and, wherein heterocyclyl is optionally substituted with oxo)); and, R₁ and R₃ are independently selected from the group consisting of hydrogen, Cl-8 alkyl, C₂-8 alkenyl, C₂-8 alkynyl (wherein alkyl, alkenyl and alkynyl are optionally substituted with a substituent selected from the group consisting of Cl-8 alkoxy, alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), (halo) 1-3, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alcohol, hydroxy, hydroxy(Cl-8)alkyl and oxo), Cl-8 alkoxy, Cl-8 alkoxy carbonyl, (halo) 1-3 (Cl-8)alcohol, Cl-8 alkylthio, aryl, heteroaryl (wherein aryl and heteroaryl are optionally substituted with a substituent selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with
a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl, halogen, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alkoxy, hydroxy and hydroxy(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), cyano, halogen, hydroxy and nitro;
or a pharmaceutically acceptable salt thereof.

[00249] A compound of Formula VIII is described in U.S. Patent No. 6,828,327, incorporated herein by reference in its entirety and for all purposes. In a further embodiment, specific examples of Compounds provided herein are set forth in U.S. Patent No. 6,828,327 as compounds 1-29, at cols. 15-17, specifically incorporated herein by reference.

[00250] In one embodiment, the Compound for use in the methods described herein is not a Compound having Formula VIII.

[00251] In a sixth embodiment, provided herein is a Compound having Formula IX:

\[
\text{Formula IX}
\]

wherein \( R_1 \) and \( R_2 \) are independently selected from the group consisting of: hydrogen, Cl-8 alkyl, C-2 alkynyl, C-2 alkynyl \{wherein alkyl, alkenyl and alkynyl are optionally substituted with one to two substituents independently selected from the group consisting of -0-(Cl-8)alkyl, -0-(Cl-8)alkyl-OH, -0-(Cl-8)alkyl-0-(Cl-8)alkyl, -0-(Cl-8)alkyl-NH_2, -0-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-(Cl-8)alkyl, -0-(Cl-8)alkyl-S02-(Cl-8)alkyl, -0-(Cl-8)alkyl-S02-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S02-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-(Cl-8)alkyl-0-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-(Cl-8)alkyl-0-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-S-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-N[(Cl-8)alkyl]_2, -0-(Cl-8)alkyl-S-S-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-S-S-
8)alkyl, --SO₂ NH₂, --S0₂ NH-(Cl-8)alkyl, --S0₂ N[(Cl-8)alkyl]₂, amino (substituted with two substituents independently selected from the group consisting of hydrogen, Cl-8 alkyl, C2-8 alkenyl, C2-8 alkenyl, --(C1-8)alkyl-OH, --(Cl-8)alkyl-0--(Cl-8)alkyl, --(Cl-8)alkyl-NH₂, --(Cl-8)alkyl-N--((Cl-8)alkyl)₂, --(Cl-8)alkyl-S-\((Cl-8)alkyl\), --C(0)--(Cl-8)alkyl, --C(0)--0--(Cl-8)alkyl, --C(0)--NH₂, --C(0)--NH--(Cl-8)alkyl, --C(0)--N[(Cl-8)alkyl]₂, --S0₂ --(Cl-8)alkyl, --S0₂ --NH₂, --S0₂ --NH--(Cl-8)alkyl, --S0₂ --N[(Cl-8)alkyl]₂, --C(N)--NH₂, aryl and aryl(Cl-8)alkyl (wherein aryl is optionally substituted with one to three substituents independently selected from the group consisting of halogen, Cl-8 alkyl, Cl-8 alkoxy, amino (substituted with two substituents selected from the group consisting of hydrogen and Cl-8 alkyl), cyano, halo, (halo)l-3 (Cl-8)alkyl, (halo)l-3 (Cl-8)alkoxy, hydroxy, hydroxy(Cl-8)alkyl and nitro), cyano, (halo)l-3, hydroxy, nitro, o xo, heterocyclyl, aryl and heteroaryl (wherein heterocyclyl, aryl and heteroaryl are optionally substituted with one to three substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, amino (substituted with two substituents selected from the group consisting of hydrogen and Cl-8 alkyl), cyano, halo, (halo)l-3 (Cl-8)alkyl, (halo)l-3 (Cl-8)alkoxy, hydroxy, hydroxy(Cl-8)alkyl and nitro), --C(0)--(Cl-8)alkyl, --C(0)--ary, --C(0)--0--(Cl-8)alkyl, --C(0)--0--aryl, --C(0)--NH--(Cl-8)alkyl, --C(0)--NH-aryl, --C(0)--N[(Cl-8)alkyl]₂, --SO₂ --(Cl-8)alkyl, --SO₂ --aryl, aryl and heteroaryl {wherein aryl and heteroaryl are optionally substituted with one to three substituents independently selected from the group consisting of Cl-8 alkyl, C2-8 alkenyl, C2-8 alkynyl, Cl-8 alkoxy, --C(0)H, --C(0)--(Cl-8)alkyl, --C(0)--0--(Cl-8)alkyl, --C(0)--NH₂, --C(NH)--NH₂, --C(0)--NH--(Cl-8)alkyl, --C(0)--N[(Cl-8)alkyl]₂, --SH, --S--(Cl-8)alkyl, --SO₂ --(Cl-8)alkyl, --SO₂ --NH₂, --SO₂ --NH--(Cl-8)alkyl, --SO₂ --N[(Cl-8)alkyl]₂, amino (substituted with two substituents independently selected from the group consisting of hydrogen, Cl-8 alkyl, C2-8 alkenyl, C2-8 alkenyl, --(C1-8)alkyl- NH₂, --C(O)--(Cl-8)alkyl, --C(O)--0--(Cl-8)alkyl, --C(O)--NH₂, --C(O)--NH--(Cl-8)alkyl, --C(O)--N[(Cl-8)alkyl]₂, --SO₂ --(Cl-8)alkyl, --SO₂ --NH₂, --SO₂ --NH--(Cl-8)alkyl, --SO₂ --N[(Cl-8)alkyl]₂ and --C(NH)--NH₂), amino-(Cl-8)alkyl- (wherein amino is substituted with two substituents independently selected from the group consisting of hydrogen, Cl-8 alkyl, C2-8 alkenyl, C2-8 alkenyl, --(Cl-8)alkyl- NH₂, --C(0)--(Cl-8)alkyl, --C(0)--0--(Cl-8)alkyl, --C(0)--NH₂, --C(0)--NH--(Cl-8)alkyl, --C(0)--N[(Cl-8)alkyl]₂, --S0₂ --NH--(Cl-8)alkyl, --S0₂ --N[(Cl-8)alkyl]² and --C(NH)--NH₂), cyano, halo, (halo)l-3 (Cl-8)alkyl-, (halo)l-3 (Cl-8)alkoxy-, hydroxy, hydroxy(Cl-8)alkyl, nitro, aryl, --(Cl-8)alkyl-aryl, heteroaryl and -(Cl-8)alkyl-heteroaryl);
with the proviso that if \( R_2 \) is selected from the group consisting of hydrogen, unsubstituted \( \text{Cl-7} \) alkyl and \(-\text{(Cl-7)alkyl-(halo)-}3\), then \( R_1 \) is selected from the group consisting of other than hydrogen, \( \text{Cl-7} \) alkyl, aryl (limited to phenyl unsubstituted or substituted with one or more substituents selected from the group consisting of halo, unsubstituted \( \text{Cl-7} \) alkyl, hydroxy, unsubstituted \( \text{Cl-7} \) alkoxy, (halo)l-3 (Cl-7)alkyl, nitro, unsubstituted amino and cyano), \(-\text{(Cl-7)alkyl-aryl} \) (wherein aryl is limited to phenyl unsubstituted or substituted with one or more substituents selected from the group consisting of halo, unsubstituted \( \text{Cl-7} \) alkyl, hydroxy, \( \text{Cl-7} \) alkoxy, (halo)l-3 (Cl-7)alkyl, nitro, unsubstituted amino and cyano), \(-\text{(Cl-7)alkyl-heteroaryl}; \)

\( Y \) and \( Z \) are independently selected from the group consisting of \( \text{O}, \text{S}, (\text{H},\text{OH}) \) and \( (\text{H},\text{H}); \)

\( \text{X} \) is selected from the group consisting of \( \text{N} \) and \( \text{CR5} \);

\( \text{R}_3 \) and \( \text{R}_4 \) are independently selected from the group consisting of hydrogen, \( \text{Cl-8} \) alkyl, C2-8 alkenyl, C2-8 alkynyl, Cl-8 alkoxy, \(-\text{C(0)H}, \text{~C(0)~}-(\text{Cl-8)alkyl}, \text{~C0}_2 \text{H}, \text{~C(0)~}0-(\text{Cl-8)alkyl}, \text{~C(0)~}0, \text{~C(0)~}0-(\text{Cl-8)alkyl}, \text{~C(0)~}0-(\text{Cl-8)alkyl}, \text{~SH}, \text{~S-}-(\text{Cl-8)alkyl}, ~\text{S0}_2 ~-(\text{Cl-8)alkyl}, \text{~S}0_2 \text{~}0-(\text{Cl-8)alkyl, ~S}0_2 \text{~}~\text{NH}_2, \text{~S}0_2 \text{~}~\text{NH}~\text{--}-(\text{Cl-8)alkyl}, \text{~S0}_2 \text{~}~\text{N[(Cl-8)alkyl]~} 2, \text{~amino (substituted with two substituents independently selected from the group consisting of hydrogen and Cl-7 alkyl); \}

\( \text{Y} \) and \( \text{Z} \) are independently selected from the group consisting of O, S, (H,OH) and (H,H);
with the proviso that one of Y and Z is O and the other is selected from the group consisting of O, S, (H,OH) and (H,H); and

\( R_5 \) is selected from the group consisting of hydrogen, halogen, C1-8 alkyl, C2-8 alkenyl, C2-8 alkynyl \{wherein alkyl, alkenyl and alkynyl are optionally substituted with one to two substituents independently selected from the group consisting of amino (substituted with two substituents selected from the group consisting of hydrogen and C1-8 alkyl), cyano, halo, hydroxy, nitro, oxo, aryl and heteroaryl\}, aryl and heteroaryl \{wherein aryl and heteroaryl are optionally substituted with one to two substituents independently selected from the group consisting of C1-8 alkyl, C1-8 alkoxy, amino (substituted with two substituents selected from the group consisting of hydrogen and C1-8 alkyl), cyano, halo, hydroxy and nitro\};
or a pharmaceutically acceptable salt thereof.

[00252] A compound of Formula IX is described in U.S. Patent No. 6,849,643, incorporated herein by reference in its entirety and for all purposes. In a further embodiment, specific examples of Compounds provided herein are set forth in U.S. Patent No. 6,828,327 as compounds 1-123, at cols. 14-19, specifically incorporated herein by reference.

[00253] In one embodiment, the Compound for use in the methods described herein is not a Compound having Formula IX.

[00254] In other embodiments, provided herein are other small molecules with GSK-3 inhibitory activity, which are set forth in U.S. Patent No. 6,924,302 (see, e.g., compounds 1-128 at cols. 20-24), incorporated herein by reference in its entirety and for all purposes.

[00255] In a seventh embodiment, provided herein are Compounds having Formula X:

\[
\text{Formula X}
\]

or a pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

\( X \) is \( \text{N} \) or \( \text{N}^{\circ}\), wherein A is O or S;

\( Y \) is S, SO, or SO\(_2\);

\( n \) is 1 or 2; and

\( Z \) is hydrogen, halo, COOH, COOR, CH\(_2\)COOH, or CH\(_2\)COOR, wherein R is alkyl.

[00256] In a particular embodiment, \( Z \) is in the 3-position. In a particular embodiment, \( Z \) is halo. In a particular embodiment, \( Z \) is 3-halo. In a particular embodiment, \( Z \) is 3-iodo.

[00257] In a particular embodiment, \( n \) is 1. In a particular embodiment, \( n \) is 2.
[00258] In a particular embodiment, Y is S. In a particular embodiment, Y is SO. In a particular embodiment, Y is S0₂.

[00259] In a particular embodiment, X is . In a particular subembodiment of this embodiment, A is O.

[00260] In a particular embodiment, Y is S, n is 1, and Z is halo. In a particular embodiment, Y is S, n is 1, and Z is 3-halo. In a particular embodiment, Y is S, n is 1, and Z is 3-iodo.

[00261] In a particular embodiment, X is . A is O, Y is S, and n is 1. In a particular embodiment, X is , A is O, Y is S, n is 1, and Z is halo. In a particular embodiment, X is , A is O, Y is S, n is 1, and Z is 3-halo.

[00262] In a specific embodiment, X is , A is O, Y is S, n is 1, and Z is 3-iodo.

[00263] In other embodiments, provided herein are Compounds having the following formulae:

![Chemical Structures](image1)

33
34
35
36

In certain embodiments of the above-described embodiments, the compounds are PEGylated.

In some embodiments, a compound described in Section 5.1 for use in the methods provided herein inhibits the activity of GSK-3P in an *in vitro* assay, such as
described in Section 5.7.2 infra. In some embodiments, a compound described in Section 5.1 for use in the methods provided herein inhibits the activity of GSK-3P in a cell-based assay, such as described in Section 5.7.2 infra. In some embodiments, a compound described in Section 5.1 for use in the methods provided herein modulates the growth of hair in an animal model described herein (see, e.g., Section 5.7.3, 10, 16, 24, and 25). In some embodiments, the compound, either alone or in combination with a therapy described in Section 5.3 infra, promotes hair growth in an animal model described herein. In alternative embodiments, the compound, either alone or in combination with a therapy described in Section 5.3 infra, reduces hair growth in an animal model described herein. In some embodiments, the compound, either alone or in combination with a therapy described in Section 5.3 infra, promotes wound healing, e.g., wound healing with reduced scarring, or improved scar revision in an animal model described herein.

5.2 FORMULATIONS AND MODES OF DELIVERY

5.2.1 FORMULATIONS

[00267] The Compounds for use in the pharmaceutical compositions and methods disclosed herein may be formulated with a pharmaceutically acceptable carrier (also referred to as a pharmaceutically acceptable excipients), i.e., a pharmaceutically acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, solvent, an encapsulating material, or a complexation agent. In one embodiment, each component is "pharmaceutically acceptable" in the sense of being chemically compatible with the other ingredients of a pharmaceutical formulation, and biocompatible, when in contact with the biological tissues or organs of humans and animals without excessive toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. See, Remington: The Science and Practice of Pharmacy, 2005, 21st ed., Philadelphia, PA: Lippincott Williams & Wilkins; Rowe et al, eds., 2005, Handbook of Pharmaceutical Excipients, 5th ed., The Pharmaceutical Press and the American Pharmaceutical Association; Ash & Ash eds., 2007, Handbook of Pharmaceutical Additives, 3rd ed., Gower Publishing Company; Gibson ed., 2009, Pharmaceutical Preformulation and Formulation, 2nd ed., Boca Raton, FL: CRC Press LLC, each of which is incorporated herein by reference.

[00268] 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 Compound 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.

[00269] The Compounds for use in the pharmaceutical compositions and methods disclosed herein 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-butanol, 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.

[00270] The Compounds for use in the pharmaceutical compositions and methods disclosed herein may also be formulated with one or more of the following additional agents. Suitable antimicrobial agents or preservatives include, but are not limited to, alkyl esters of p-hydroxybenzoic acid, hydantoins derivatives, propionate salts, phenols, cresols, mercurials, phenoxyethanol, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoates, thimerosal, benzalkonium chloride (e.g., benzethonium chloride), butyl, methyl- and propylparabens, 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. 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-β-cyclodextrin, sulfobutylether-β-cyclodextrin, and sulfobutylether 7-β-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 the scalp or face may additionally be formulated so that it has easy rinsing, minimal skin/eye irritation, no damage to existing hair, has a thick and/or creamy feel, pleasant fragrance, low toxicity, good biodegradability, and a slightly acidic pH (pH less than 7), since a basic environment weakens the hair by breaking the disulfide bonds in hair keratin.

5.2.2 MODES OF ADMINISTRATION

The Compounds for use in the pharmaceutical compositions disclosed herein can be formulated 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. The Compounds for use in the methods and uses disclosed herein can be administered 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 Compounds (or combination treatment) can also be formulated as modified release dosage forms, including delayed-, extended-, prolonged-, sustained-, pulsatile-, controlled-, accelerated-, fast-, targeted-, 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.). The Compounds can be administered by a health care practitioner or by the subject. In some embodiments, the subject administers the Compound to him or herself.

5.2.2.1 TOPICAL ADMINISTRATION

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 Compound in the skin or to deliver a dose of the Compound systematically. In some embodiments, topical administration of a Compound is combined with another treatment described herein, such as, but not limited to, a technique of integumental perturbation described in Sections 5.3.5 and 5.3.6 infra.

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, 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 2005/1077 10, 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 the Compound encapsulated in a polymer. In another embodiment, the 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.

The selected formulation will penetrate into the skin and reach the hair follicle. Thus, in some embodiments, the stratum corneum and/or epidermis are removed by a method
of integumental perturbation described herein (including by wounding or scar revision, 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 Compound will be used to crosslink the polymer, with release of the Compound controlled by the rate of degradation of the hydrogel.

[00277] 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, antimicrobial 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.

[00278] 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, benzoinedated 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 (OAV) emulsions, including cetyl alcohol, glyceryl monostearate, lanolin, wool alcohol (acetylated 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 semisolid, 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-polyoxypropylerie copolymers, and polyvinylalcohol; cellulose 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.

Other means of topical administration, including mechanical means

Other means of topical administration of the Compounds disclosed herein are also contemplated. Each of these methods of topical administration may be used alone to administer Compounds or in combination with one or more other treatments as described in Section 5.3 infra.

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 JetPeel™ (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.

[00283] In some embodiments, the device for topical administration of a Compound is an automatic injection device worn continuously but delivers the Compound intermittently. In some embodiments, the device for topical administration of a Compound 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 a Compound delivers the Compound at a controlled depth in the skin so that it reaches hair follicles, but entry into the circulation is minimized.

[00284] Other methods for administration of the Compounds described herein, used alone or in combination with other treatments described in Section 5.3 (e.g., in combination with integmental 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 integmental perturbation 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 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 integmental 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.
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 perturbed area. In some embodiments, the stratum corneum and epidermis are already removed, e.g., by a method of integumental perturbation described herein, and thus permits effective use of the mechanism using lowered pressure and velocity requirements to achieve dermal delivery.

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

In another embodiment, the Compound can be administered using a two-chamber sprayer device, wherein the Compound 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 Compound 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 Compound will be sprayed as a dry powder that is adherent to the underlying tissue.

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

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.

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.

[00292] In another embodiment, a non-fractional C0₂ or Erbium-YAG laser is combined with drug spraying either without skin disruption, in conjunction with skin disruption, or following skin disruption.

[00293] 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 disruption, or following skin perturbation.

[00294] 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 without skin perturbation, in conjunction with skin perturbation, or following skin perturbation. 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 without skin perturbation, in conjunction with skin perturbation, or following skin perturbation.

[00295] In another embodiment, fractional ablative laser treatment of the skin (e.g., an Erbium-YAG laser used at 2940 nm or a C0₂ laser used at 10,600 nm) is combined with 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 C0₂ laser used at 10,600 nm) is combined with 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.

[00296] In some embodiments, topical administration comprises administration of Compound-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 the Compound. In one embodiment,
particles with different release properties are be delivered simultaneously to achieve pulse delivery.

[00297] In another embodiment, topical administration comprises administration of a Compound-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 Compound-containing formulation through hollow needles.

[00298] In another embodiment, topical administration comprises administration of a Compound-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 Compound-containing formulation is incorporated.

[00299] In another embodiment, topical administration comprises administration of a Compound-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 Compound in a sustained fashion over time.

[00300] An example of a device that can be used to deliver the therapeutic compound to the skin site is depicted in Figures 29-42. 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.

[00301] 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.

[00302] As can be seen in Figure 31, 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 31, 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.

Figures 32 and 33 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.

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.

Figures 34-42 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 34-37 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.

[00306] To use the two liquid component drug cartridge 40 with the drug sprayer 2, as can be seen in Figures 35-37, 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 36 and 37, 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.

[00307] 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 32 and 33) to move or drive the plungers 80 towards the front end 46 of the drug cartridge 40. Thus, as can be seen in Figure 37, 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.

[00308] 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 38-42, 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.
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.

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 38-42, 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 39-42, 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 32 and 33) to move the plunger 120. Initially, the plunger 120 is retracted in the direction shown by arrow 126 in Figure 41 causing the second piston 106 to move in a corresponding manner. Because of the airtight 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 41, 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 42. 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.

In certain embodiments, the first liquid component 62 is a solution comprising a GSK-3 inhibitor 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 GSK-3 inhibitor concentration in the GSK-3 inhibitor 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 GSK-3 inhibitor solution can be a water-based solution.

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 GSK-3 inhibitor as described herein (see, e.g., Section 5.1).

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.

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.

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.

[00319] 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.

[00320] 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.

(i) DELIVERY OF CROSS-LINKED MICROSPHERES

[00321] In certain embodiments, the drug spraying device disclosed herein enables the sustained release of GSK-3 inhibitor, without the use of highly hydrophobic, occlusive matrices. In particular, the drug spraying device enables the delivery of GSK-3 inhibitor 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.

[00322] Without being bound by theory, the drug spraying device enables the administration of GSK-3 inhibitor 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 GSK-3 inhibitor) will be sequestered in the hydrogel, releasing GSK-3 inhibitor in a sustained manner. Thus, the issue of phagocytosis of the microspheres is overcome.

[00323] In certain embodiments, to deliver cross-linked microspheres, the solid component 88 comprises a polymer macromonomer (Polymer 1) (a polymer that can further crosslink with another component) and microspheres containing a GSK-3 inhibitor. The liquid component 86 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.

[00324] 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 15 (Section 20).

(ii) DELIVERY OF CROSS-LINKED BIODEGRADABLE SCAFFOLD

[00325] In certain embodiments, the drug spraying device disclosed herein enables the sustained release of GSK-3 inhibitor and uptake by the skin through a scab. In particular, the drug spraying device enables the delivery of GSK-3 inhibitor such that the delivery system is incorporated into the scab. This can be accomplished by placing a GSK-3 inhibitor 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.

[00326] In certain embodiments, the solid component 88 comprises a polymer macromonomer (Polymer 1) (a polymer that can further crosslink with another component) and the liquid component 86 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 GSK-3 inhibitor. 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, GSK-3 inhibitor 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.

[00327] 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."

[00328] 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 (Section 21).

(iii) DELIVERY OF DRUG COMBINATIONS

[00329] 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).

[00330] In certain embodiments, the first liquid component 62 is a first formulated drug and the second liquid component 64 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 GSK-3 inhibitor 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 62 could contain micronized GSK-3 inhibitor suspended in a FDA-approved liquid excipient and the second liquid component 64 can be a dissolved GSK-3 inhibitor in an aqueous sprayable gel. Co-spraying both forms of GSK-3 inhibitor provides instantly-bioavailable, GSK-3 inhibitor and a sustained form of GSK-3 inhibitor made available as the micronized GSK-3 inhibitor dissolves.

[00331] 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 (Section 22).
(iv) CLEANSING AND DRUG DELIVERY

[00332] 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 a GSK-3 inhibitor. Any wound-cleansing solution known to the skilled artisan can be used with these embodiments.

[00333] 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 18 (Section 23).

[00334] 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.

5.2.2.2 PARENTERAL ADMINISTRATION

[00335] Administration can be parenterally by injection, infusion, or implantation, for local or systemic administration. Parenteral administration, as used herein, includes intravenous, intraarterial, 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, antimicrobial 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.

5.2.2.3 ORAL ADMINISTRATION

[0036] Pharmaceutical compositions comprising the Compounds disclosed herein for oral administration can be provided in solid, semisolid, or liquid dosage forms for oral administration. As used herein, oral administration also includes buccal, lingual, and sublingual 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, effervescent 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.

5.2.3 EX VIVO DELIVERY

[00337] The Compound treatments, such as intermittent and pulse Compound treatments, may also be administered to skin-derived cells or skin tissue ex vivo. For example, a Compound 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 a Compound treatment 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.

[00338] 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.

5.2.4 MODIFIED RELEASE FORMS

[00339] The Compounds disclosed herein 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 Compound 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, or, in the case of immunostimulatory adjuvants, prolonged exposure to the immune system).

5.2.4.1 MATRIX CONTROLLED RELEASE DEVICES

The modified release dosage form can be fabricated using a matrix controlled release device known to those skilled in the art. See, Takada et ai, 1999, in Encyclopedia of Controlled Drug Delivery, Mathiowitz E, ed., Vol. 2, Wiley.

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, carrageenans, 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); polylactides; 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.

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/vinylolxyethanol 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.

[00344] 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.

[00345] 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.

5.2.4.2 OSMOTIC CONTROLLED RELEASE DEVICES

[00346] 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).

[00347] 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-swelling hydrophilic polymers, which are also referred to as "osmopolymers" and "hydrogels." Suitable water-swelling 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-hydroxy ethyl 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.

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.

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.

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.

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, polysulfones, polyethersulfones, 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. 5,798,191. Such hydrophobic but water-vapor permeable membrane are typically composed of hydrophobic polymers such as polyalkenes, polyethylene, polypropylene, polytetrafluoroethylene, polyacrylic acid derivatives, polyethers, polysulfones, polyethersulfones, 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 by 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 hydroxyethyl cellulose, and other pharmaceutically acceptable excipients or carriers.

5.2.4.3 **IN SITU GELING DRUG DELIVERY SYSTEMS**

[00357] In one embodiment, the 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 Compound-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 Compound-loaded solution will thicken into a gel, releasing the Compound-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=polylactic 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 Compound-loaded solution can be injected as a liquid, to form an in situ depot within the tissue. In another variation of the concept, the Compound-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 the Compound for 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.

5.2.4.4 **MULTIPARTICULATE CONTROLLED RELEASE DEVICES**

[00358] The 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 mm in diameter. Such multiparticulates can be made by the processes known
to those skilled in the art, including microfluidization, membrane-controlled emulsification,
oil-in-water, water-oil-water and oil-in oil emulsification and homogenization processes,
complex coacervation, wet-and dry-granulation, extrusion/spheronization, roller-compaction,
melt-congealing, and by spray-coating seed cores. See, for example, Ghebre-Sellasse, ed.,
1994, Multiparticulate Oral Drug Delivery, Marcel Dekke; and Ghebre-Sellassie ed., 1989,
Pharmaceutical Pelletization Technology, Marcel Dekker.
[00359] 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.

5.2.4.5 TARGETED DELIVERY

[00360] The Compounds for use herein may be formulated with a carrier that delivers the
Compound to the site of action, for example, 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 therapy with the Compound and/or ensure that the Compound
reaches only follicles of particular tissues. The carrier may be an aptamer targeted to a
particular protein or cell type in the follicle, an antibody or antigen-binding fragment thereof,
a virus, virus-like particle, virosome, liposome, micelle, microsphere, nanoparticle, or any
other suitable compound.

[00361] 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.

[00362] 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. For hair follicle drug delivery, microspheres and nanospheres have been utilized, to deliver drugs into the hair follicle. Entry into 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 the hair follicle can result in a short residence time of the delivery systems 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 the follicle. 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.

5.2.4.6 LOCAL DELIVERY

[00363] In order to circumvent side effects, the dosage of systemically administered Compound is tightly controlled. Another way in which such side effects may be circumvented is to deliver the Compound locally to the site where hair growth modulation is desired.

[00364] The Compounds described herein may be delivered locally to any part of the subject in which modulation of hair growth is desired, including, e.g., the head (e.g., the scalp, cheek, chin, upper lip, lower lip, ears, nose, eyelashes, or eyebrow), neck, abdomen, chest, breast, e.g., the nipples, back, arms, armpits (axillary hair), stomach, genital area, buttocks, legs, hands, or feet of a subject. In one embodiment, Compound is applied or administered to wounded or scarred skin.

[00365] Such local delivery of the Compound can be achieved by topical administration, transdermal, intradermal, subcutaneous (depot effect), or by intramuscular, intravenous and oral routes of delivery in formulations for targeting systemically delivered Compound to desired follicles. Such modes of delivery are discussed supra.

5.2.5 DELIVERY VIA SCAFFOLDS

[00366] In some embodiments, enhancement of hair follicle formation, and 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 Compound treatment described herein in combination with 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.

[00367] The scaffold for use in combination with a Compound 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.

[00368] 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.

[00369] 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.

[00370] 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 an impermeable granular structure that the keratinocytes cannot "crawl upon."

[00371] 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.

[00372] 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-3 inhibitor (such as one or more Compounds described herein, or another GSK-3 inhibitor), BMP inhibitor, or PPAR antagonist.

[00373] 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 5.3, especially Sections 5.3.4 and 5.3.7 to 5.3.10. 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 integumentally perturbed or wounded site.

[00374] In some embodiments, the Compound itself is incorporated within the mesh scaffold. In some embodiments, the Compound is incorporated within one or more layers of a multilayered mesh scaffold. For example, in one embodiment the mesh scaffold contains the Compound in alternating layers, which may achieve a pulsatile delivery of Compound. In some embodiments, the Compound is incorporated in microspheres in the scaffold, enabling a controlled release of Compound from the scaffold.

[00375] In another embodiment, the mesh scaffold can be fibrin gels that additionally contain Compound. A fibrin network is the first scaffold that a cell encounters as it performs its role in healing wounds due to 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 Compound, fibrinogen and thrombin, that "gels" in situ. One issue that is
encountered is the ability of Compound to diffuse through the fibrin "scab" - making the
drug part of the scab solves this issue.

[00376] In another embodiment, the mesh scaffold is a synthetic biodegradable dressing
and Compound delivery system that also acts as a "sponge" and absorbs the exudates/bloods
from a wound or otherwise integumentally perturbed site. 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
Compound. The release rate of Compound 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 Compound. Changing the ratio of PLA to PLG
will change the release profile of the Compound 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 Compound 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 Compound to be released. In some embodiments, most of the Compound 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.

[00377] 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 5.2.4 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.
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.

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

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.

In a particular embodiment, a combinatorial strategy that uses a biodegradable scaffold combined with administration of a Compound formulation described herein (alone or in combination with another treatment, such as described in Section 5.3, especially Sections 5.3.4 and 5.3.7 to 5.3.10) is applied, which may result in the in situ generation of embryonic stem cells or recruitment of cells required for healing following wounding or other form of integumental perturbation. This approach may be used together with another form of integumental perturbation described in Sections 5.3.5 and 5.3.6 (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**

[00382] 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.

[00383] 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 or other form of integumental perturbation. 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

5.2.5.2 BIOMIMETIC PROPERTIES OF THE SCAFFOLD

[00384] 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.

[00385] 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

[00386] 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.

[00387] 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

[00388] 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.

[00389] 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

5.2.5.5 OXYGEN PERMEABILITY OF THE SCAFFOLD

[00390] 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

[00391] 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

[00392] In another embodiment, a loose, dry, highly poroisis network or scaffold or mesh is placed in the bleeding site of the wound or otherwise integumentally perturbed site to gently absorb the blood and the cell adhesion proteins released at the site. 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.

[00393] A dry scaffold has the added advantage of absorbing the blood at the wound or otherwise integumentally perturbed 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.

[00394] 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.
In one embodiment, a dry, sterile biodegradable scaffold is placed onto the freshly formed wound or perturbed skin site. 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

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.

In some embodiments, the active agents (e.g., Compound 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., a Compound and a 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 or integumental perturbation is large and uniform coverage is needed.

In some embodiments, the active agent-containing spray-on hydrogel is applied on the wound or otherwise integumentally perturbed 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.

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.

In a variation of the foregoing embodiments, the wound or otherwise integmentally perturbed site may be covered with a breathable, non-occlusive spray-on hydrogel to cover the site from infection during healing.
5.3 TREATMENT REGIMENS

In the embodiments described herein, the Compound or formulation thereof can be administered topically, subcutaneously, orally, etc. Regardless of the route of administration used for Compound delivery, the dosing regimen should be adjusted so that maximum benefit is achieved while reducing potential side effects.

In some embodiments, a Compound is administered in a formulation (e.g., a topical form) such that the maximum level of Compound measured in the blood is below the IC50 for GSK-3p inhibition. In some embodiments, a Compound that is topically administered reaches a maximum level greater or equal to the IC50 for GSK-3p inhibition in skin. In one such embodiment, the Compound never reaches a level greater than the IC50 for GSK-3p in blood.

In some embodiments, the target concentration of Compound should be maintained in the skin or blood, and preferably the skin, 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 8 days; at least 9 days; at least 10 days; at least 11 days; at least 12 days; at least 13 days; at least 14 days; at least 15 days; at least 16 days; at least 19 days; or at least 21 days; and, in certain embodiments, not more than 28 days. This can be accomplished using, e.g., repeated applications of the Compound or a single application of a sustained release or extended release Compound formulation. For example, either a single pulse protocol or intermittent treatments can be used to achieve the target concentration of Compound 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 the Compound treatments, such as intermittent Compound treatments or the single pulse protocol. In some embodiments, it is preferable to allow the concentration of Compound to decline between dosages, in order to achieve a pulsatile effect.

In some embodiments, topical administration of a Compound is preferred over oral or subcutaneous administration. Depending on the formulation used, a topically administered Compound may achieve a higher concentration of Compound in skin than in the blood, thereby reducing the risk of toxicity that may be associated with elevated blood levels of Compound. Conversely, and depending on the formulation used, a subcutaneously or orally administered Compound may be preferred in order to achieve a controlled release of Compound from the blood to the skin.
[00405] Regardless of the route of administration, care should be taken to avoid toxicity. In this regard, a dosage should be chosen that maximizes efficacy while minimizing toxicity. Such a dosage may be chosen using the assays described in Section 5.7. Patients should be monitored for toxic side effects according to standard clinical practice. In some embodiments, Compound 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 Compound. It may be possible to predict dosage requirements for an individual patient 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 or 48 or 96 hours later (when Compound is generally eliminated) which serves as the control value. Once the dose is established for a patient, routine monitoring for toxicity is recommended.

[00406] In some embodiments, an effective amount of Compound is administered such that the target concentration of Compound in plasma or serum, as measured 30 minutes to 1 hour after the Compound treatment, is 0.1-1.0 nM, 1.0-10 nM, 10-50 nM, 50-100 nM, 100-500 nM, 0.5-1.0 μM, 1.0 μM-2.0 μM, 2.0-2.5 μM, 2.5-3.0 μM, or 3.0 μM or greater. In some embodiments, an effective amount of Compound is administered such that the plasma or serum Compound concentration measured either 8 hours, 16 hours, 1 day, 2 days, 3 days, 1 week, 2 weeks, or 1 month after the Compound treatment is 0.1 to 1 nM, 0.5 to 1.5 nM, 1 to 10 nM, 10 to 50 nM, 50 to 100 nM, 100 to 150 nM, 150 to 200 nM, 250 to 300 nM, 100 to 250 nM, 100 to 500 nM, 200 to 400 nM, 500 to 1000 nM; or 1000 to less than 10000 nM. In one embodiment, the plasma or serum Compound concentration reaches at least 1 nM. In another embodiment, the peak plasma level never reaches greater than 1 μM. Serum Compound concentration may be measured using methods of liquid chromatography and/or mass spectrometry, which are well known in the art.

[00407] In some embodiments, an amount of Compound is administered such that the target concentration of Compound in the skin is 0.1 nM to 1 nM, 1 nM to 10 nM, 10-100 nM, 100-500 nM, 500-1000 nM, 1 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 nM, 10 to 100 nM, 100 to 200 nM, or 500 to 1000 nM. In some embodiments, the concentration of Compound achieved in the skin is greater than 0.1 nM. In some embodiments, the concentration of Compound achieved in the skin is greater than 1 nM. In some embodiments, the concentration of Compound achieved in the skin is greater than 100 nM. In some embodiments, the concentration of Compound
achieved in the skin is greater than 500 nM. In one embodiment, the concentration of Compound achieved in the skin is approximately 10-100 nM. In one embodiment, the concentration of Compound achieved in the skin is approximately 100-1000 nM. In one embodiment, the concentration of Compound achieved in the skin is approximately 1 µm to 10 µm. In one embodiment, the concentration of Compound achieved in the skin is approximately 10-100 µm. In other embodiments, an amount of Compound is administered such that the concentration of Compound delivered to the skin is 0.0000001 mg/ml to 0.000001 mg/ml, 0.000001 mg/ml to 0.00001 mg/ml, 0.00001 mg/ml to 0.0001 mg/ml, 0.0001 mg/ml to 0.01 mg/ml, 0.01 mg/ml to 0.1 mg/ml, 0.1 mg/ml to 1 mg/ml, 1 mg/ml to 10 mg/ml. In some embodiments, the concentration of Compound delivered to the stratum corneum is 0.0000001 mg/ml to 0.000001 mg/ml, 0.000001 mg/ml to 0.00001 mg/ml, 0.00001 mg/ml to 0.0001 mg/ml, 0.0001 mg/ml to 0.01 mg/ml, 0.01 mg/ml to 0.1 mg/ml, 0.1 mg/ml to 1 mg/ml, 1 mg/ml to 10 mg/ml. One of skill in the art would be able to measure Compound concentrations in skin using techniques known in the art, for example, mass spectroscopy, e.g., inductively coupled plasma mass spectroscopy (ICP-MS), of the LC/MS/MS assay used herein.

[00408] In other embodiments, the Compound 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.

5.3.1 FORMS FOR ADMINISTRATION

[00409] Specific, non-limiting, formulations of Compound for topical and oral administration are provided in Sections 5.3.1.1 and 5.3.1.2 below.

5.3.1.1 TOPICAL FORMS FOR ADMINISTRATION

[00410] In the embodiments described in the subsections that follow, the Compound can be applied topically, e.g., as a cream, gel, ointment, or other form for topical administration as described in Section 5.2.2.1 supra. The Compound can be formulated as a pharmaceutical composition for topical administration.

[00411] In some embodiments, topical Compound is administered twice daily. In some embodiments, topical Compound is administered once daily. In some embodiments, the form of the Compound for topical administration (e.g., gel, cream, ointment, salve, etc.) comprises, w/w, 0.000001%, 0.00001%, 0.0001% Compound, 0.001% Compound, 0.01% Compound,
0.1% Compound, 0.5% Compound, 1% Compound, and 10% Compound. In some embodiments, the form of the Compound for topical administration comprises, w/w, 0.000001% to 0.00001% Compound, 0.00001% to 0.0001% Compound, 0.0001% to 0.001% Compound, 0.001% to 0.01% Compound, 0.01% to 0.1% Compound, 0.1% to 1.0% Compound, 1.0% to 5% Compound, 5% to 10% Compound, or 10% to 15% Compound. In one embodiment, the form of the Compound for topical administration is 0.00001% to 1.0% w/w Compound.

[00412] In some embodiments, a topical formulation is formulated such that 0.01 mg Compound per kg of patient weight (mg/kg) is administered, or 0.02 mg/kg, 0.05 mg/kg, 0.1 mg/kg, 0.2 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2 mg/kg, 5.0 mg/kg, 10 mg/kg, 15 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, 1000 mg/kg, or 1500 mg/kg is administered. In some embodiments, the topical formulation contains a dose in the range of about 0.01 mg/kg to about 0.1 mg/kg, 0.02 mg/kg to about 0.2 mg/kg, 0.1 mg/kg to about 1 mg/kg, 0.2 mg/kg to about 2 mg/kg, 0.01 mg/kg to about 2 mg/kg, 0.1 mg/kg to about 100 mg/kg, about 1 mg/kg to about 20 mg/kg, about 2 mg/kg to about 10 mg/kg, about 100 mg/kg to about 1 g/kg, about 125 mg/kg to about 500 mg/kg, or about 150 mg/kg to about 300 mg/kg of Compound. In some embodiments, the topical formulation contains a dose in the range of about 0.01 mg/kg to about 1 mg/kg. In some embodiments, the topical formulation contains a dose in the range of about 0.02 mg/kg to about 2 mg/kg.

[00413] The concentration of Compound in a particular topical formulation to deliver the intended dose of Compound will depend on the release properties of the Compound, the hydrophobicity of the Compound, the partition coefficient of the Compound, etc. The amount of Compound to generate a topical formulation with one of the aforementioned concentrations of Compound is readily deducible by one of ordinary skill in the art, and depends upon several factors including, e.g., its salt form, stability, release properties, its hydrophobicity or hydrophilicity, etc.

5.3.1.2 ORAL FORMS FOR ADMINISTRATION

[00414] In some embodiments, an oral formulation is administered. The Compound can be formulated for oral administration. In some embodiments, the oral formulation is administered, as determined by the medical practitioner, once daily; in other embodiments,
the oral formulation is administered twice daily. In some embodiments, an oral formulation containing 0.01 mg Compound per kg of patient weight (mg/kg), 0.05 mg/kg, 0.1 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 5.0 mg/kg, 10 mg/kg, 15 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, 1000 mg/kg, or 1500 mg/kg is administered. In some embodiments, the oral formulation contains a dose in the range of about 0.1 mg/kg to about 100 mg/kg, about 1 mg/kg to about 20 mg/kg, about 2 mg/kg to about 10 mg/kg, about 100 mg/kg to about 1 g/kg, about 125 mg/kg to about 500 mg/kg, or about 150 mg/kg to about 300 mg/kg of Compound.

[00415] 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 Compound and the concentration of efficacy of the formulation.

### 5.3.2 PULSE TREATMENT

[00416] A pulse Compound treatment can be administered one time, or multiple times at intervals of time. For example, in one embodiment, the Compound is administered one time per week. 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.

In some embodiments, a pulse Compound treatment is administered before wounding or other form of integumental perturbation. In some embodiments, a pulse Compound treatment is administered at the time of wounding or other form of integumental perturbation. In some embodiments, a pulse Compound treatment is administered following wounding or other form of integumental perturbation.

[00417] In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered before scab formation. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse
Compound treatment is administered during scab formation. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered periscab detachment. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered immediately after scab detachment. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered up to 6 hours after scab detachment. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered 6-12 hours after scab detachment. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered 12-18 hours after scab detachment. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered 18-24 hours after scab detachment. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered 1 day after scab detachment. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered 2 days after scab detachment. In one embodiment, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered 3 days after scab detachment. In some embodiments, in which a pulse Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the pulse Compound treatment is administered within 2 days, 3 days, 5 days, 7 days, 10 days, 2 weeks, 3 weeks, or 4 weeks after wounding or other form of integumental perturbation.
In one embodiment, the pulse Compound treatment is administered at the time of wounding or other form of integumental perturbation and then maintained for 1 or 2 or 3 or 4 or 5 days thereafter (in some embodiments, a scab forms during this time). In some embodiments, a pulse Compound treatment is administered as soon as the scab falls off and maintained for 3 or 4 or 5 days. In one embodiment, the pulse Compound 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 Compound 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 Compound 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 Compound treatment is administered as soon as the scab falls off and maintained for 19 or 21 days. In some embodiments, the pulse Compound treatment is administered in order to modulate the neopidermis that forms underneath the scab. In some such embodiments, the pulse Compound treatment is administered at the time of wounding or other form of integumental perturbation and is maintained 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 Compound is short, for example, limited to one or a few days just following scab detachment, or even continued only for as long as the scab is still attached. The timing of wounding or other form of integumental perturbation and Compound administration is preferably monitored and adjusted so that optimal results are achieved.

In some embodiments, a pulse treatment is combined with a form of wounding or other form of integumental perturbation that does not lead to formation of a scab. In one such embodiment, the pulse Compound treatment is administered before wounding or other form of integumental perturbation. In another such embodiment, the pulse Compound treatment is administered at the time of wounding or other form of integumental perturbation. In some embodiments, a pulse Compound treatment is administered following wounding or other form of integumental perturbation. In some embodiments, in which a pulse Compound treatment is administered following wounding or other form of integumental perturbation that does not lead to formation of a scab, the pulse Compound 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, 5 days, 7 days, 10 days, 2 weeks, or 3 weeks after the wound or other form of integumental perturbation.
The Examples in Sections 6 to 25 provide exemplary protocols for carrying out the aforementioned embodiments, although the invention is not meant to be so limited.

### 5.3.3 INTERMITTENT TREATMENTS

An intermittent Compound 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.

In one embodiment, Compound 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 days. In some embodiments, Compound 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 7 days; and in some embodiments not more than 14 days. In one embodiment, the holiday is one week. In some embodiments, holidays are interspersed for at least 14 days, 21 days, 28 days, or longer.

In some embodiments, an intermittent Compound treatment is begun before wounding or other form of integumental perturbation. In some embodiments, an intermittent Compound treatment is begun at the time of wounding or other form of integumental perturbation. In some embodiments, an intermittent Compound treatment is begun following wounding or other form of integumental perturbation.

In one embodiment, in which an intermittent Compound treatment is begun following a wound or other form of integumental perturbation that leads to formation of a scab, the intermittent Compound treatment is begun following wounding or other form of integumental perturbation. In one embodiment, in which an intermittent Compound treatment is begun following a wound or other form of integumental perturbation that leads to formation of a scab, the intermittent Compound treatment is begun before scab formation. In one embodiment, in which a wound or other form of intermittent Compound treatment is begun following a wound or other form of integumental perturbation that leads to formation
of a scab, the intermittent Compound treatment is begun during scab formation. In one embodiment, in which an intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound in the intermittent Compound treatment is before scab detachment. In one embodiment, in which an intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is immediately after scab detachment. In one embodiment, in which the intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is immediately after scab detachment. In one embodiment, in which the intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is up to 6 hours after scab detachment. In one embodiment, in which the intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is 6-12 hours after scab detachment. In one embodiment, in which the intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is 12-18 hours after scab detachment. In one embodiment, in which the intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is 18-24 hours after scab detachment. In one embodiment, in which the intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is 1 day after scab detachment. In one embodiment, in which the intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is 2 days after scab detachment. In one embodiment, in which the intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is 3 days after scab detachment. In one embodiment, in which the intermittent Compound treatment is administered following a wound or other form of integumental perturbation that leads to formation of a scab, the first administration of Compound is administered immediately after scab detachment, followed by another administration each
day for several days to 1 week. In some embodiments, in which an intermittent Compound
treatment is begun following a wound or other form of integumental perturbation that leads to
formation of a scab, Compound treatment is begun within 3 days, 5 days, 7 days, 10 days, 2
weeks, or 3 weeks after integumental perturbation.

[00425] In one embodiment, the intermittent Compound treatment is begun at the time of
wounding or other form of integumental perturbation and then administered daily (or twice
daily) for 1 or 2 or 3 or 4 or 5 days thereafter (in some embodiments, a scab forms during this
time). In some embodiments, the intermittent Compound treatment is begun as soon as the
scab falls off, and administered daily for 5 days. In some embodiments, the intermittent
Compound 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 Compound 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
Compound 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 Compound treatment is begun as soon as the scab falls off and
maintained for 19 or 21 days. In some embodiments, the intermittent Compound treatment is
to modulate the neoeipidermis that forms underneath the scab. In some such embodiments,
the intermittent Compound treatment is begun at the time of wounding or other form 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 Compound is short, for example, limited to daily
doses for one or a few days just following scab detachment, or even continued only for as
long as the scab is still attached. The timing of wounding or other form of integumental
perturbation and Compound administration is preferably monitored and adjusted so that
optimal results are achieved.

[00426] In some embodiments, an intermittent Compound treatment is combined with a
form of wounding or other form of integumental perturbation that does not lead to formation
of a scab. In one such embodiment, the intermittent Compound treatment is begun before
wounding or other form of integumental perturbation. In another such embodiment, the
intermittent Compound treatment is begun at the time of wounding or other form of
integumental perturbation. In some embodiments, an intermittent Compound treatment is
begun following wounding or other form of integumental perturbation. In some
embodiments, in which an intermittent Compound treatment is begun following a wound or
other form of integumental perturbation that does not lead to formation of a scab, the intermittent Compound 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, 5 days, 7 days, 10 days, 2 weeks, or 3 weeks after integumental perturbation.

[00427] The Examples in Sections 6 to 25 provide exemplary protocols for carrying out the aforementioned embodiments, although the invention is not meant to be so limited.

5.3.4 COMBINATION TREATMENTS

[00428] Treatment with one or more Compounds in combination with conventional methods for enhancing hair growth or removal of unwanted hair, or for enhancing wound healing or scar revision enhances the effectiveness of the foregoing methods. 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. Most drugs for hair loss aim to retain the existing hair follicles in their active cycling states, or to rejuvenate telogen hair follicles to actively cycling anagen states. However, a dosage form that encourages the growth of "new" hair follicles, combined with one that retains the hair follicles in their actively cycling states offer significant value to the individual who is balding, and may encourage hair follicle formation that in turn promotes wound healing in a wounded individual or an individual for whom a scar revision is desired. The combined modality of treatment could involve alternating treatment of each dosage form or concurrent or simultaneous treatment.

[00429] The one or more Compounds may be administered 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 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).

[00430] The one or more Compounds may be administered concurrently with or separately from the additional compound(s), or may be administered and/or delivered to the tissue site with different pharmacokinetics. In one embodiment, the combination treatment comprises one or more Compounds and an additional compound(s) formulated together. The Compound 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.

[00431] In another embodiment, the combination treatment comprises one or more Compounds and an additional compound(s) formulated separately. The separate formulations may be administered concurrently, sequentially, or in alternating sequence. For example, the one or more Compounds may be administered sequentially, or concurrently with another compound such as finasteride or minoxidil, to achieve the desired effect of hair retention and growth.

[00432] In some embodiments, the combination treatment comprises administration of one or more Compounds 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, viroscope), 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 chlorohydrine, aluminum chlorohydrine PEG, aluminum chlorohydrine PG, aluminum dichlorohydrate, aluminum dichlorohydrine PEG, aluminum dichlorohydrine PG, aluminum sesquichlorohydrine, aluminum sesquichlorohydrine PEG, aluminum sesquichlorohydrine PG, aluminum sulfate, aluminum zirconium octachlorohydrate, aluminum zirconium octachlorohydrine GLY (abbreviation for glycine), aluminum zirconium pentachlorohydrate, aluminum zirconium pentachlorohydrine GLY, aluminum zirconium tetrachlorohydrate, aluminum zirconium trichlorohydrate, aluminum zirconium tetrachlorohydrate GLY, and aluminum zirconium trichlorohydrine GLY, potassium aluminum sulphate, (also known as
alum (KA₁(S₀₄)₂12H₂O), aluminum undecylenoyl collagen amino acid, sodium aluminum lactate+ aluminum sulphate (Na₂HAl(OOCCHOHCH₂)₂(OH)₆ + A₁₂(S₀₄)₃), sodium aluminum chlorohydroxylactate, aluminum bromohydrate (Al₂Br(OH)snH₂O), aluminum chloride (AlC₁₃H₂O), 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 = C₆H₁₁ 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 Behzophenone™), and preservatives), 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 carboxy alkylates, cerulenin or a cerulcin analog, including pharmaceutically acceptable salts or solvates thereof, another fatty acid synthase inhibitor, such as tricosanol or analogs thereof, a polyphenol extracted from green tea (EGCG), available from Sigma Corporation (St. Louis, Missouri), or a-methylene-y-butyrolactone), a massage agent, an exfoliant, an anti-itch agent, a pro-inflammatory agent, an immunostimulant (e.g., 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), a cell cycle regulator, a hormonal agonist, 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, cortisone, prednisone, dehydrotestosterone, glucocorticoids, mineralocorticoids, estrogen, testosterone, progestins), antigestagens (e.g., mifepristone, onapristone), an antiandrogen (e.g., cyproterone acetate), an antiestrogen, an antihistamine (e.g., mepyramine, diphenhydramine, and antazoline), an anti-inflammatory (e.g., corticosteroids, NTHEs, and COX-2 inhibitors), a retinoid (e.g., 13-cis-retinoic acid, adapaiene, all-trans-retinoic acid, and etretinate), an immunosuppressant (e.g., cyclosporine, tacrolimus, rapamycin, everolimus, and pimecrolimus), an antibiotic, an anti-cancer agent (such as, e.g., fluorouracil (5-FU or f5U) or other pyrimidine analogs, methotrextate, cyclophosphamide, vincristine), a mood stabilizer (e.g., valproic acid or carbamazepine) an antimetabolite, an anti-viral agent, and an antimicrobial (e.g., benzyl benzoate, benzalkonium chloride, benzoic acid, benzyl alcohol, butylparaben, ethylparaben, methylparaben, propylparaben, camphorated metacresol, camphorated phenol, hexylresorcinol, methylbenzethionium chloride, cetrimide,
chlorhexidine, chlorobutanol, chlorocresol, cresol, glycerin, imidurea, phenol,
phenoxethanol, phenylethylalcohol, phenylmercuric acetate, phenylmercuric borate,
phenylmercuric nitrate, potassium sorbate, sodium benzoate, sodium propionate, sorbic acid,
and thiomersal (thimerosal)).

[00433] In some embodiments, the combination treatment comprises Compound treatment,
such as, e.g., intermittent or a pulse Compound 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
Compound treatment, such as, e.g., intermittent or a pulse Compound treatment, in
combination with one or more non-narcotic analgesics, selected from the group of, e.g.,
aceclofenac, acetaminophen, acetylsalicylic acid; aspirin, carbamazepine,
dihydroxyaluminum acetylsalicylate, fenoprofen, fluproquazone, ibufenac, indomethacin,
ketorolac, magnesium acetylsalicylate, morpholine salicylate, naproxen, phenacetin, phenyl
salicylate, salacetamide, salicin, salicylamide, sodium salicylate, and tolfenamic acid. Other
pain treatments that may be used in combination with the Compound treatments described
herein include nerve blocks or non-traditional pain medications, such as, e.g., Lyrica
(pregabalin) or Neurontin (gabapentin).

5.3.5 COMBINATION TREATMENTS COMPRISING
INTEGUMENTAL PERTURBATION

[00434] In some embodiments, the combination treatment comprises administration of one
or more Compounds provided herein in combination with integumental perturbation (e.g., by
wounding) and, optionally, also comprises another treatment known in the art or described
herein. Integumental perturbation can be achieved by any means known in the art or
described herein, such as, for example, using chemical or mechanical means. In one
embodiment, integumental perturbation comprises disrupting the skin of the subject (for
example, resulting in the induction of re-epithelialization of the skin of the subject). In some
embodiments, a certain area of the epithelium is partially or wholly disrupted. In some
embodiments, a certain area of both the epithelium and stratum corneum are partially or
wholly disrupted. For a discussion of skin disruption and re-epithelialization, including
methods for disrupting skin and inducing and detecting re-epithelialization, see PCT
Publication Nos. WO 2008/042216 and WO 2006/105109, each of which is incorporated
herein by reference. Integumental perturbation can be used to induce, for example, a burn,
excision, dermabrasion, full-thickness excision, or other form of abrasion or wound.

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 CO₂-based, or Erbium-YAG-based, neodymium:yttrium aluminum
garnet (Nd:YAG) laser, etc.). Integumental perturbation can also be achieved through, for
every, 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 ai,
epidermis can be achieved, for example, through the application of an electrical current, or
through electroporation or RF ablation. Electric or magnetic means can also include the
induction of an electric or a magnetic field, or an electromagnetic field. For example, an
electrical current can be induced in the skin by application of an alternating magnetic field. A
radiofrequency power source can be coupled to a conducting element, and the currents that
are induced will heat the skin, resulting in an alteration or disruption of the skin.
Integumental perturbation can also be achieved through surgery, for example, a biopsy, a skin
transplant, hair transplant, cosmetic surgery, etc.

In some embodiments, integumental perturbation is by laser treatment.
Exemplary laser treatments for integumental perturbation include or fractional laser (e.g.,
Fraxel), laser ablation, Erbium-YAG laser, Ultrapulse CO₂ fractional laser, Ultrapulse CO₂
ablative laser, Smooth Peel Full-ablation Erbium laser (Candela), neodymium:yttrium aluminum
garnet (Nd:YAG) laser, as described, for example, in the examples of Sections 6-8,
below. In one embodiment, a laser treatment is chosen in which the integumental
perturbation achieved most resembles that achieved by dermabrasion (for example, a
dermabrasion method described herein). In a preferred embodiment, integumental
perturbation by laser treatment is by a fractional laser. See, e.g., the laser treatments
described in U.S. Provisional Application Nos. 61/262,820, 61/262,840, 61/262,831, each of
which is incorporated herein by reference in its entirety. 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)). Treatment with an Erbium-YAG laser at 1540 or 1550 nm is typically non-ablative, and pinpoint bleeding typical of laser treatment is not observed since the stratum corneum is left intact. The column of dead (epidermal and/or dermal) cells in the path of the laser treatment is termed a "coagulum." In another embodiment, integumental perturbation by laser treatment is by a fractional laser, using, e.g., a C0₂ laser at 10,600 nm. Treatment with a C0₂ laser at 10,600 nm is typically ablative, and typically leads to the appearance of pinpoint bleeding.

[00437] A standard C0₂ or Erbium-YAG laser can be used to create superficial and, optionally, broad based, integumental perturbation similar to dermabrasion (discussed below). Although the pinpoint bleeding clinical endpoint may not be achieved due to the coagulation properties of (particularly non-ablative) lasers, use of a laser has an advantage making it possible to select the specific depth of skin disruption to effectively remove the stratum corneum and epidermis, or portions thereof.

[00438] In one embodiment, the laser treatment is ablative. For example, full ablation of tissue is generated by the targeting of tissue water at wavelengths of 10,600 nm by a CO₂ laser or 2940 nm by an Erbium-YAG laser. In this mode of laser treatment, the epidermis is removed entirely and the dermis receives thermal tissue damage. The depth of tissue ablation may be a full ablation of the epidermis, or a partial ablation of the epidermis, with both modes causing adequate wounding to the skin to induce the inflammatory cascade requisite for regeneration. In another variation, the depth of ablation may extend partially into the dermis, to generate a deep wound. The denuded skin surface is then treated with one or more Compounds; alternatively, the one or more Compounds can be delivered into the skin after the initial re-epithelialization has occurred already, to prevent clearance and extrusion of the Compound-containing depots from the tissue site by the biological debris-clearance process. In one embodiment, one or more Compounds is delivered by a sustained release depot that is comprised of biocompatible, bioabsorbable polymers that are compatible to tissue.

[00439] The standard full thickness excision model is created using scissors or with a scalpel in animal models (see, also, the examples of Sections 10, 16, 24, and 25 infra). This procedure, while contemplated for use herein (see, e.g., the example of Sections 10, 16, 24, and 25 infra), carries with it the risk of scarring. However, various fractional laser modalities could be used to achieve a similarly deep disruption on a grid pattern. A fractional laser can be use to "drill," for example, 1-mm diameter holes with a 1-mm hole spacing (the fractional laser can make holes of smaller dimensions). Although the skin is completely removed
within the 1-mm hole, the surrounding intact skin prevents scarring and therefore the full thickness excision model is invoked within each hole.

[00440] In some embodiments, the laser treatment is ablative and fractional. For example, fractional tissue ablation can be achieved using a CO\textsubscript{2} 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 some such embodiments, the lasing beam creates micro-columns of thermal injury into the skin, at depths up to 4 mm and vaporizes the tissue in the process. Ablative treatment with a fractional laser leads to ablation of a fraction of the skin leaving intervening regions of normal skin intact to rapidly repopulate the epidermis. Approximately 15%-25% of the skin is treated per session. The density of micro thermal zones (MTZ) can be varied to create a dense "grid" of injury columns surrounded by intact skin and viable cells. The density of the grid on the treatment area plays an important role. The denser the grid, the more the thermal injury and the type of injury begins to approximate full ablation. Therefore, it is appreciated that there may be an "optimum" MTZ density that is appropriate for use in the methods disclosed herein. In one embodiment, one or more Compounds is delivered into the dermis immediately after wounding, or after initial re-epithelialization has occurred.

[00441] In another embodiment, the mode of laser treatment is non-ablative, wherein the stratum corneum and the epidermis are intact after treatment, with the dermis selected for the deep thermal treatment required for the requisite injury to tissue. This can be accomplished by cooling the epidermis during the laser treatment. For example, one could use the timed cooling of the epidermis with a cryogen spray while the laser delivers deep thermal damage to the dermis. In this application, the depth of treatment may be 1 mm to 3 mm into the skin. One could also use contact cooling, such as a copper or sapphire tip. Lasers that are non-ablative have emission wavelengths between 1000-1600 nm, with energy fluences that will cause thermal injury, but do not vaporize the tissue. The non-ablative lasers can be bulk, wherein a single spot beam can be used to treat a homogenous section of tissue. In some embodiments, multiple treatments are required to achieve the desired effect. In one embodiment, one or more Compounds is delivered deep into the dermis in polymeric micro-depots and released in a sustained fashion. Lasers that are non-ablative include the pulsed dye laser (vascular), the 1064 Nd:YAG laser, or the Erbium-YAG laser at 1540 nm or 1550 nm (e.g., the Fraxel® laser). Use of an Erbium-YAG laser at around 1540 nm or around 1550 nm, as opposed to its use at 2940 nm, "coagulates" zones of dermis and epidermis (forming a "coagulum") and leaves the stratum corneum essentially intact.
In another embodiment, the mode of laser treatment is fractional and non-ablative. Treatment with a fractional, non-ablative laser leads to perturbation of a fraction of the skin, leaving intervening regions of normal skin intact to rapidly repopulate the epidermis. Approximately 15%-25% of the skin is treated per session. As in any non-ablative process, the skin barrier function is maintained, while deep thermal heating of dermis can occur. Thus, zones of dermis and epidermis are coagulated and the stratum corneum is left essentially intact. This process has been coined "fractional photothermolysis" and can be accomplished, e.g., using the Erbium-YAG laser with an emission at or around 1540 nm or 1550 nm. In one embodiment, one or more Compounds is delivered immediately after the tissue injury, deep into the dermis. In another embodiment, a combination of bulk and fractional ablation modes of tissue injury are used.

In a specific embodiment, the mode of laser treatment for, e.g., a Caucasian male 30-50 years old, is fractional and non-ablative using an Erbium-YAG laser at 1550 nm, with the following settings: 50-70 J/cm², treatment level 8-10 (density of the "dots"), with 8 passes. In this regard, the laser device can be equipped with a touch pad screen that offers the operator a menu of options for setting the parameters for operating the laser to promote hair growth. For example, the device can be programmed to offer the operator selections for hair growth vs. removal, choice of skin color, hair follicle density, power settings, etc.

In another embodiment, a combination treatment comprising use of a laser includes administration to the skin of a compound absorbing light at wavelengths between 1000-1600 nm for the purpose of efficient channeling of light to heat energy. This method of channeling energy may cause micro-zones of thermal injury within the skin. The compound may be delivered to the skin homogenously in the treatment zone, then subsequently irradiated with a non-ablative laser to efficiently capture the vibrational energy of the infrared beam. This method may result in evenly distributed and deep thermal injury, without causing tissue vaporization.

In another embodiment, a combination treatment comprising use of a laser includes administration of one or more Compounds that is encapsulated in matrices that are highly hydrophilic and charged, for example, linked to the dermis by covalent or ionic bonding to prevent the matrices from being cleared by phagocytosis, as part of the wound healing process.

In another embodiment, a combination treatment comprising use of a laser includes the step of placing a biocompatible, synthetic skin substitute on the newly created 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 Compound formulation described herein. This method of treatment may enable treating a large bald area on the scalp 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 one or more Compounds 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 Compound or other compound application may prevent the Compound or other compound from being cleared away from the wound site as the wound heals.

In some embodiments, a fractional like hole pattern (similar to that achieved with a fractional laser or full thickness excision) is achieved with using an array of punch biopsy needles. For example, 1-mm punch biopsies can be arranged with 1-mm hole spacing. When inserted into the scalp or other area of skin to be treated, the cored skin samples can be removed and, thus, an effect approximating the full thickness excision model is invoked within each hole. Similarly, and for smaller holes, microneedles (e.g., 19 or 21 gauge needles) and/or micro-coring needles could be used.

In some embodiments, integumental perturbation is by dermabrasion (also referred to herein as "DA"), a well-established dermatological procedure that has been used for decades as a skin resurfacing technique (Grimes, 2005, Microdermabrasion. Dermatol Surg 31:1351-1354). While the popularity of mechanical dermabrasion has decreased in recent years with the advent of laser-based procedures, dermabrasion is still used for removing facial scars resulting from acne and other trauma. Small, portable mechanical dermabrasion equipment uses interchangeable diamond fraises operated at different rotation speeds to remove the epidermis and dermis to differing skin depth levels. Adult human skin treated with dermabrasion completely re-epithelializes in 5-7 days with minor redness lasting up to a few weeks. Dermabrasion may be carried out using any technique known in the art or as described in the examples of Section 6, 7, 9, 10, 16, 17, 18, and 24, infra. For example, as described in the examples of Sections 6, 7, 9, 10, 16, 17, 18, and 24 dermabrasion may be carried out using standard DA with aluminum oxide crystals using the Aseptico Econo-Dermabrader, Advance Microderm DX system, or M2-T system; standard DA with Bell Hand Engine with diamond fraise; wire brush; etc.
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, dermabrasion may be carried out using an abrasive wheel to achieve larger globules of bleeding and frayed collagen.

For example, dermabrasion may be carried out using an abrasive wheel to, in some embodiments, achieve pinpoint bleeding. In other embodiments, dermabrasion may be carried out using an abrasive wheel to achieve larger globules of bleeding and frayed collagen. Non-powered devices such as abrasive cloths can also be used to achieve the dermabrasion, with the optional achievement of the same endpoint.

In some embodiments, DA is accomplished using a device typically used for microdermabrasion (also referred to herein as "MDA"). For example, in such DA protocols, a microdermabrasion device is used to remove a greater depth and/or area of skin than is typical for microdermabrasion. In some embodiments, the microdermabrasion device is used under sterile conditions. In some embodiments, dermabrasion is achieved by using a device for microdermabrasion to the point where treatment is stopped upon the observation of pinpoint bleeding, which signals the removal of the stratum corneum and epidermis into the papillary dermis. In other embodiments, dermabrasion is achieved by using a device for microdermabrasion to the point where treatment is stopped upon the observation of larger globules of bleeding and frayed collagen, which signals the removal of the stratum corneum and epidermis into the papillary and reticular dermis. In some embodiments, this extended use is reduced by using a microdermabrasion device with increased output pressure and increased abrasion particle size, which may accelerate the skin removal process.

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.

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 removes the entire epidermis. 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 deep as the hypodermis.

In some embodiments, integumental perturbation by one or more of the aforementioned methods is to a skin depth of 30 µm. 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 30-100 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 30-200 µm. In some embodiments, integumental perturbation is to a skin depth of 60-200 µm. In some embodiments, integumental perturbation is to a skin depth of 100-200 µm. In some embodiments, integumental perturbation is to a skin depth of 100-150 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.

5.3.6 COMBINATION TREATMENTS COMPRISING INTEGUMENTAL PERTURBATION FOR WOUND HEALING AND SCAR REVISION.

The present invention is also 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) harnesses their regenerative capacity and provides a transformational approach to scar revision and the management of wounds. The approaches described herein permit
scar revision under sterile and controlled conditions that recreates and harnesses the fetal
skin’s plastic and regenerative capacity.

[00456] The physical disruption of the skin (integumental perturbation) provides a signal for the formation of new hair follicles. Wounding is itself a form of integumental perturbation. Consequently, scar revision (which involves wounding) also provides a signal for hair follicle neogenesis and/or migration into the wound site. For instance, as discussed in Section 2.7.2 above, a current method for scar revision is serial expansion. We believe that serial expansion is one example of the regenerative capacity of skin, since the physical tension induced by balloons induces integumental perturbation and results in either migration of hair follicles into the wound site or division and differentiation of hair follicle stem cells and formation of new hair follicles. Other current methods of scar revision have similarly involved integumental perturbation. One method involves the surgical excision of the wound and surrounding normal tissue. The newly formed wound is then re-closed by primary intent. In some cases, a jagged surgical incision is created so that the lines of tension of the skin are parallel to the incisions (since perpendicular incisions to the lines of tension heal poorly.) Another method involves dermabrasion to remove epidermis and papillary dermis. This treatment has been used for acne (atrophic scars), but is not widely used today. Needling is an antiquated technique that employs repeated "needle" injury to the scar to "loosen" it. In subcision, a technique developed by Norman and David Orentreich that has not been widely accepted, cuts are made "under" scars to loosen the connective tissue that might be anchoring scar.

[00457] Laser treatment, such as by pulsed dye laser and, more recently, nonablative fractional laser, has also been reported to improve the appearance of surgical scars. See Tierney et al, 2009, *Dermatol Surg* 35:172-180 (incorporated herein by reference in its entirety). We have found that another example of the regenerative capacity of skin underlies the capacity of fractional lasers to effect resurfacing and mediate wound revision. Fractional laser treatment of scarred tissue creates areas of small micro-injuries with intact epidermis in-between, and the integumental perturbation of the laser activates hair follicle deposition into the injury sites, either by migration from the intact epidermis or by inducing hair follicle neogenesis in the wound. In one embodiment, laser-induced wounding of columns (the nonablative coagulum is a preferred embodiment) triggers the regenerative capacity of the intervening normal skin stem cells. This technique may have utility in, for example, revising small scars (to improve texture, pigmentation and other features).
Without being bound by any theory, one advantage of using combinations comprising integumental perturbation is that the perturbation provides a signal for hair follicle deposition and/or deposition of other adnexal structures into the wound site, e.g., by their migration and/or by generation of new hair follicles (hair follicle neogenesis) or adnexal structures. Again while not being bound by any theory, whether or not a wound heals by scarring may depend on the efficiency of hair follicle or other adnexal structure deposition into the wound. If these structures, e.g., hair follicles, are not timely deposited into the healing wound, the process will result in a scar. Thus, wound healing without scarring (and scar revision) may be effected by improving the efficiency of adnexal structure (e.g., hair follicles) deposition into the wound or by slowing wound healing in order to allow sufficient time for deposition of these structures into the wound site.

In certain embodiments, enhancement of wound healing or scar revision is accomplished by Compound 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 some embodiments, the Compound treatment is administered to skin that has been damaged and which no longer contains follicles. In such embodiments, the Compound treatment may restore follicle production in that area of skin. In one such embodiment, an area of skin containing a wound that has not healed correctly, such as a scar (e.g., a keloid scar), is administered a Compound treatment in order to restore hair follicles and/or hair growth to that area of skin. These effects may be accomplished by modulating the dosage of Compound.

In certain other embodiments, enhancement of wound healing or scar revision is accomplished by a combination of integumental perturbation and a Compound treatment, such as a pulse or intermittent Compound treatment. In some embodiments, the combination treatment comprises intermittent or a pulse Compound treatment in combination with integumental perturbation or, optionally, also comprises another treatment known in the art or described herein. Combinations comprising integumental perturbation are preferred for skin that is not already acutely wounded, since wounding itself is a form of integumental perturbation. Integumental perturbation can be achieved by any means known in the art or described herein, such as, for example, using chemical or mechanical means.

### DISCUSSION

The Compound treatments described herein potentiate the formation of new hair follicles. Integumental perturbation produces in the affected skin tissue an increase in the
number of hair follicle stem cells and in the plasticity of hair follicle cells, such that resident hair follicles may be reprogrammed. Accordingly, and without being bound by any theory for how the invention works, integumental perturbation in combination with one or more Compounds provides an environment for the formation of a large number of follicles with desired properties.


[00463] FSCs originate from one or more of the following: (i) existing follicles ("follicle derived follicle stem cells" or "FDFSC") (see, e.g., Toscani et al., 2009, Dermatol Surg. 2009; (ii) the skin ("tissue derived follicle stem cells" or "TDFSC") (see, e.g., Ito M, 2007, Nature 447:3 16-320); (iii) bone marrow ("bone marrow derived follicle stem cells" or "BMDFSC") (see, e.g., Fathke et al., 2004, Stem Cells 22:812-822; and Rovo et al., 2005, Exp Hematol. 33:909-911); and/or (iv) mesenchymal stem cells such as adipocyte stem cells.

[00464] FSCs generate new hair follicles that preserve the type of hair follicle that is typical for each location of skin or scalp. For example, FSCs from the coronal scalp of a male with MPHL typically generate atrophic follicles with vellus or club hairs. In contrast, FSCs from the occipital scalp of the same male typically generate follicles with terminal hair that are not subject to involution in response to DHT.

[00465] However, if external signals are provided that interfere with this "default" program, the FSCs responsible for follicle formation may be reprogrammed. FSCs in the process of asymmetric division and subsequent differentiation are susceptible to signals (such as estrogen or testosterone) that alter the determinism of their differentiation program. For example, FSCs from the coronal scalp of a male with MPHL, under the influence of estrogen, can generate follicles with terminal hair that are not subject to involution in response to DHT. Such follicles have characteristics usually associated with: (i) pre-alopecia follicles in the coronal scalp; (ii) female-type follicles on the coronal scalp; or (iii) occipital scalp type follicles. Alternatively, by antagonizing estrogen or testosterone, the assumption of the default hair pattern in a particular skin area may be prevented. For example, a female's
unwanted moustache hair may be reduced by perturbing the skin of the upper lip and administering a testosterone antagonist.

[00466] Thus, treatment with one or more Compounds in combination with integumental perturbation provides a window during which a third treatment that alters the follicle development program may be administered in order to significantly change the number and quality of follicles in a particular area of skin. In some embodiments, the third treatment (e.g., estrogen or testosterone modulator, such as those described in Poulos & Mirmirani, 2005, Expert Opin. Investig. Drugs 14:177-184 (incorporated herein by reference) is administered simultaneously with integumental perturbation. In some embodiments, the third treatment is administered after integumental perturbation. In some embodiments, the third treatment is administered 1 day, 2 days, 3 days, 5 days, 7 days, 10 days, or 2 weeks after integumental perturbation. In one embodiment, the third treatment is administered at the time of integumental perturbation and then daily for 5 days thereafter (in some embodiments, a scab forms during this time). In some embodiments, the third treatment is administered daily for 5 days beginning as soon as the scab falls off. In some embodiments, the third treatment is administered in order to modulate the neoepidermis that forms underneath the scab. In some such embodiments, the third treatment is administered at the time of integumental perturbation and 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 the third treatment 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. The timing of the integumental perturbation, Compound administration, and the third treatment is preferably monitored and adjusted so that optimal results are achieved.

[00467] The following sections contain non-limiting examples of combination treatments that are specific for, respectively, (i) enhancing hair growth and/or treating conditions associated with hair loss; (ii) removal of unwanted hair and/or treating conditions associated with excessive hair growth; (iii) modulation of wound healing; and (iv) improvement of scars and scar revisions. The treatments described in these sections may optionally be combined with the aforementioned treatments and/or with one another. For example, in some embodiments, a combination of two or three or four treatments is used in order to achieve optimal results.
5.3.7 COMBINATION TREATMENTS FOR ENHANCING HAIR GROWTH

[00468] In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with a treatment that enhances hair growth and/or treats a disease or condition associated with excessive hair loss. Any treatment that enhances hair growth and/or treats a disease or condition associated with excessive hair loss that is known in the art or yet to be developed is contemplated for use in such combination treatments.

[00469] In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with one or more antiandrogens, such as, e.g., finasteride (e.g., marketed as Propecia or Proscar), fluconazole, spironolactone, flutamide, diazoxide, 17-alpha-hydroxyprogesterone, 11-alpha-hydroxyprogesterone, ketoconazole, RU58841, dutasteride (marketed as Avodart), fluridil, or QLT-7704, an antiandrogen oligonucleotide, or others described in Poulos & Mirmirani, 2005, Expert Opin. Investig. Drugs 14:177-184, the contents of which is incorporated herein by reference. Commonly used dosage forms of finasteride that may be used in such combination therapies are, for example, oral finasteride at 1 mg/day. See, e.g., Physicians' Desk Reference, 2009, 63rd ed., Montvale, NJ: Physicians' Desk Reference Inc., entries for Propecia® and Proscar® at pages 2095-2099 and 2102-2106, respectively, which are incorporated herein by reference in their entirities. The regular dosages may be increased or decreased as directed by the physician. For example, a lower dosage may be used over a shorter duration owing to the synergistic effect of the combination with the one or more Compounds.

[00470] In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with one or more channel openers (e.g., potassium channel opener, e.g., an ATP-sensitive potassium channel (KATP opener), or an activator of such a channel), such as, e.g., minoxidil (e.g., marketed as Rogaine or Regaine), diazoxide, or phenytoin. Commonly used dosage forms of minoxidil that may be used in such combination therapies are topical solutions comprising 2% minoxidil or 5% minoxidil, for example, topical minoxidil foam 5%. The regular dosages may be increased or decreased as directed by the physician. For example, a lower dosage may be used over a shorter duration owing to the synergistic effect of the combination with the one or more Compounds.

[00471] In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with a prostaglandin F2a analog. Non-limiting examples of prostaglandin F2a analogs include Bimatoprost, Latanoprost, Travoprost, Unoprostone, Tafluprost, Dinoprost, AS604872, BOL303259X, PF3 187207, and Carboprost.
In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with a 5α-reductase inhibitor. Non-limiting examples of 5α-reductase inhibitors include Finasteride, Dutasteride, Turosteride, Bexlosteride, Izonsteride, Epristeride, Epigallocatechin, Azelaic acid, FCE 28260, and SKF 105,111.

In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with an antiandrogen (e.g., finasteride, 5-alpha reductase inhibitors) and a channel opener (e.g., minoxidil).

In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with one or more antiandrogens, such as ketoconazole, or a prostaglandin or a prostaglandin agonist, or a prostaglandin F2 alpha or analog thereof (such as, e.g., bimatoprost, travoprost, or latanoprost), DoxoRx, Docetaxel, FK506, GP1 1046, GP1 1151 I, LGD 1331, ICX-TRC, MTS-0 1, NEOH 101, HYG-102440, HYG-4 10, HYG-420, HYG-430, HYG-440, spironolactone, CB-03-01, RK-023, Abatacept, Viviscal®, MorrF, ASC-J9, NP-619, AS101, Metron-F-1, MK-396, PSK 3841, Targretin Gel 1%, MedinGel, Dinoprost, Carboprost, PF3 187207, BOL303259X, AS604872, THG1 1331, PF-277343, PF-3004459, Raptiva, herbs (such as, e.g., saw palmetto, glycine soja, Panax ginseng, Castanea Sativa, Arnica Montana, Hedera Helix Geranium Maculatum), triamcinolone acetonide (e.g., suspension of 2.5 to 5 mg/ml for injection), a topical irritant (e.g., anthralin) or sensitizer (e.g., squaric acid dibutyl ester [SADBE] or diphenyl cyclopropene [DPCP]), botulinum toxin-A, clomipramine, unsaturated fatty acids (e.g., gamma linolenic acid), a fatty acid derivative, thickeners (such as, e.g., carbomer, glycol distearate, cetearyl alcohol), caffeine or coffee, a hair loss concealer, bimatoprost (trade name: Latisse), niacin, nicotinate esters and salts, adenosine, methionine, or CaCl2. In some embodiments, the combination therapy comprises treatment with one or more Compounds in combination with nitroxide spin labels (e.g., TEMPO and TEMPOL). See United States Patent 5,714,482, which is incorporated herein by reference.

In some embodiments, the combination treatment comprising treatment with one or more Compounds to enhance hair growth in a female subject is not finasteride or ketoconazole. In some embodiments, the combination therapy comprising treatment with one or more Compounds to enhance hair growth in a pregnant, female subject is not finasteride or ketoconazole.

In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with a copper peptide(s), preferably applied topically, or another compound with superoxide dismutation activity. In some embodiments, the
combination therapy comprises treatment with one or more Compounds in combination with an agent that increases nitric oxide production (e.g., arginine, citrulline, nitroglycerin, amyl nitrite, or sildenafil (Viagra)). In preferred embodiments, such compounds are administered further in combination with a catalase or catalase mimetic, or other antioxidant or free radical scavenger.

[00477] In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with one or more agents that counteract hair follicle cell senescence (also referred to herein as "anti-senescence agents"), 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.

[00478] In some embodiments, the combination treatment comprises administration of Compound in combination with one or more agents that induce an immune response or cause inflammation, such as, e.g., tetanus toxoid, topical non-specific irritants (anthralin), or sensitizers (squaric acid dibutyl ester [SADBE] and diphenyl cyclopropanone [DPCP]). While not intending to be bound by any theory, it is thought that by contacting these agents to the skin, lymphocytes and hair follicle stem cells may be recruited to skin. In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with a chemical or mechanical (such as those discussed infra) treatment that induces an inflammatory process in the skin. While not intending to be bound by any theory, inducing inflammation in the site where hair growth is desired helps to recruit stem cells to the tissues that drive the formation of new follicles.

[00479] In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with an antiapoptotic compound. In one embodiment, the antiapoptotic compound is not a Wnt or a Wnt agonist.

[00480] In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with one or more of stem cell therapy, hair cloning, hair transplantation, scalp massage, a skin graft, hair plugs, or any surgical procedure aimed at hair restoration.

[00481] In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with use of a laser device or other mode of accomplishing "photo-biostimulation" of the hair follicles. For example, the Hairmax Lasercomb or the
Leimo laser are non-limiting examples of devices that can be used to stimulate growth of hair and can be used in combination with the methods described herein.

In certain embodiments, treatment with one or more Compounds in combination with the aforementioned methods for enhancing hair growth prevents, delays, or reverses scalp hair loss in MPHL and/or diffuse hair thinning associated with aging. In some embodiments, treatment with one or more Compounds, alone or in combination with the aforementioned methods for enhancing hair growth, synchronizes hair follicle cells in the cell cycle. In a specific embodiment, the one or more Compounds is administered to arrest hair follicle cells in G2/M phase, which synchronizes them; then the Compound treatment is removed; and then their re-entry into the cell cycle and mitotic division is stimulated with other drugs (which leads to anagen follicles and an increased number of follicles). In another embodiment, the Compound treatment arrests hair follicle cells in late prophase or metaphase, which synchronizes them; the Compound treatment is removed; and then their re-entry into the cell cycle and mitotic division is stimulated with other drugs (which leads to anagen follicles and an increased number of follicles). In another embodiment, the Compound treatment arrests hair follicle stem cells in G2/M phase, which synchronizes them; then the Compound treatment is removed; and then their re-entry into the cell cycle and mitotic division is stimulated with other drugs (which leads to anagen follicles and an increased number of follicles). In another embodiment, the Compound treatment arrests hair follicle stem cells in late prophase or metaphase, which synchronizes them; the Compound treatment is removed; and then their re-entry into the cell cycle and mitotic division is stimulated with other drugs (which leads to anagen follicles, and an increased number of follicles).

In some embodiments, treatment with one or more Compounds, alone or in combination with the aforementioned methods for enhancing hair growth, synchronizes hair follicle cells in the Follicle Cycle. In one such embodiment, the treatment regimen induces follicles to enter anagen. In another embodiment, the treatment regimen prevents follicles from entering catagen. In one embodiment, the treatment regimen induces follicles in telogen to enter exogen, or induces follicles in exogen to enter anagen.

In certain embodiments, treatment with one or more Compounds in combination with the aforementioned methods for enhancing hair growth improves the effectiveness of these methods, making the treatment more effective, efficient, cost-effective, and/or user friendly. For example, fewer treatments may be required. In certain embodiments, one of the previously described hair growth enhancement treatments on its own is not cosmetically
satisfactory, the benefits are too short-lived, or the hair that results from the treatment is vellus hair, or other thin or patchy hair, or has inadequate pigmentation. When one of these treatments is combined with treatment with one or more Compounds, the hair that results may be more cosmetically satisfactory, longer lasting, terminal hair or head hair (depending on the type of hair intended as opposed to vellus hair, and/or thicker, more uniform, and properly pigmented hair. In certain embodiments, more than one hair will emerge from each follicle, leading to the appearance of thicker hair.

[00485] In certain embodiments, one of the aforementioned treatments to enhance hair growth is administered following integumental perturbation (as described herein) and treatment with one or more Compounds. In one exemplary embodiment, integumental perturbation is followed by treatment with one or more Compounds, which is then followed by one of the aforementioned treatments to enhance hair growth. In another exemplary embodiment, integumental perturbation accompanies treatment with one or more Compounds, which is then followed by one of the aforementioned treatments to enhance hair growth. In another embodiment, integumental perturbation is prior to treatment with one or more Compounds administered together with one of the aforementioned treatments to enhance hair growth.

[00486] In addition to the examples in Sections 6-10, 16, and 17 the following embodiment is illustrative of the methods described herein. Integumental perturbation is achieved by either treatment with a fractional Erbium-YAG laser to epidermal or dermal depth, a fractional CO₂ laser to epidermal or dermal depth, or dermabrasion as described herein. This is followed by the stimulation of follicle formation by treatment with one or more Compounds. Then, the follicles (or Follicle Stem Cells) are reprogrammed, e.g., a miniaturizing male temporal scalp follicle (or Follicle Stem Cell) is changed to a non-miniaturizing female-type temporal scalp follicle (or Follicle Stem Cell) using one or more of the following drugs: estrogen, finasteride, dutasteride (Avodart™). Alternatively, the follicle type can be reprogrammed, e.g., a miniaturizing male temporal scalp follicle can be changed to a non-miniaturizing male occipital scalp-type follicle, using a drug such as valproate.) Then, terminal hair growth is stimulated by the application of low energy light (using, e.g., LaserMax or IPL) or minoxidil.

[00487] In certain embodiments, enhancement of hair growth is accomplished by a combination of integumental perturbation, as described herein, and treatment with one or more Compounds without one of the aforementioned treatments for enhancing hair growth. In some embodiments, the combination of integumental perturbation and treatment with one
or more Compounds of an area of skin that already contains hair-producing follicles (preferably, terminal hair) increases production of hair in that area of skin. In some embodiments, the combination of integumental perturbation and Compound treatment is administered to skin that has been damaged and which no longer contains follicles. In such embodiments, the combination of integumental perturbation and Compound treatment may restore follicle production in that area of skin. In one such embodiment, an area of skin containing a wound that has not healed correctly, such as a scar (e.g., a keloid scar), is administered a combination treatment of integumental perturbation and one or more Compounds in order to restore hair follicles and/or growth to that area of skin.

[00488] In certain embodiments, enhancement of hair growth is accomplished by treatment with one or more Compounds alone. In some embodiments, treatment with one or more Compounds of an area of skin that already contains hair-producing follicles increases production of hair in that area of skin. In some embodiments, the one or more Compounds is administered to skin that has been damaged and which no longer contains follicles. In such embodiments, treatment with the one or more Compounds may restore follicle production in that area of skin. In one such embodiment, an area of skin containing a wound that has not healed correctly, such as a scar (e.g., a keloid scar), is administered one or more Compounds in order to restore hair follicles and/or hair growth to that area of skin. These effects may be accomplished by modulating the dosage of the one or more Compounds.

[00489] 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, for example, if branched hair follicles or multiple shafts per pore were produced by the combination and not by either alone.

5.3.7.1 **COMBINATION TREATMENTS FOR SCARRING ALOPECIA**

[00490] In addition to the combination treatments for enhancing hair growth described herein, the following are exemplary combination treatments comprising Compound treatment, including intermittent Compound treatment and pulse Compound treatment, for enhancing hair growth in a patient having scarring alopecia. In certain embodiments, the combination treatment, e.g., comprising an intermittent or pulse Compound treatment, is specific for a particular subtype of scarring alopecia.

[00491] In some embodiments, the combination treatment for enhancing hair growth in a patient having scarring alopecia comprises Compound treatment, such as, e.g., intermittent
Compound treatment or a pulse Compound treatment, in combination with one or more anti-inflammatory medications and antimalarial drugs. Medications that may be administered orally include hydroxychloroquine, doxycycline, mycophenolate mofetil, cyclosporine, or corticosteroids. Medications that may be administered topically include corticosteroids (such as, e.g., betamethasone, e.g., Luxiq®), tacrolimus, pimecrolimus, or Derma-Smoothe/FS scalp oil. Medications that may be administered by injection include triamcinolone acetonide (a corticosteroid), which may be injected into inflamed, symptomatic areas of the scalp. In particular, non-limiting, embodiments, such combinations are used in the treatment of a patient with the lymphocytic group of cicatricial alopecias, including lichen planopilaris, frontal fibrosing alopecia, central centrifugal alopecia, and pseudopelade (Brocq).

[00492] In some embodiments, the combination treatment for enhancing hair growth in a patient having scarring alopecia comprises Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, in combination with one or more antibiotics, such as oral or topical antibiotics. In some embodiments, the combination treatment comprises one or more retinoids, such as isotretinoin, or methotrexate, tacrolimus, cyclosporin, or thalidomide. In particular, non-limiting embodiments, such combinations are used in enhancing hair growth in a patient with the neutrophilic group of cicatricial alopecias (e.g., folliculitis decalvans, tufted folliculitis, and dissecting cellulitis), and successful treatment enhances hair growth while reducing or eliminating microbes that are involved in the inflammatory process.

[00493] In some embodiments, a combination treatment for a patient with the mixed group of cicatricial alopecias (e.g., folliculitis keloidalis) may include antimicrobials, isotretinoin, and anti-inflammatory medications.

[00494] In some embodiments, the combination treatment for enhancing hair growth in a patient with scarring alopecia comprises Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, in combination with a form of integumental perturbation described herein. For example, in some embodiments, an affected area of the skin is transplanted with hair follicles from an unaffected area. In some embodiments, surgical techniques for replacing tissue comprising scarred hair follicles with tissue from another area of the skin (e.g., scalp) comprising unaffected hair follicles are used. Surgical treatment for cosmetic benefit is an option in, for example, some cases after the disease has been inactive for one to two or more years. Hair restoration surgery or scalp reduction may be considered in these instances. Thus, in some embodiments, the Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, is
administered in combination with scar revision, such as by skin graft, serial expansion of surrounding skin, or laser treatment. In some embodiments, the Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound 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).

[00495] In some embodiments, a Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, is administered in combination with a treatment that 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 Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, is administered in combination with a treatment of wounds (e.g., surgical wounds) that minimizes scarring, by, for example, administering physical therapy to a subject, reducing infection, 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.

[00496] In a specific embodiment, the combination treatment for a patient with scarring alopecia comprises controlled integumental perturbation using a fractional ablative laser, followed by twice daily topical administration of Compound for 14 days. In certain embodiments, the Compound treatment is begun on the same day as the laser treatment. In one embodiment, the patient has primary cicatricial alopecia. In a specific embodiment, the patient has lichen planopilaris or frontal fibrosing alopecia.
In other specific embodiments, treatment with Compound is performed using a formulation as described in Section 5.2 above. In certain embodiments, treatment with Compound is commenced on the same day as the integumental perturbation and is continued once, twice, three times, four times, or five times daily for 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, or 21 days.

5.3.7.2 COMBINATION TREATMENTS TO INCREASE HAIR FOLLICLES FOR THE MODULATION OF WOUND HEALING

This invention is based, in part, on the discovery that there is a correlation between the deposition of adnexal structures, such as new hair follicles, in wounded areas and wound healing. Thus, without being bound by any theory for how the invention works, the Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, may promote wound healing, particularly wound healing with reduced scarring, and scar revision by, at least in part, promoting the entry of hair follicles into the wound as it heals, which in turn leads to healing skin with normal adnexal structures and, therefore, less scarring. This may occur by inducing the generation of new hair follicles and/or promoting migration of hair follicles into the wound site.

The Compound treatments, including intermittent and pulse Compound treatments, described herein may (i) promote hair follicle neogenesis (e.g., de novo formation of hair follicles from tissue or bone-marrow derived stem cells or disintegration of preexisting follicles into cells that mix together and reform the hair follicle); and/or (ii) promote branching (e.g., with the assistance of stem cells from dissociated hair follicles) and division of existing hair follicles. Without being bound by any theory, the mechanism by which hair follicles enter the wound site depends on the type of skin that is wounded and the type of wound. For example, in one embodiment, a superficial wound is healed by the assistance of hair follicles remaining in the wound. In another embodiment of a more severe wound, the hair follicles disintegrate and are reorganized and reformed by the presence of stem cells that enter the wound. In another embodiment of severe wounding, such as seen in full thickness excision wounding, hair follicle neogenesis promotes wound healing.

5.3.8 COMBINATION TREATMENTS FOR INHIBITING HAIR GROWTH OR REMOVAL OF UNWANTED HAIR

In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with one or more techniques of depilation (removal of the
part of the hair above the surface of the skin) or epilation (removal of the entire hair, including the part below the skin) of a part of the skin affected by unwanted hair. One or more Compounds can be used in combination with any form of epilation or depilation known in the art. Any treatment that inhibits the growth of unwanted hair, removes unwanted hair and/or treats a disease or condition associated with unwanted hair that is known in the art or yet to be developed is contemplated for use in such combination treatments. For example, see U.S. Patent No. 6,050,990, issued April 18, 2000, which is incorporated herein by reference in its entirety.

In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with one or more prescription drugs such as, e.g., eflomithine (trade name: Vaniqa, which is formulated as eflomithine hydrochloride, 13.9%) or another ornithine decarboxylase inhibitor, and/or 5-fluorouracil (5-FU, Efudex 5% cream). In some embodiments, the one or more Compounds in combination with epidermal growth factor (EGF) or a mimetic thereof.

In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with one or more polyamine derivatives and/or analogs including pharmaceutically acceptable salts and solvates thereof; inhibitors of ornithine decarboxylase, such as difluoromethyl ornithine (DFMO), methylglyoxalbisguanylylhydrazone (MGBG), hydrozino ornithine (HAVA), and mixtures thereof; N-acetyl cysteines (NAC); neutralized salts of a non-hydroxy C₂-C⁴⁰ dicarboxylic acids, preferably malonate salts; and mixtures thereof. See, e.g., International Patent Application Publication No. WO 2005/120451, published December 22, 2005, which is incorporated herein by reference in its entirety.

In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with Stryphnodendron adstringens (Martius) Coville bark extract, which suppresses terminal hair. See Vicente et al., 2009, J Eur Acad Dermatol Venereol 23:410-414. In another embodiment, the combination therapy comprises the use of fennel. See Javidnia et al., 2003, Phytomedicine 10:455-458.

In some embodiments, the combination treatment for hair removal comprises treatment with one or more Compounds in combination with one or more antiandrogen drugs, such as cyproterone acetate, ketoconazole, or spironolactone (the latter marketed under the trade names Aldactone, Novo-Spiroton, Aldactazide, Spiractin, Spirotone, Verospiron or Berlactone). In one embodiment, such therapy is for use in female subjects affected by
idiopathic hirsutism. In some embodiments, such anti-androgen combination therapy is not for use in female subjects predisposed to, at risk for, or suffering from, cancer.

In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with a pro-apoptotic compound or a cytotoxic agent (such as, e.g., those described in U.S. Patent No. 6,050,990, which is incorporated herein by reference). In one embodiment, the pro-apoptotic compound is not a Wnt antagonist.

In some embodiments, the combination treatment for hair removal comprises treatment with one or more Compounds in combination with one or more of hair bleaching, shaving, waxing, sugaring, threading, plucking, use of an abrasive material, laser, electrolysis or electrology, use of an epilation device (e.g., commercially available devices such as Emjoi®, Epilady®, Duet, Legend, and EpiGirl, While Philips Norelco’s Satinelle Epilator, and similar products by Panasonic, Braun, and Norelco), use of friction, exfoliation, burning, intense pulsed light ("IPL"; e.g., Flashlamp or EpiLight), use of a mechanical device, or use of a chemical depilatory such as thioglycolic acid (e.g., Nair®), use of turmeric optionally with other ingredients like besan powder and milk, or enzymes such as the Epiladerm-Complex.

In certain embodiments, treatment with one or more Compounds in combination with the aforementioned methods for hair removal or inhibiting hair growth improves the effectiveness of these methods, making the treatment more effective, efficient, cost-effective, and/or user friendly. For example, fewer treatments may be required. In certain embodiments, one of the previously described hair removal or growth inhibition treatments on its own is not cosmetically satisfactory, the benefits are too short-lived, or the hair that remains or grows back after the treatment is too pigmented, thick or course. When one of these treatments is combined with treatment with one or more Compounds, the results may be more cosmetically satisfactory, longer lasting, and the hair that remains or grows back are fewer in number, thinner (e.g., vellus hair only), less pigmented, and/or shorter. In certain embodiments, a subject previously had one or more hair follicles with numerous hairs emerging from it, and after the combination therapy, only one hair, preferably a vellus hair, emerges from each follicle, leading to the appearance of thinner hair.

In particular embodiments, treatment with one or more Compounds in combination with the aforementioned methods for hair removal or inhibiting hair growth prevents, delays, or reverses terminal hair growth on female axilla, female face, female legs, male ears, male nose, or male back.
In particular embodiments, treatment with one or more Compounds in combination with the aforementioned methods for hair removal or inhibiting hair growth synchronizes hair follicle cells in the Cell Cycle. In one such embodiment, the treatment with one or more Compounds arrests hair follicle cells in G2/M phase, which synchronizes them; the Compound treatment is removed; and then their entry into the cell cycle and mitotic division is stimulated with other drugs that expose their DNA and renders them susceptible to cytotoxic drugs or apoptosis-inducing drugs that cause them to enter catagen and involute. In another embodiment, the treatment with one or more Compounds arrests hair follicle cells in late prophase or metaphase, which synchronizes them; the Compound treatment is removed; and then their entry into the cell cycle and mitotic division is stimulated with other drugs, which exposes their DNA and renders them susceptible to cytotoxic drugs or apoptosis-inducing drugs, causing them to enter catagen and involute. In another embodiment, the treatment with one or more Compounds treatment arrests hair follicle stem cells in G2/M phase, which synchronizes them; the Compound treatment is removed; and then their entry into the cell cycle and mitotic division is stimulated with other drugs which exposes their DNA and renders them susceptible to cytotoxic drugs or apoptosis-inducing drugs, causing the stem cells to enter catagen and involute. In another embodiment, the treatment with one or more Compounds arrests hair follicle stem cells in late prophase or metaphase, which synchronizes them; the Compound treatment is removed and then their entry into the cell cycle and mitotic division is stimulated with other drugs which expose their DNA and renders them susceptible to cytotoxic drugs or apoptosis-inducing drugs, and thus they enter catagen and involute.

In particular embodiments, treatment with one or more Compounds in combination with the aforementioned methods for hair removal or inhibiting hair growth synchronizes hair follicles in the Follicle Cycle. For example, the treatment with one or more Compounds may induce follicles to leave anagen, enter catagen and involute, or enter exogen. The treatment with one or more Compounds may inhibit follicles from entering anagen, or maintain follicles in telogen, or maintain follicles in exogen.

In certain embodiments, one of the aforementioned treatments to inhibit hair growth or remove unwanted hair is administered following integumental perturbation (as described herein) and treatment with one or more Compounds. In one exemplary embodiment, integumental perturbation is followed by treatment with one or more Compounds, which is then followed by one of the aforementioned treatments to inhibit hair growth or remove unwanted hair. In another exemplary embodiment, integumental
perturbation accompanies treatment with one or more Compounds, which is then followed by
one of the aforementioned treatments to inhibit hair growth or remove unwanted hair. In
another embodiment, integumental perturbation is prior to treatment with one or more
Compounds, administered together with one of the aforementioned treatments to inhibit hair
growth or remove unwanted hair.

[00512] In certain embodiments, inhibition of hair growth or removal of unwanted hair is
accomplished by a combination of integumental perturbation, as described herein, and
treatment with one or more Compounds without one of the aforementioned treatments for
inhibition of hair growth or removal of unwanted hair. In some embodiments, the
combination of integumental perturbation and treatment with one or more Compounds of an
area of skin that already contains follicles that do not produce hair or that produce only vellus
hair further reduces hair in that area of skin.

[00513] In certain embodiments, inhibition of hair growth or removal of unwanted hair is
accomplished by treatment with one or more Compounds alone. In some embodiments,
treatment with one or more Compounds of an area of skin that already contains follicles that
do not produce hair or that produce only vellus hair further reduces hair in that area of skin.
These effects may be accomplished by modulating the dosage of Compound(s).

5.3.9 COMBINATION TREATMENTS FOR MODULATION OF
WOUND HEALING

[00514] In some embodiments, a combination treatment comprises the Compound
treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, in
combination with another treatment that modulates wound healing, including any treatment
described herein or known in the art to modulate wound healing.

[00515] In one embodiment, the Compound treatment, such as, e.g., intermittent
Compound treatment or a pulse Compound treatment, is administered in combination with a
treatment that enhances one or more of the steps of wound healing discussed in Section 2.4.1
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.

[00516] In some embodiments, the Compound treatment, such as, e.g., intermittent
Compound treatment or a pulse Compound 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.

In some embodiments, the Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, is administered in combination with a pain reliever, antibiotic and antibacterial 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.

In some embodiments, the Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound 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.

In some embodiments, the Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, is administered in combination with a treatment that reduces the time it takes for a 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 Compound treatments, including intermittent or pulse Compound 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, Bl, 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).

In another embodiment, the Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound 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 Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound 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.

In one embodiment, the Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound 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 Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound 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.

[00522] 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 Compound 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 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 compound (a Compound disclosed herein or other compound) application may prevent the Compound or other compound from being cleared away from the wound site as the wound heals.

5.3.10 COMBINATION TREATMENTS FOR IMPROVEMENT OF SCARS AND SCAR REVISIONS

[00523] In some embodiments, the Compound treatment, such as, e.g., intermittent Compound treatment or a pulse Compound treatment, is administered in combination with a treatment that improves wound healing, in order to reduce the appearance or extent of
scarring. In some embodiments, a Compound treatment, such as, e.g., an intermittent Compound treatment or a pulse Compound 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 Compound treatment, such as, e.g., an intermittent Compound treatment or a pulse Compound treatment, is administered in combination with scar revision, such as by skin graft, serial expansion of surrounding skin, or laser treatment as described in Section 5.3.5 above. In some embodiments, the Compound treatment, such as, e.g., an intermittent Compound treatment or a pulse Compound 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 TGFβ2 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).

[00524] In some embodiments, the Compound treatment, such as, e.g., an intermittent Compound treatment or a pulse Compound treatment, is administered in combination with a treatment that 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 Compound treatment, such as, e.g., an intermittent Compound treatment or a pulse Compound treatment, is administered in combination with a treatment of wounds that minimizes scarring, by, for example, administering physical therapy to a subject (e.g., range-of-motion exercises), reducing infection, 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.
5.3.11 REGIMENS FOR COMBINATION TREATMENTS

[00525] For any of the combination treatments described above, in specific embodiments, the treatment with one or more Compounds can be administered prior to, concurrently with, or subsequent to the administration of a second (or third, or more) treatment.

[00526] In one embodiment, the treatment with one or more Compounds 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.

[00527] In another embodiment, the treatment with one or more Compounds and other treatment are administered at exactly the same time.

[00528] In yet another embodiment, the treatment with one or more Compounds and the other treatment are administered in a sequence and within a time interval such that the treatment with one or more Compounds and the other treatment can act together to provide an increased benefit than if they were administered alone. In another embodiment, the treatment with one or more Compounds 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 treatment with one or more Compounds 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 treatment with one or more Compounds and the other treatment can be administered at the same or different sites of the subject's body. When administered simultaneously, the treatment with one or more Compounds 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.

[00529] In various embodiments, the treatment with one or more Compounds 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 treatment with one or more Compounds and other treatment are administered 2 to 4 days apart, 4 to 6 days apart, 1 week a part, 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 treatment with one or more Compounds 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.

[00530] In one embodiment, the treatment with one or more Compounds and the other treatment are administered within the same patient visit. In one embodiment, the treatment with one or more Compounds is administered prior to the administration of the other treatment. In an alternate embodiment, treatment with one or more Compounds is administered subsequent to the administration of the other treatment.

[00531] In certain embodiments, the treatment with one or more Compounds and the other treatment are cyclically administered to a subject. Cycling treatment involves the administration of the one or more Compounds 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 one or more Compounds 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 one or more Compounds 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 treatment with one or more Compounds 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 one or more Compounds or the other treatment formulation is used, and/or depending on the progress of the treatment course.

[00532] As described in the examples in Sections 6, 17, and 18, infra, in some embodiments, subjects discontinue their current treatment (e.g., topical minoxidil, finasteride, efalornithine), and the one or more Compounds is applied for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the one or more Compounds is discontinued and treatment with the current treatment is re-started. In some embodiments, the subject is treated with 10 cycles of the protocol: alternating topical Compound (e.g., for 1 week) with the other treatment (e.g., for 3 weeks).
For embodiments in which the treatment with one or more Compounds accompanies hair transplantation (e.g., follicular unit extraction), an area of scalp that was pre-treated with one or more Compounds and, e.g., minoxidil or finasteride, is used as a source for transplanted follicles. After hair follicle implantation, treatment with one or more Compounds at the wounds(s) from which transplanted tissue was obtained and/or the site of implantation is initiated for one week, and then discontinued and followed by treatment with, e.g., minoxidil or finasteride for three weeks.

Other regimens for combination treatments for use in the methods described herein include those described in Section 5.3 supra.

5.3.1.1 INTEGUMENTAL PERTURBATION-
COMPOUND-MINOXIDIL TREATMENT

Provided herein is a method to induce hair growth on the scalp. More specifically, provided herein is a method to induce hair growth on the scalp of a male or female subject with androgenetic alopecia. Even more specifically, provided herein is a method to induce hair growth on the scalp of a male or female subject having androgenetic alopecia with the presence of a vertex transition zone defined as an area possessing both terminal and miniaturized hairs, Hamilton-Norwood type 3V, 4, 5, 5A, or 5V, and Fitzpatrick skin type 1-4. In certain embodiments of the invention, the method for inducing hair growth comprises: (a) integumental perturbation; (b) a period of treatment with a GSK-3 inhibitor as disclosed herein (see, e.g., Section 5.1); and (c) a period of treatment with minoxidil. More specifically, the method for inducing hair growth comprises in the following order: (a) integumental perturbation; (b) a period of treatment with a GSK-3 inhibitor; (c) a period without treatment with a GSK-3 inhibitor and without treatment with minoxidil; and (d) a period of treatment with minoxidil. Without being bound by theory, in areas of hair loss in male or female subjects with androgenetic alopecia, controlled cutaneous perturbation using dermabrasion plus the topical application of a GSK-3 inhibitor results in follicular neogenesis, and the subsequent treatment of the induced neofollicles with minoxidil results in more numerous and/or thicker hair shafts.

Integumental perturbation can be performed by mechanical means, chemical means, or electromagnetic means (see, e.g., Section 5.3.5). In a specific embodiment, dermabrasion is used as a means of integumental perturbation. Even more specifically, dermabrasion can be carried out using a hand-held dermabrader with a standard grit diamond
fraise to achieve pinpoint capillary bleeding (estimated depth 100 microns, not anticipated to cause scarring).

[00537] Treatment with a GSK-3 inhibitor can be performed using a formulation of a GSK-3 inhibitor as described in, e.g., Section 5.2.1. Modes of administration of the GSK-3 inhibitors are set forth in Section 5.2.2 above. In a specific embodiment, treatment with a GSK-3 inhibitor is commenced on the same day as the integumental perturbation and is continued once, twice, three times, four times, or five times daily for 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, or 21 days.

[00538] In certain embodiments, treatment with a GSK-3 inhibitor can be combined with another treatment as described in Section 5.3.4.

[00539] In some embodiments, treatment with a GSK-3 inhibitor is followed by a period of 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 without topical treatment (i.e., without treatment with a GSK-3 inhibitor and without treatment with minoxidil).

[00540] In some embodiments, subsequent to the period without topical treatment, treatment with minoxidil is commenced wherein minoxidil is applied once, twice, three times, four times, or five times daily for at least 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 month, 7 months, 8 months, 9 months or at least 10 months. In specific embodiments, 1%, 2%, 3%, 4%, 5%, 6%, 7%, or 8% minoxidil foam can be used (e.g., ROGAINE).

[00541] In certain embodiments, the method for inducing hair growth further comprises administration of finasteride (PROPECIA). For example, finasteride can be administered orally at a dose of 1 mg/day. In certain embodiments, administration of finasteride commences concurrently with the topical treatment with minoxidil.

[00542] In certain embodiments, the method for inducing hair growth further comprises administration of dutasteride or any other 5-alpha-reductase inhibitor known to the skilled artisan. For example, dutasteride can be administered orally at a dose of 1 mg/day. In certain embodiments, administration of dutasteride commences concurrently with the topical treatment with minoxidil.

[00543] In certain embodiments, a method for inducing hair growth on the scalp of a male or female subject with androgenetic alopecia comprises:

[00544] (a) Dermabrasion (estimated depth 100 microns) at Day 0;

[00545] (b) Topical administration of a GSK-3 inhibitor twice daily from Day 0 to Day 6;
(c) Period without topical treatment from Day 7 to Day 27;
(d) Topical administration of 5% minoxidil foam commencing at Day 28 for at least 5 months.

In certain embodiments, a method for inducing hair growth on the scalp of a male or female subject with androgenetic alopecia comprises:

(a) Dermabrasion (estimated depth 100 microns) at Day 0;
(b) Commencing at Day 0, topical administration of a GSK-3 inhibitor twice daily for about 7 days;
(c) Period without topical treatment for about 21 days;
(d) Immediately following step (c), topical administration of 5% minoxidil foam for at least 5 months.

In certain embodiments, the present invention provides a kit comprising in separate containers a GSK-3 inhibitor and minoxidil foam. The GSK-3 inhibitor can be a Compound as disclosed herein. The minoxidil foam can be 1%, 2%, 3%, 4%, 5%, 6%, 7%, or 8% minoxidil foam (e.g., ROGAINE). In certain embodiments, the kit further comprises means for integumental perturbation (e.g., a handheld dermabrasion device).

Any method known to the skilled artisan can be used to demonstrate that hair growth has been induced. For example, detectable hairs can be quantified by photography. Further, changes in the hair shaft thickness of photographically detectable hairs can be determined. Further, the permanence of the hair growth is monitored over a time period of at least 3 months, 6 months, 9 months, 1 year, 1.5 years, 2 years, 2.5 years, 3 years, 4 years, or at least, 5 years.

5.4 INDICATIONS AND TREATMENT REGIMENS FOR ALOPECIA

Human hair patterning consists of gender specific changes that occur over the life of subjects and vary in degree between individuals and more generally between humans of different racial and ethnic backgrounds. Before puberty, males and females have similar patterns of scalp hair and the rest of their bodies are covered with largely invisible vellus hair. The forearms and legs grow thin, fine terminal hair gradually even before puberty. In males and females puberty is associated with terminal hair growth in the axilla, and anogenital regions. During puberty, both males and females grow terminal hair over forearms and legs, but males have quantitatively more growth in these regions. Males after puberty grow terminal hair over the moustache/beard, chest, and back regions. Later, males manifest varying degrees of loss of terminal hair on the scalp (vertex/corona and frontal/
temporal/parietal regions) in a process called "MPHL alopecia." Both males and females with genetic susceptibilities manifest diffuse scalp terminal hair thinning that is called "androgenetic alopecia." Males manifest various degrees of terminal hair growth on ears, in the nose (nares) and of eyebrows. After menopause, females manifest moustache/beard hair growth. Both males and females manifest diffuse scalp hair thinning with age. Both females and males with genetic susceptibility manifest hair color changes with age.

00556 The sex hormones, androgens and estrogens, play important roles in much of human hair patterning. A high ratio of androgen to estrogen activity drives both the process of transforming vellus hair to terminal hair (in puberty, e.g. axilla and anogenital regions; male moustache and beard) and in transforming terminal hair (and large terminal follicles) to vellus hair (and miniaturized follicles) in MPHL alopecia. Sex hormones are synthesized by the skin locally where they exert intracrine or paracrine actions. (Reviewed in, Zouboulis CC, Chen WC, Thornton MJ, Qin K, Rosenfield, 2007, "Sexual hormones in human skin" Horm Metab Res 39:85-95) The local level and activity of each sex steroid depends upon the activity of androgen- and estrogen-synthesizing enzymes and of appropriate androgen- and estrogen receptors in specific cell types. Androgens are regulated by five major enzymes and changes in the expression of isoenzymes; or changes in the expression of androgen receptor and androgen receptor transactivating factors regulate hair patterning. Estrogens are produced in sebaceous glands which express cytochrome P450 aromatase ("aromatase") that converts androgen to 17-beta-estradiol (E2). Estrogens act on Estrogen Receptor alpha (ERalpha) and Estrogen Receptor alpha (ERbeta) in human skin are expressed in site specific localizations. In addition to regulating hair patterning and growth, androgens have effects in sebaceous gland growth and differentiation, epidermal barrier homeostasis and wound healing; and estrogens regulate skin aging, pigmentation, hair growth, sebum production and skin cancer. (Ohnemus et al., 2006, Endocr Rev. 27(6):677-706, "The hair follicle as an estrogen target and source.")

00557 The location of specialized human hair follicles allow us to introduce a classification (Table 1 and Figure 1) of those follicles that produce terminal hair which highlights the distinctive features including type of hair produced (length, curl); appearance/disappearance during life and regulation by sex steroids. This classification makes several assumptions in grouping together as similar types certain male and female follicles that may not be justified by future study. For example, except for scalp hair, humans have only small amounts of visible hair until puberty, when specialized hair follicles in the pubic (anogenital) and armpit (axillary) regions begin producing terminal hair. Some believe
that pubic and axillary specialized follicles change their activity in response to only androgens in both males and females and that the hair follicles in the female pubic and axillary regions are extremely sensitive to androgens. The classification system is useful to show the variety and unique characteristics of specialized hair follicles in humans that can be modified using the methods described herein.

**Table 1. Types of Human Hair Follicles Producing Terminal Hair**

- Pubic (anogenital) - puberty driven, not androgen or estrogen specific, curly intermediate length
- Armpit (axillary) - puberty driven, not androgen or estrogen specific, curly intermediate length
- Scalp - throughout life, thins with aging
- Vertex - induced by androgens in males to miniaturize and involute
- Crown- induced by androgens in genetically susceptible males to miniaturize and involute
- Temples- induced by androgens in genetically susceptible males to miniaturize and involute
- Beard/Moustache- induced by androgens to at puberty, suppressed by estrogens, curly intermediate length
- Chest/back- induced by androgens to at puberty, suppressed by estrogens, short length
- Eyebrow - throughout life, thickens/grows with aging in men, straight short length
- Nose surface - on the surface of the nose; thickens/grows with aging in males, short length
- Nose/nostrils (nares) - in the lumen; increases at puberty, thickens/grows with aging in males, short length
- Ear (auricle) - appears with aging in males, short length
- Leg- induced by androgens in males at puberty, suppressed by estrogens but not as completely as beard, short length
- Arm- induced by androgens in males & females at puberty, suppressed by estrogens but not as completely as beard, short length
- Eyelid/eyelashes - throughout life, single units
5.4.1 PATIENT POPULATIONS FOR ALOPECIA AND HAIR GROWTH

[00558] A candidate subject for treatment with one or more Compounds for promoting hair growth is any subject suffering from hair loss, hair thinning, balding, or who has or has had a disease or condition associated therewith, or who wishes to enhance the growth or thickness of hair.

[00559] The subject may be any subject, preferably a human subject, including male, female, intermediate/ambiguous (e.g., XO), and transsexual subjects. In certain embodiments, the subject is a human adolescent. In certain embodiments, the subject is undergoing puberty. In certain embodiments, 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 elderly. In certain embodiments, the subject is 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 20 to 60 years old. In some embodiments, the subject is a male 30 to 60 years old. In some embodiments, the subject is a male 40 to 60 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.

[00560] In one embodiment, the treatment with one or more Compounds is delivered to an area in which hair growth is desired, for example, the scalp or face (e.g., the eyebrow, eyelashes, upper lip, lower lip, chin, cheeks, beard area, or mustache area) or another part of the body, such as, e.g., the chest, abdomen, arms, armpits (site of axillary hair), legs, or genitals. In some embodiments, hair restoration to a wounded or scarred part of the skin is desired. In one embodiment, the scar is caused by surgery, such as a face lift, skin graft, or hair transplant.
The subject may have a disease or disorder of balding or hair loss (including hair thinning), such as forms of non-scarring (non-cicatricial) alopecia, such as androgenetic alopecia (AGA), including MPHL or FPHL (e.g., thinning of the hair, i.e., diffuse hair loss in the frontal/parietal scalp), or any other form of hair loss caused by androgens, toxic alopecia, alopecia areata (including alopecia universalis), scarring (cicatricial) alopecia, pathologic alopecia (caused by, e.g., medication, trauma stress, autoimmune diseases, malnutrition, or endocrine dysfunction), trichotillomania, a form of hypotrichosis, such as congenital hypotrichosis, or lichen planopilaris, or any other condition of hair loss or balding known in the art or described infra.

In some embodiments, the subject has hair loss caused by a genetic or hereditary disease or disorder, such as androgenetic alopecia.

In some embodiments, the subject has hair loss caused by anagen effluvium, such as occurs during chemotherapy (with, e.g., 5-fluorouracil, methotrexate, cyclophosphamide, vincristine). In addition to chemotherapy drugs, Anagen effluvium can be caused by other toxins, radiation exposure (including radiation overdose), endocrine diseases, trauma, pressure, and certain diseases, such as alopecia areata (an autoimmune disease that attacks anagen follicles.)

Telogen effluvium is caused frequently by drugs like lithium and other drugs like valproic acid and carbamazepine. In addition to psychiatric drugs, telogen effluvium can be induced by childbirth, traction, febrile illnesses, surgery, stress, or poor nutrition. (See, Mercke et al., 2000, Ann. Clin. Psych. 12:35-42).

In some embodiments, the subject has hair loss caused by or associated with medication, such as chemotherapy (e.g., anti-cancer therapy or cytotoxic drugs), thallium compounds, vitamins (e.g., vitamin A), retinoids, anti-viral therapy, or psychological therapy, radiation (such as the banding pattern of scalp hair loss that may be caused by radiation overdose), trauma, endocrine dysfunction, surgery, physical trauma, x-ray atrophy, burning or other injury or wound, stress, aging, an autoimmune disease or disorder, malnutrition, an infection (such as, e.g., a fungal, viral, or bacterial infection, including chronic deep bacterial or fungal infections), dermatitis, psoriasis, 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 disease or disorder associated with or that
causes balding or hair loss known in the art or described infra.

[00566] In some embodiments, the subject has hair thinning, or "shock loss," or a bald
patch caused by prior use as a source of tissue or follicles for hair transplantation or follicular
unit transplantation.

[00567] In some embodiments, a candidate subject is a subject who wishes to enhance hair
growth, for example, to have more hair, faster-growing hair, longer hair, and/or thicker hair.
In some embodiments, the candidate is a subject who wishes to increase hair pigmentation.
In some embodiments, the subject is not affected by a condition of excessive hair loss.

5.4.2 SCARRING ALOPECIA

[00568] In some embodiments, the subject has scarring (cicatricial) alopecia. Forms of
cicatricial alopecia that may be treated in accordance with the methods described herein
include primary cicatricial alopecia (PCA) and secondary cicatricial alopecia. Primary
cicatricial alopecias that may be treated in accordance with the methods described herein
include lymphocyte-mediated PCAs, such as lichen planopilaris (LPP), frontal fibrosing
alopecia (FFA), central centrifugal cicatricial alopecia (CCCA), and pseudopelade (Brocq);
neutrophil-mediated PCAs, such as folliculitis decalvans and tufted folliculitis; and PCAs
involving a mixed inflammatory infiltrate, such as occurs in dissecting cellulitis and
folliculitis keloidalis.

[00569] In some embodiments, in a candidate subject for Compound treatment (e.g., with
intermittent or pulse Compound treatment) for scarring alopecia, the area affected by the
scarring alopecia is no longer increasing. In some embodiments, in a candidate subject for
Compound treatment (e.g., with intermittent or pulse Compound treatment) for scarring
alopecia, hair loss has in the affected area has ceased. In some embodiments, a candidate
subject for Compound treatment (e.g., with intermittent or pulse Compound treatment) for
scarring alopecia is clinically quiescent with respect to the inflammatory activity that may be
associated with the condition. In one embodiment with respect to a subject having a
lymphocyte-mediated PCA, inflammation is measured as the number of T lymphocytes
and/or T lymphocyte subsets as detected in lesional skin, e.g., by immunoperoxidase cell
surface staining using monoclonal antibodies. In another embodiment with respect to a
subject having a lymphocyte-mediated PCA, lymphocytic inflammation (which may be found
along with necrotic keratinocytes) is detected by histologic examination of the scalp. In
another embodiment, direct immunofluorescence staining techniques are employed to detect
antibody deposits in the affected tissue. In certain embodiments, clinical evaluation of the scalp is performed to determine clinical quiescence of the inflammation. Symptoms of itching, burning, pain, or tenderness usually signal ongoing activity. Signs of scalp inflammation include redness, scaling, and pustules. In certain embodiments, a scalp biopsy can be performed to demonstrate active inflammation or its absence. In certain embodiments, a hair "pull test" is performed to identify areas of active disease in which follicles are easily pulled out, and thus, inflammation is still ongoing. The pulled hairs can be mounted on a slide and the hair bulbs are viewed with a microscope to determine how many are growing hairs and how many are resting hairs. In addition, if pustules are present, cultures may be performed to identify which microbes, if any, may be contributing to the inflammation. In certain embodiments, a subject is clinically quiescent if hairs cannot be easily pulled out, if itching, burning, pain, tenderness, redness, scaling, and / or pustules are absent from the affected area.

[00570] In some embodiments, a method described herein is used to enhance hair growth in a patient with scarring alopecia. In some embodiments, the patient has a secondary cicatricial alopecia. In some embodiments, the patient has a form of primary cicatricial alopecia, such as lymphocyte-mediated PCAs, such as lichen planopilaris (LPP), frontal fibrosing alopecia (FFA), central centrifugal cicatricial alopecia (CCCA), and pseudopelade (Brocq); neutrophil-mediated PCAs, such as folliculitis decalvans and tufted folliculitis; and PCAs involving a mixed inflammatory infiltrate, such as occurs in dissecting cellulitis and folliculitis keloidalis.

[00571] Cicatricial alopecias affect both men and women, most commonly adults, although all ages may be affected. In general, they are rare. There have been a few reports of cicatricial alopecia occurring in a family. However, the majority of patients with cicatricial alopecia have no family history of a similar condition. Lichen planopilaris may affect middle-aged women most commonly. Central centrifugal alopecia may affect black women most commonly. Frontal fibrosing alopecia is seen most commonly in post-menopausal women. Thus, in certain embodiments, in addition to the subjects described in Section 5.4.1 supra, a candidate subject for Compound treatment (e.g., with intermittent or pulse Compound treatment) for scarring alopecia is a black woman (e.g., of African-American descent), a middle-aged woman, or a post-menopausal woman.

[00572] In some embodiments, the invention provides a method for enhancing hair growth in a patient with scarring alopecia comprising controlled integumental perturbation using a fractional ablative laser, followed by twice daily topical administration of Compound for 14
days. In certain embodiments, the Compound treatment is begun on the same day as the laser treatment. In certain embodiments, treatment with Compound is commenced on the same day as the integumental perturbation and is continued once, twice, three times, four times, or five times daily for 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, or 21 days.

[00573] In a specific embodiment, the invention provides a method for enhancing hair growth in a patient with lichen planopilaris comprising controlled integumental perturbation using a fractional ablative laser, followed by twice daily topical administration of Compound for 14 days. In certain embodiments, the Compound treatment is begun on the same day as the laser treatment. In certain embodiments, treatment with Compound is commenced on the same day as the integumental perturbation and is continued once, twice, three times, four times, or five times daily for 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, or 21 days.

[00574] In another specific embodiment, the invention provides a method for enhancing hair growth in a patient with frontal fibrosing alopecia comprising controlled integumental perturbation using a fractional ablative laser, followed by twice daily topical administration of Compound for 14 days. In certain embodiments, the Compound treatment is begun on the same day as the laser treatment. In certain embodiments, treatment with Compound is commenced on the same day as the integumental perturbation and is continued once, twice, three times, four times, or five times daily for 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, or 21 days.

5.4.3 ANDROGENETIC ALOPECIA

[00575] Both males and females develop diffuse hair loss in the frontal/parietal scalp called "thinning," which begins between 12 and 40 years of age. Perhaps more than males, females notice (and complain of) diffuse hair thinning progressively in middle age more than males, perhaps because diffuse alopecia is more noticeable and problematic for females because they do not suffer from MPHL and retain the frontal hairline. In females, thinning is known as "Female Pattern Hair Loss (FPHL)" and is caused or exacerbated by androgens. (Price VH, 2003, J Investig Dermatol Symp Proc. 8(I):24-7, Androgenetic alopecia in women).
Minoxidil is FDA approved for both males and females. Kopexil is a product similar to minoxidil, but with fewer side effects and which has been proposed for use in treating hair loss in female patients. Finasteride, while not approved for females, does provide a benefit. See, PDR entry for Propecia, which is incorporated herein by reference. One or more Compounds can be used in combination with minoxidil or other channel openers, finasteride, dutasteride, flutamide, or other antiandrogens, laser therapy or other mode of photo-stimulation of hair follicles, dermabrasion, hair transplantation or other surgical treatment for treatment, or any other treatment provided in Section 5.3 supra. These treatments can be administered during the Compound treatment "holidays." Alternatively, these treatments can be administered prior to or subsequent to treatment with one or more Compounds.

5.4.4 MALE PATTERN HAIR LOSS (MPHL)

After puberty, males begin to lose the scalp hair over the vertex, crown and frontal/parietal areas in a relatively characteristic pattern that is a continuum (described by Hamilton Norwood scale). The loss of scalp hair in men is called MPHL and is known to be a process driven by the androgen, dihydrotestosterone (DHT), which can be inhibited and to some extent reversed by finasteride which inhibits the conversion of testosterone to DHT. Minoxidil can also delay or reverse MPHL.

Minoxidil and finasteride treatment are more effective at delaying the progression of MPHL than in reversing it. These agents are generally not effective a year or more after hair loss has occurred, consistent with some kind of terminal senescence or involution of the follicle (as will be discussed below). These treatments can be administered during the Compound treatment "holidays." Alternatively, these treatments can be administered prior to or subsequent to treatment with one or more Compounds.

Most drugs for hair loss aim to retain the existing hair follicles in their active cycling states, or to rejuvenate telogen hair follicles to actively cycling anagen states. However, a treatment that encourages the growth of "new" hair follicles, combined with one that retains the hair follicles in their actively cycling states offer significant value to the individual who is balding. Moreover, since treatment with one or more Compounds synchronizes the hair/Follicle Cycle, a treatment intended to enhance hair growth or remove hair will be more effective, efficient, cost-effective, and user friendly. For example, fewer treatments may be required. The hair that results may be more cosmetically satisfactory, longer lasting, more terminal hair and/or thicker, more uniform, and properly pigmented hair.
5.4.5 AGING

[00580] Aging of humans results in programmed hair patterning. Diffuse hair loss, including thinning of the occipital scalp occurs in aging.

[00581] It is believed that hair loss in postmenopausal women is related to the loss of estrogens (and/or a decrease in the estrogen/androgen ratio). Accordingly, in some embodiments, the combination treatments disclosed herein for age-related hair loss comprise a combination of treatment with one or more Compounds and estrogen replacement therapy or androgen inhibition therapy.

[00582] Aging also results in change of follicle cycle control. In males, eyebrows grow longer and nares hair grow longer suggesting that the lengths of telogen and anagen are no longer regulated as closely. In other words, with aging there is a loss of the function of suppressing terminal hair growth. We propose that these changes are due to repopulation of follicles with new follicles that have been seeded by follicle stem cells (FSCs) and educated by nurse cells. Over time, however, an accumulation of errors in nurse cell education occurs, and the number of stem cells derived from bone marrow decreases. While nurse cells also educate tissue stem cells, they are essential for BMSC. However, the timing of follicle cycle programming has not been transmitted with fidelity, so the lengths are not typical of the original follicles.

[00583] Treatment with one or more Compounds may be used in combination with any of the treatments described in Section 5.3 in order to treat age-related hair loss. These treatments can be administered during the Compound treatment "holidays." Alternatively, these treatments can be administered prior to or subsequent to treatment with one or more Compounds.

5.4.6 HAIR COLOR CHANGES

[00584] Hair color changes in both males and females becoming progressively grayer (mixture of gray hair; white hair and black hair) and whiter. Color change is patterned, since scalp hair changes earlier than body beard hair or body hair. Beard hair may also change color in a pattern that follow a moustache line, before ultimately turning uniformly gray (typically a mixture of white and black hair). This is due to decreased melanin content in the hair shaft (supplied by melanocytes associated with hair follicles).

[00585] Using the treatments described herein can result in rejuvenated follicles that do not follow the hair pattern types and recruit/attract melanocytes and as a result produce
colored hair. These treatments can be administered during the Compound treatment "holidays." Alternatively, these treatments can be administered prior to or subsequent to treatment with one or more Compounds.

5.4.7 FACTORS THAT REGULATE SEX HORMONE SENSITIVITY OF HAIR FOLLICLE CELLS

[00586] Cytokines regulate the activity of Dermal Papillae, which is believed to be the target of androgen regulation of hair growth. Interleukin-1 alpha decreases responses to androgen in cultured dermal papilla cells (Boivin et al., 2006, Exp Dermatol. 15:784-793). TGF-betal may mediate androgen-induced hair growth suppression, since in culture, human dermal papilla cells (DPCs) from androgenetic alopecia (AGA) subjects that transiently expressing androgen receptor were co-cultured with keratinocytes (KCs), and secreted TGF-betal that inhibited KC growth (Inui et al., 2003, J Investig Dermatol Symp Proc. 8:69-71).

[00587] In certain embodiments, adjuvants and/or other stimulators of local cytokines are used in conjunction with the treatment with one or more Compounds. Without being bound by any theory, one rationale for administering adjuvants and/or other stimulators of local cytokines in conjunction with the treatment with one or more Compounds is that the production of local cytokines may induce changes in the follicle cell cycle and recruit new FSCs to follicles.

[00588] Melatonin is a protein hormone secreted by the pineal gland modulates hair growth, pigmentation and/or molting in many species. Human scalp hair follicles in anagen are important sites of extra-pineal melatonin synthesis. Melatonin may also regulate hair Follicle Cycle control, since it inhibits estrogen receptor-alpha expression (Fischer et al., 2008, Pineal Res. 44:1-15). These treatments can be administered during the Compound treatment "holidays". Alternatively, these treatments can be administered prior to or subsequent to treatment with one or more Compounds.

5.4.8 TREATMENTS FOR DELAYING OR REVERSING HUMAN HAIR PATTERNING

[00589] Given the regulation of human hair patterning by sex steroids, it is believed that humans evolved hair patterning to provide social signals in interactions such as mating and dominance. However, current fashion motivates many men to prevent, delay or reverse male MPHL. Minoxidil (an antihypertensive drug that opens the K+ channel) and antiandrogens, such as finasteride, dutasteride or ketoconazole, are reasonably effective in stimulating the growth of vellus and miniaturized hair in certain MPHL conditions. Kopexil (e.g.,
Keranique), is a modified form of minoxidil that has been proposed to have fewer side effects, and therefore has been proposed for treatment of hair loss in females. Finasteride is not approved for use in females. However, patient dissatisfaction with statistically significant, but cosmetically insignificant increase in hair counts contribute to poor compliance and unsatisfactory outcomes. Minoxidil use is further complicated by the fact that it is messy, leaves a residue, and requires daily application. In addition, the side effects from persistent finasteride or minoxidil treatment - such as sexual dysfunction - are another reason why subjects may benefit from a reduced treatment duration or treatment at a lower dose as may be enabled by the combination treatments described herein. Importantly, both minoxidil and finasteride are effective only for as long as it is taken; the hair gained or maintained is lost within 6-12 months of ceasing therapy. See, e.g., Rossi, ed., 2004, Australian Medicines Handbook. Adelaide: Australian Medicines Handbook. Even when effective, these drugs do no create hair follicles of the kind that were there before balding, and the resultant hair follicles are smaller and less dense.

[00590] Women also suffer from hair thinning and hair loss due to a variety of factors; for example, certain conditions, such as, e.g., polycystic ovary, result in male-pattern facial and body hair on females, which motivates them to remove or reduce hair. Many women also desire the prevention, delay or reversal of "female-pattern baldness," which may result from a variety of factors, for example, the aging process.

[00591] Similarly, current fashion motivates many women to remove facial, axillary and leg hair ("unwanted hair") by shaving, laser, depilation, plucking, wax, electrolysis, and medications such as Vaniqa (eflomithine hydrochloride 13.9%) (as well as others). Competitive advantages motivate swimmers, body builders, and certain other athletes to remove body hair, and current fashion motivates many men to reduce or eliminate back hair and hair from other parts of the body such as nose hair, ear hair, and eyebrow hair.

[00592] These treatments can be administered during the Compound treatment "holidays." Alternatively, these treatments can be administered prior to or subsequent to treatment with one or more Compounds.

[00593] Androgen receptor inhibitors are also useful for stimulating scalp hair growth and inhibiting beard and moustache hair (Hu LY, et al., 2007, Bioorg Med Chem Lett. 2007 17:5983-5988). These treatments can be administered during the Compound treatment "holidays." Alternatively, these treatments can be administered prior to or subsequent to treatment with one or more Compounds.
5.5 INDICATIONS AND TREATMENT REGIMENS FOR UNWANTED HAIR

[00594] A candidate subject for treatment with one or more Compounds (e.g., an intermittent treatment, that is, alternating treatment with one or more Compounds with "vacation/holiday" periods) for inhibiting hair growth or removing hair is any subject who has or has had a condition, disease or disorder associated with excess hair or unwanted hair, or who wishes to inhibit the growth of hair or remove hair. In some embodiments, permanent hair removal is desired.

[00595] The subject may be any subject, preferably a human subject, including male, female, and transsexual subjects. In certain embodiments, the subject is a human adolescent. In certain embodiments, the subject is undergoing puberty. In certain embodiments, 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 elderly. In certain embodiments, the subject is 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 not a female subject. In some embodiments, the subject is not pregnant or expecting to become pregnant. In some embodiments, the subject is not breastfeeding.

[00596] In some embodiments, the one or more Compounds is delivered to an area of unwanted hair, for example, the head (e.g., the eyebrow, nose and nares, upper lip, lower lip, chin, cheeks, ears, or forehead) or another part of the body, such as, e.g., the chest, breast (e.g., the nipples), abdomen, neck, back, arms, armpits (site of axillary hair), legs, hands, feet, buttocks, or genitals. In some embodiments, hair removal from a wounded or scarred area of the skin is desired. In some embodiments, hair removal from darkly pigmented skin or a darkly pigmented area of the skin, such as a mole, freckle, or the genital area, is desired.

[00597] In particular embodiments, subjects who are candidates for treatment with one or more Compounds include those afflicted with hypertrichosis (excess hair not localized to the androgen-dependent areas of the skin), including generalized congenital hypertrichosis (congenital hypertrichosis lanuginosa), acquired generalized hypertrichosis (acquired
hypertrichosis lanuginosa), patterned acquired hypertrichosis, localized congenital hypertrichosis, localized acquired hypertrichosis, paradoxical hypertrichosis, and "werewolf syndrome," pili multigemini, excess hair in androgen-dependent areas of the skin, idiopathic hirsutism, post-menopausal facial hair, axillary hair, back hair, ear hair, or any other disease, disorder, or form of unwanted hair or excessive hair as discussed infra and/or known in the art.

[00598] In some embodiments, the subject has excess hair caused by a genetic or hereditary disease or disorder. In some embodiments, the subject has excess hair growth caused by or associated with medication, such as chemotherapy (e.g., anti-cancer therapy, anti-viral therapy, psychological therapy), steroid therapy (e.g., systemic androgenic steroid therapy or corticosteroid therapy), antihypertensive drugs (e.g., minoxidil), or cyclosporine, radiation, trauma, endocrine dysfunction (such as, e.g., adrenal virilism, basophilic adenoma of the pituitary, masculinizing ovarian tumors, Stein-Leventhal syndrome), porphyria cutanea tarda, surgery, burning or other wound, stress, aging, an autoimmune disease or disorder, malnutrition (e.g., lanugo), an infection (such as, e.g., a fungal, viral, or bacterial infection), dermatitis, psoriasis, eczema, pregnancy, menopause, allergy, or any other form of excessive hair growth known in the art. Any other disease or disorder associated with unwanted hair or excessive hair known in the art is also contemplated.

[00599] In some embodiments, a candidate subject is any subject who wishes to remove or prevent excess hair or unwanted hair, or who wishes to inhibit the growth of hair or remove hair, for example, to have less hair, slower-growing hair, shorter hair, and/or thinner hair. In some embodiments, the candidate is a subject who wishes to decrease hair pigmentation. In some embodiments, the subject is not affected by a condition or disease or disorder associated with excessive hair.

5.5.1 REGIONS OF INCREASED TERMINAL HAIR GROWTH IN ADULT MALES

[00600] In addition to MPHIL and post-puberty male pattern body hair growth, males also manifest varying degrees of growth of new or increased terminal hair in middle age (over 35 years). Males begin to grow terminal hair on the ears, particularly around the auricular canal; and have increased hair growth (length and density) in the nares, and increased hair growth of eyebrows (hair length and curl.) In some cases, males develop terminal hairs on the skin covering the central prominence of the nose.
The mechanisms of these transformations have not been studied carefully, but appear to be androgen driven since they are uncommon in females and more prominent in certain races/ethnic backgrounds (for example Scottish and Welsh men). However, some females experience the growth of ear hair as a side-effect of topical minoxidil treatment.

Any combination treatment described in Section 5.3 may be used to reduce unwanted terminal hair in adult males. In some embodiments, the combination treatment comprises treatment with one or more Compounds in combination with a drug such as, e.g., eflornithine (trade name: Vaniqa, which is formulated as eflornithine hydrochloride, 13.9%) or 5-fluorouracil (e.g., 5-FU, Efudex 5% cream). In some embodiments, the combination treatment for hair removal comprises treatment with one or more Compounds in combination with one or more antiandrogen drugs. A combination treatment for hair removal may also comprise treatment with one or more Compounds with one or more of hair bleaching, shaving, waxing, sugaring, threading, plucking, use of an abrasive material, laser, electrolysis or electrology, use of an epilation device, use of friction, exfoliation, burning, intense pulsed light ("IPL"; e.g., Flashlamp or EpiLight), use of a mechanical device (e.g., epilators such as Epilady, Emjoi, etc.), or use of a chemical depilatory (e.g., Nair ®).

These treatments for use in combination with one or more Compounds can be administered during the Compound treatment "holidays." Alternatively, these treatments can be administered prior to or subsequent to treatment with one or more Compounds.

5.5.2 REGIONS OF INCREASED TERMINAL
HAIR GROWTH IN ADULT FEMALES

After menopause, females frequently experience increased hair growth to varying degrees on the face in the moustache/beard pattern. In females generally, estrogens suppress moustache/beard hair. In addition, women treated with androgens or with certain medical conditions retain the potential to develop beard and moustache hair. Estrogens (particularly estrogen-progesterone oral birth control pills) are believed to inhibit hair growth primarily by suppressing ovarian androgen production, however action on skin estrogen receptors may also play a role in these activities. Cyproterone acetate and spironolactone and other antiandrogens (e.g., ketoconazole) are effective as anti-androgens in reducing unwanted hair on females (e.g., idiopathic hirsutism), although there is wide variability in individual responses.

Vaniqa (eflornithine hydrochloride 13.9%) and other ornithine decarboxylase inhibitors, polyamine derivatives, as well as 5-fluorouracil (5-FU, Efudex 5% cream) and certain antiandrogens inhibit hair growth. These drugs (described in Section 5.3 supra) and
other drugs described in Section 5.3 can be used with, or as an adjunct to laser hair removal
or to electrolysis, depilatory creams, plucking and waxing. Other products that suppress
terminal hair have been described: (a) Vicente RA, et al, 2009, J Eur Acad Dermatol
Venereol. 23(4):410-4, "Double-blind, randomized, placebo-controlled trial of a cream
containing the Stryphnodendron adstringens (Martius) Coville bark extract for suppressing
terminal hair growth"; (b) Javidnia et al., 2003, Phytomedicine 10(6-7):455-8.
"Antihirsutism activity of Fennel (fruits of Foeniculum vulgare) extract. A double-blind
placebo controlled study." These treatments can be administered during the Compound
treatment "holidays." Alternatively, these treatments can be administered prior to or
subsequent to treatment with one or more Compounds.

5.6 PATIENT POPULATIONS AND INDICATIONS
FOR WOUND HEALING AND SCAR REVISION

[00606] A candidate subject for a Compound treatment (e.g., intermittent treatment, that is,
alternating Compound treatment with "vacation/holiday" periods or a pulse treatment)
described herein is any subject at risk for, has, or has had a wound or scar.

[00607] The subject may be any subject, 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 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 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.

[00608] In one embodiment, the Compound treatment (e.g., intermittent or pulse Compound treatment) is delivered to an area 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.

[00609] 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), 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, and surgery, such as described in Multiplicity & 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.

[00610] 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.

[00611] In some embodiments, the methods described herein are used to treat a radiation scar, acne scar, cutenage 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, as exemplified in the examples of Section 7.

[00612] In some embodiments, the 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 ai, 2009, Dermatol. Surg. 35:1873-
1879, which is incorporated herein by reference in its entirety. Without being bound by any
treatment, Compound treatment may benefit this process by facilitating the "recipient
dominance" phase (that temporally follows "donor dominance"). It is postulated that
Compound treatments, e.g., pulse or intermittent Compound treatments, 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).

[00613] 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.
In a particular embodiment, the subject for whom Compound treatment, e.g., pulse or intermittent Compound treatment, 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 moderated 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 - 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

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 or radiation overdose), 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 infra.

5.7 METHODS FOR EVALUATING EFFICACY OF TREATMENT

5.7.1 TOXICITY AND EFFICACY ASSAYS

[00617] The toxicity and/or efficacy of the compositions comprising a Compound can be determined by standard pharmaceutical procedures in cell culture or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is known as the therapeutic index and it can be expressed as the ratio LD50/ED50. Dosage regimens that exhibit large therapeutic indices are preferred. While dosage regimens that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets the Compound to the preferred site on the skin (e.g., using a topical formulation) in order to minimize potential damage to other tissue, thereby reducing side effects.

[00618] Data obtained from the in vitro assays and animal studies described herein can be used in formulating a dosage range of the Compound for use in human subjects. The dosage the Compound lies preferably within a range of skin concentrations, and possibly circulating concentrations, that include the ED50 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 treatment according to the methods described herein, the therapeutically effective dose can be estimated initially from in vitro (e.g., cell culture) assays. A dose may be formulated in animal models to achieve a skin concentration that includes the IC50 and a circulating plasma concentration that includes, or preferably is lower than, the IC50 (i.e., the concentration of Compound that achieves a half-maximal inhibition of GSK-3P) as determined in cell culture. Such information can be used to more accurately determine useful doses in human subjects. Levels in whole blood or plasma may be measured, for example, by high performance liquid chromatography or any other method described herein. Levels in skin may be measured, for example, by an LC/MS/MS assay described herein.

[00619] Further, any assays known to those skilled in the art can be used to evaluate the efficacy of treatment with a Compound, either alone or in a combination treatment described herein.
5.7.2 ASSAYS FOR GSK-3BETA INHIBITION

In some embodiments, a Compound described herein reduces or inhibits the activity of GSK-3p. The effect of a Compound on GSK-3p activity may be assessed using any assay known in the art.

In one embodiment, a Compound is a direct inhibitor of GSK-3P, i.e., the Compound reduces or inhibits GSK-3P substrate phosphorylation. In one such embodiment, the Compound acts as a competitive inhibitor of ATP binding to GSK-3p. For example, in one embodiment, a Compound’s effect on GSK-3P is assessed using an in vitro GSK-3P inhibition assay in which GSK-3P, a substrate, and Mg^{2+}-ATP are incubated in the presence of the Compound, and the effect on GSK-3P activity is compared to a control assay conducted in the absence of the Compound. See, e.g., Ring et al., 2003, Diabetes, 52:588-594, incorporated herein by reference in its entirety, for an example of such an assay. Cell-based assays are also provided in Ring et al., which may be used to determined the ability of a Compound to inhibit GSK-3p. Another cell-based assay, using crude cell extracts, for measuring the effect of a Compound on GSK-3P is provided in Ryves et al., 1998, Analytical Biochemistry 264:124-127, which is incorporated herein by reference in its entirety.

In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 2 mM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 1 mM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 0.5 mM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 0.1 mM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 50 µM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 10 µM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 1 µM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 0.5 µM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 100 nM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 50 nM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 20 nM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of less than 10 nM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of between 1 and 10 nM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of between 10 and 50 nM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of between 50 and 100 nM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of between 100 and 500 nM. In some embodiments, the Compound inhibits GSK-3P with an IC_{50} of between 0.5
µM and 1 µM. In some embodiments, the Compound inhibits GSK-3P with an IC₅₀ of between 1 µM and 10 µM. In some embodiments, the Compound inhibits GSK-3P with an IC₅₀ of between 10 µM and 100 µM. In some embodiments, the Compound inhibits GSK-3P with an IC₅₀ of between 100 µM and 500 µM. In some embodiments, the Compound inhibits GSK-3P with an IC₅₀ of between 0.5 mM and 1 mM. In some embodiments, the Compound inhibits GSK-3P with an IC₅₀ of between 1 mM and 2 mM. It will be appreciated by one of skill in the art that a Compound is expected to have a lower IC₅₀ in vitro than in vivo, for example, differing by one order of magnitude, or two or three or more orders of magnitude.

In some embodiments, a Compound is a direct inhibitor of GSK-3P, i.e., the Compound reduces or inhibits GSK-3P activation (one non-limiting example is competition with ATP binding). In some embodiments, a Compound is a selective inhibitor of GSK-3P, for example, compared to the Compound’s effect on other kinases. In some embodiments, the Compound is an indirect inhibitor of GSK-3P (one non-limiting example is prevention of dephosphorylation of GSK-3P that is required for GSK-3P activation). For example, in some embodiments, the Compound is a GSK-3P phosphatase inhibitor.

5.7.3 ANIMAL MODELS

Human skin and hair have features that are relatively unique among terrestrial mammals. 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.

However, it is noted that the Compounds described herein may be evaluated for their potential use in humans using the animal models described in this section and in the examples in Sections 10, 16, 24, and 25.

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

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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.

5.7.3.1 **HUMAN SKIN XENOGRAFT MODELS**

[00627] 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 the treatment with one or more Compounds described herein, as well as the combination treatments described in Section 5.3 supra. Although any method for producing human skin xenografts known in the art may be used, an exemplary model is described in the example of Section 10 below.

[00628] 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.

[00629] In some embodiments, the safety and efficacy of a Compound treatment (e.g., intermittent or pulse Compound treatment), optionally as part of a combination treatment described in Section 5.3 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.

[00630] Success of treatment aimed at improving hair growth can be measured by:

- increased terminal hair formation
- follicle synchronization so that the overall hair density appears to be greater compared to previous asynchronous hair growth
- increased anagen or decreased telogen
- follicle regeneration
- increased proliferation of dermal papilla
- increased recruitment or proliferation of stem cells to the follicle
- generation of new hair follicles
- increased diameter of existing or new hair (increased thickness of hair shafts)
• increased number of hair follicles at a more mature stage of development
• increased hair weight.

Success of a Compound treatment (e.g., intermittent or pulse Compound treatment) for wounding healing or scar revision 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.

Any method known in the art may be used to evaluate the safety and efficacy of a Compound treatment protocol, or of the combination treatments described in Section 5.3. In one embodiment, a human skin xenograft model is used. For example, one or more Compounds may be administered with a full thickness excision, laser, inflammatory stimulus, or dermabrasion procedure for integumental perturbation described in the examples of Sections 6 to 10, 16, 17, 18, 19, 24, or 25 below. A synergistic effect of an treatment with one or more Compounds on another treatment for restoring or enhancing hair growth (described in, e.g., Section 5.3) may be measured as an improvement over a control subject receiving only one of the two treatments (i.e., the treatment with one or more Compounds alone or the second treatment alone).

In an alternative embodiment, the treatment with one or more Compounds is used in combination treatments (e.g., described in Section 5.3 supra) to reduce unwanted hair growth. Success of such treatments can be measured using an animal model, e.g., the human xenograft mouse model described herein, by:
• decreased terminal hair formation
• follicle synchronization so that synergies are achieved when the hair growth retardant is sequentially applied
• decreased anagen or increased telogen
• inhibition of follicle regeneration.
• decreased hair weight, thickness, or density
• decreased number of hair follicles at a mature stage of hair follicle development.

[00634] An additive or a synergistic effect of treatment with one or more Compounds on another treatment for removing unwanted hair (described in, e.g., Section 5.3) may be measured as an improvement over a control subject receiving only one of the two treatments (i.e., the treatment with one or more Compounds alone or the second treatment alone).

5.7.3.2 OTHER ANIMAL MODELS

[00635] Another animal model for use in evaluating treatment that may more closely mimic the biology of human skin and hair 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 and in the examples in Sections 10, 16, 24, and 25 below 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. Other animal models that may be of use in evaluating the treatments described herein include pig, cat, or stumptailed macaque models.

5.7.4 METHODS FOR EVALUATING TREATMENT IN HUMANS

[00636] The safety and efficacy of the treatment with one or more Compounds described herein may also be measured in human subjects according to methods known in the art. See, e.g., International Patent Application Publication No. WO 2005/084621, published September 15, 2005, the contents of which is incorporated by reference herein in its entirety. For example, one or more Compounds may be administered in combination with minoxidil, finasteride, laser therapy, or a dermabrasion procedure for integumental perturbation, as described in the Examples of Sections 6-9, 17, 18, or 19 below.

[00637] Success of treatment aimed at improving hair growth can be measured by:
• increased terminal hair formation (e.g., measuring new hair growth as an increased number of fibers in an affected area of the skin, or increased thickness (e.g., diameter) or length of hair fibers)
• follicle synchronization so that the overall hair density appears to be greater compared to previous asynchronous hair growth (e.g., measured examination of a biopsy)
• increased anagen or decreased telogen (e.g., measured examination of a biopsy)
• increased follicle regeneration (e.g., measured examination of a biopsy)
• increased proliferation of dermal papilla (e.g., measured examination of a biopsy)
• increased recruitment or proliferation of stem cells to the follicle (e.g., measured examination of a biopsy)
• generation of new hair follicles (e.g., measured examination of a biopsy or by confocal microscope), by assessing number of hair follicles and/or by assessing morphological development stages of hair follicles compared to baseline or a negative control).

[00638] For example, success of a Compound treatment (e.g., intermittent or pulse Compound treatment) for wound healing (including wound healing with reduced scarring) or scar revision 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 Compound
• 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.

[00639] In some embodiments, the treatment with one or more Compounds increases hair count 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. In some embodiments, the treatment with one or more Compounds increases terminal hair 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. In some embodiments, the treatment with one or more Compounds increases hair thickness 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 in hair count, terminal hair, or hair thickness 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
treatment with one or more Compounds.

[00640] A synergistic effect of treatment with one or more Compounds on another
treatment for restoring or enhancing hair growth (described in, e.g., Section 5.3) may be
measured as an improvement over a control subject receiving only one of the two treatments
(i.e., treatment with one or more Compounds alone or the second treatment alone).

[00641] In an alternative embodiment, the treatment with one or more Compounds is used
in combination treatments (e.g., described in Section 5.3 supra) to reduce unwanted hair
growth. Any appropriate method for testing the safety and efficacy of such treatments may
be used, for example, as described in the examples of Sections 6 to 25 below. Success of
such treatments can be measured by:

- decreased terminal hair formation (e.g., measuring hair growth as a decreased number
  of fibers in an affected area of the skin, or decreased thickness (e.g., diameter) or
  length of hair fibers)
- follicle synchronization so that synergies are achieved when the hair growth retardant is
  sequentially applied
- decreased anagen or increased telogen
- inhibition of follicle regeneration.

[00642] In some embodiments, the treatment with one or more Compounds decreases hair
count 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. In
some embodiments, the treatment with one or more Compounds decreases terminal hair 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. In some
embodiments, the treatment with one or more Compounds decreases hair thickness 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 in hair count, terminal hair, or hair thickness may be measured after 1 day, 2
days, 3 days, 5 days, 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6
months, or one year or longer after initiation of the treatment with one or more Compounds.
A synergistic effect of treatment with one or more Compounds on another treatment for removing unwanted hair (described in, e.g., Section 5.3) may be measured as an improvement over a control subject receiving only one of the two treatments (i.e., the treatment with one or more Compounds alone or the second treatment alone).

5.7.4.1 METHODS FOR EVALUATING TREATMENT OF SCARRING ALOPECIA

The safety and efficacy of a Compound treatment (e.g., intermittent or pulse Compound treatment) for scarring alopecia may be measured using the methods described in herein. In some embodiments, successful treatment is determined as an increase in the number of photographically detected hairs. In other embodiments, successful treatment is evaluated by a skin biopsy for hair follicle structures and scar attributes. In other embodiments, treatment is continued until the symptoms and signs of scalp inflammation are controlled, and progression of the condition has been halted. In certain embodiments, scalp inflammation is measured by biopsy of the scalp. For example, in some embodiments, treatment is continued until, e.g., itching, burning, pain, and tenderness have cleared, scalp redness, scaling, and/or pustules are no longer present, and the hair loss has not extended. Commonly, cicatricial alopecias may reactivate after a quiet period, and treatment may have to be repeated at the reemergence of symptoms or signs of the condition.

5.7.5 IN VITRO MODELS

Skin explant model. The efficacy of the Compounds 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.

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.
6. **EXAMPLE 1: TREATMENT PROTOCOLS FOR MODULATION OF HAIR GROWTH**

6.1 **INTERMITTENT TREATMENT OF HAMILTON-NORWOOD TYPE VI MALE-PATTERN ALOPECIA USING A COMPOUND PROVIDED HEREIN WITH TOPICAL MINOXIDIL**

[00647] A male human subject, 30 years old, with Hamilton-Norwood type VI male-pattern alopecia presents complaining of continued hair loss despite treatment with topical minoxidil foam 5%, oral finasteride 1 mg/day. The subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical minoxidil and to apply the Compound to affected area of the scalp for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical minoxidil foam is re-started and he is evaluated after three weeks.

[00648] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers; and the patient's subjective evaluation of hair growth. The treated area of affected scalp is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g. G2, M, etc.): new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

[00649] The subject is treated with 10 cycles of the protocol: alternating topical Compound (1 week) with minoxidil foam treatment (3 weeks in which finasteride treatment is continued). Response to therapy is measured by the methods described above.

6.2 **INTERMITTENT TREATMENT OF HAMILTON-NORWOOD TYPE VI MALE-PATTERN ALOPECIA USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL MINOXIDIL**

[00650] A Caucasian male human subject, 30 years old, with Fitzgerald Type II skin (wherein Type I is the lightest and Type VI is the darkest (see Weller et al., 2008, Clinical Dermatology, 4th ed., Maiden, MA: Blackwell Publishing, pp. 268)), with Hamilton-Norwood type VI male-pattern alopecia presents complaining of continued hair loss despite treatment with topical minoxidil foam 5%, oral finasteride 1 mg/day. The bald and transitional areas of the subject's scalp are prepared by shaving and then treated with a fractional and non-ablative Erbium-YAG laser with an emission at 1540-1 550 nm (set to 50-70 J/cm², treatment level of 8-10, and 8 passes) and the subject is provided with a topical
preparation of a Compound provided herein and instructed to discontinue topical minoxidil and to apply the Compound to the treated area of the scalp for one week. After one week, treatment with the Compound is discontinued and treatment with topical minoxidil foam is re-started and he is evaluated after three weeks.

[00651] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers; and the patient’s subjective evaluation of hair growth. The treated area of affected scalp is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

[00652] The subject is optionally treated with 10 more cycles, e.g., to increase hair density, for example: 1 week of topical Compound followed by 3 weeks of minoxidil foam treatment, with optional laser treatment. Response to therapy is measured by the methods described above.

6.3 INTERMITTENT PRE-TREATMENT OF "DONOR" SCALP AREAS WITH A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL MINOXIDIL IN PREPARATION FOR A HAIR TRANSPLANT PROCEDURE IN A PATIENT WITH HAMILTON-NORWOOD TYPE VII MALE-PATTERN ALOPECIA

[00653] A male human subject, 30 years old, with Hamilton-Norwood type VII male-pattern alopecia presents complaining of continued hair loss despite treatment with topical minoxidil foam 5%, oral finasteride 1 mg/day.

6.3.1 TREATMENT A

[00654] Pre-treatment: In preparation for a hair transplant procedure, the subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical minoxidil and to apply the Compound to the to the occipital and deep temporal areas area of the scalp from which follicles will be harvested for transplant for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical minoxidil foam is re-started and he is evaluated after three weeks.

[00655] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers; and
the patient's subjective evaluation of hair growth. The treated area of affected scalp is biopsied and studied for new follicle growth, bifurcating follicles undergoing follicle division; follicles growing new hair fibers.

[00656] The subject is treated with 10 cycles of the protocol: alternating topical Compound (1 week) with minoxidil foam treatment (3 weeks). Response to therapy is measured by the methods described above.

[00657] Post-transplantation: The area of scalp that was pre-treated with the Compound and minoxidil is used as a source for the transplanted follicles. After hair follicle implantation, treatment with the Compound is initiated for one week, and then discontinued and followed by treatment with topical minoxidil foam for three weeks.

[00658] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers; and the patient's subjective evaluation of hair growth.- The treated area of affected scalp is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

[00659] The subject is treated with 10 cycles of the protocol: alternating topical Compound (1 week) with minoxidil foam treatment (3 weeks).

6.3.2 TREATMENT B

[00660] The subject is provided a treatment in which the bald crown scalp receives in order: (i) Erbium-YAG laser; (ii) a Compound provided herein; and (iii) estrogen during the phase where follicle stem cells are reorganizing and reforming hair follicles. The estrogen treatment reprograms the follicle stem cells to alter their sensitivity/response to androgens. In effect, this could be described as rendering follicle stem cells either (a) "female-type" with respect to crown scalp follicle cells or (b) "occipital scalp type" in terms of their lack of response to androgens by involution.
6.4 INTERMITTENT PRE-TREATMENT OF "DONOR" SCALP AREAS USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL MINOXIDIL IN PREPARATION FOR A HAIR TRANSPLANT PROCEDURE IN A PATIENT WITH HAMILTON-NORWOOD TYPE VII MALE-PATTERN ALOPECIA

[00661] A Caucasian male human subject, 30 years old, with Fitzgerald Type II skin (wherein Type I is the lightest and Type VI is the darkest (see Weller et al., 2008, Clinical Dermatology, 4th ed., Maiden, MA: Blackwell Publishing, pp. 268)), with Hamilton-Norwood type VII male-pattern alopecia presents complaining of continued hair loss despite treatment with topical minoxidil foam 5%, oral finasteride 1 mg/day.

[00662] Pre-treatment: In preparation for a hair transplant procedure, the subject is administered a fractional and non-ablative laser therapy using an Erbium-YAG laser with an emission at 1540-1550 nm (set to 50-70 J/cm², treatment level of 8-10, and 8 passes) and the subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical minoxidil and to apply the Compound to the to the occipital and deep temporal areas area of the scalp from which follicles will be harvest for transplant for one week. After one week, treatment with the Compound is discontinued and treatment with topical minoxidil foam is re-started and he is evaluated after three weeks.

[00663] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers. The treated area of affected scalp is biopsied and studied for new follicle growth, bifurcating follicles undergoing follicle division; follicles growing new hair fibers.

[00664] The subject is optionally treated with 10 more cycles, e.g., to increase hair density, for example: topical Compound (1 week) with minoxidil foam treatment (3 weeks), with optional laser treatment. Response to therapy is measured by the methods described above.

[00665] Post-transplantation: The area of scalp that was treated with the Compound and minoxidil is used as a source for the transplanted follicles. After hair follicle implantation, treatment with Compound is initiated for one week, and then discontinued and followed by treatment with topical minoxidil foam for three weeks.

[00666] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers. The treated area of affected scalp is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages.
of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

[00667] The subject is treated with 10 cycles of the protocol: alternating Erbium-YAG laser treatment followed by topical Compound (1 week) with minoxidil foam treatment (3 weeks).

6.5 INTERMITTENT TREATMENT OF A POST-MENOPAUSAL PATIENT WITH DIFFUSE THINNING OF SCALP HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL MINOXIDIL

[00668] A female human subject, 65 years old, complains of diffuse thinning of scalp hair growth after menopause. She describes continued hair loss despite treatment with topical minoxidil foam 2% and oral finasteride 1 mg/day. The subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical minoxidil and to apply the Compound to affected area of the scalp for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical minoxidil foam is re-started and she is evaluated after three weeks.

[00669] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers. The treated area of affected scalp is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

[00670] The subject is treated with 10 cycles of the protocol: alternating topical Compound (1 week) with minoxidil foam treatment (3 weeks). Response to therapy is measured by the methods described above.

6.6 INTERMITTENT TREATMENT OF A POST-MENOPAUSAL PATIENT WITH DIFFUSE THINNING OF SCALP HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL MINOXIDIL

[00671] A Caucasian female human subject Fitzgerald Type 11 skin, 65 years old, complains of diffuse thinning of scalp hair growth after menopause. She describes continued hair loss despite treatment with topical minoxidil foam 2% and oral finasteride 1 mg/day. The subject is administered a fractional and non-ablative laser therapy using an Erbium-YAG
laser with an emission at 1540-1550 nm (set to 50-70 J/cm², treatment level of 8-10, and 8 passes) and the subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical minoxidil and to apply the Compound to the treated area of the scalp for one week. After one week, treatment with the Compound is discontinued and treatment with topical minoxidil foam is re-started and she is evaluated after three weeks.

[00672] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers. The treated area of affected scalp is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

[00673] The subject is optionally treated with 10 more cycles, e.g., to increase hair density, for example: topical Compound (1 week) followed by minoxidil foam treatment (3 weeks), with optional laser treatment. Response to therapy is measured by the methods described above.

6.7 INTERMITTENT PRE-TREATMENT OF "DONOR" SCALP AREAS OF A POST-MENOPAUSAL PATIENT WITH DIFFUSE THINNING OF SCALP HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL MINOXIDIL IN PREPARATION FOR A HAIR TRANSPLANT PROCEDURE

[00674] A female human subject, 65 years old, with Fitzgerald Type II skin complains of diffuse thinning of scalp hair growth after menopause. She describes continued hair loss despite treatment with topical minoxidil 2% foam and oral finasteride 1 mg/day.

[00675] Pre-treatment: In preparation for a hair transplant procedure, the subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical minoxidil and to apply the Compound to the occipital and deep temporal areas of the scalp from which follicles will be harvested for transplant for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical minoxidil foam is re-started and she is evaluated after three weeks.

[00676] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers. The treated area of affected scalp is biopsied and studied for new follicle growth, bifurcating follicles undergoing follicle division; follicles growing new hair fibers.
[00677] The subject is treated with 10 cycles of the protocol: alternating topical Compound (1 week) with minoxidil foam treatment (3 weeks). Response to therapy is measured by the methods described above.

[00678] Post-transplantation: The area of scalp that was pre-treated with the Compound and minoxidil is used as a source for the transplanted follicles. After hair follicle implantation, treatment with the Compound is initiated for one week, and then discontinued and followed by treatment with topical minoxidil foam for three weeks.

[00679] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers. The treated area of affected scalp is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g., G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

[00680] The subject is treated with 10 cycles of the protocol: alternating topical Compound (1 week) with minoxidil foam treatment (3 weeks).

6.8 INTERMITTENT PRE-TREATMENT OF "DONOR" SCALP AREAS OF A POST-MENOPAUSAL PATIENT WITH DIFFUSE THINNING OF SCALP HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL MINOXIDIL IN PREPARATION FOR A HAIR TRANSPLANT PROCEDURE

[00681] A Caucasian female human subject (with Fitzgerald Type II skin, wherein Type I is the lightest and Type VI is the darkest), 65 years old, complains of diffuse thinning of scalp hair growth after menopause. She describes continued hair loss despite treatment with topical minoxidil 2% foam and oral finasteride 1 mg/day.

[00682] Pre-treatment: In preparation for a hair transplant procedure, the subject is administered a fractional and non-ablative laser therapy using an Erbium-YAG laser with an emission at 1540-1550 nm (set to 50-70 J/cm², treatment level of 8-10, and 8 passes) and the subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical minoxidil and to apply the Compound to the occipital and deep temporal areas area of the scalp from which follicles will be harvested for transplant for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical minoxidil foam is re-started and she is evaluated after three weeks.
Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers. The treated area of affected scalp is biopsied and studied for new follicle growth, bifurcating follicles undergoing follicle division; follicles growing new hair fibers.

The subject is optionally treated with 10 more cycles, e.g., to increase hair density, for example: alternating Erbium-YAG laser treatment plus topical Compound (1 week) with minoxidil foam treatment (3 weeks). Response to therapy is measured by the methods described above.

Post-transplantation: The area of scalp that was pre-treated with the Compound and minoxidil is used as a source for the transplanted follicles. After hair follicle implantation, treatment with the Compound is initiated for one week, and then discontinued and followed by treatment with topical minoxidil foam for three weeks, with or without laser treatment.

Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of scalp); thickness of fibers; length of hair fibers. The treated area of affected scalp is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g., G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

The subject is optionally treated with 10 cycles of the protocol: alternating topical Compound (1 week) with minoxidil foam treatment (3 weeks), with or without laser treatment.

6.9 INTERMITTENT TREATMENT OF POST-MENOPAUSAL FACIAL HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL EFLORNITHINE

A female human subject, 50 years old, complains of moustache hair growth after menopause. Vaniqa® (eflornithine hydrochloride 13.9%) is being applied without much relief. The subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical eflornithine and to apply the Compound to the affected area of the face for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical eflornithine is re-started and she is evaluated after three weeks.
Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of facial skin); thickness of fibers; length of hair fibers; and the patient’s subjective evaluation of hair growth inhibition. The treated area of affected face is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

The subject is optionally treated with 10 more cycles, e.g., to decrease hair density, for example: alternating topical Compound (1 week) with eflomithine foam treatment (3 weeks). Response to therapy is measured by the methods described above.

6.10 INTERMITTENT TREATMENT OF POST-MENOPAUSAL FACIAL HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL EFLORNITHINE

A Caucasian female human subject (with Fitzgerald Type II skin, wherein Type I is the lightest and Type VI is the darkest), 50 years old, complains of moustache hair growth after menopause. Vaniqa® (eflomithine hydrochloride 13.9%) is being applied without much relief. The subject is administered a fractional and non-ablative laser therapy using an Erbium-YAG laser with an emission at 1540-1550 nm (set to 50-70 J/cm², treatment level of 8-10, and 8 passes) and the subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical eflomithine and to apply the Compound to the treated area of the face for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical eflomithine is re-started and she is evaluated after three weeks.

Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of facial skin); thickness of fibers; length of hair fibers. The treated area of affected face is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

The subject is optionally treated with 10 more cycles, e.g., to decrease hair density, for example: topical Compound (1 week) followed by with eflomithine foam treatment (3 weeks), with or without laser treatment. Response to therapy is measured by the methods described above.
6.11 INTERMITTENT TREATMENT OF POST-MENOPAUSAL FACIAL HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL 5-FLUOROURACIL

[00694] A female human subject, 50 years old, complains of moustache hair growth after menopause. Vaniqa (eflornithine hydrochloride 13.9%) is being applied without much relief. The subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical efloithine and to apply the Compound to the affected area of the face for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical 5-fluorouracil (5-FU, Efudex 5% cream) is started and she is evaluated after three weeks.

[00695] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of facial skin); thickness of fibers; length of hair fibers. The treated area of affected face is biopsied and studied for new follicle growth, bifurcating follicles undergoing follicle division; follicles growing new hair fibers.

[00696] The subject is treated with 10 cycles of the protocol: alternating topical Compound (1 week) with 5-FU cream treatment (3 weeks). Response to therapy is measured by the methods described above.

6.12 INTERMITTENT TREATMENT OF POST-MENOPAUSAL FACIAL HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL 5-FLUOROURACIL

[00697] A Caucasian female human subject (with Fitzgerald Type II skin, wherein Type I is the lightest and Type VI is the darkest), 50 years old, complains of moustache hair growth after menopause. Vaniqa (eflornithine hydrochloride 13.9%) is being applied without much relief. The subject is administered a fractional and non-ablative laser therapy using an Erbium-YAG laser with an emission at 1540-1550 nm (set to 50-70 J/cm², treatment level of 8-10, and 8 passes) and the subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical efloithine and to apply the Compound to the treated area of the face for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical 5-fluorouracil (5-FU, Efudex 5% cream) is started and she is evaluated after three weeks.

[00698] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of facial skin); thickness of fibers; length of hair fibers.
The treated area of affected face is biopsied and studied for new follicle growth, bifurcating follicles undergoing follicle division; follicles growing new hair fibers.

The subject is optionally treated with 10 more cycles, e.g., to decrease hair density, for example: topical Compound (1 week) followed by 5-FU cream treatment (3 weeks), with or without laser treatment. Response to therapy is measured by the methods described above.

6.13 INTERMITTENT TREATMENT OF AXILLARY HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL EFLORNITHINE

A female human subject, 30 years old, complains of axillary hair growth and irritation from using razors to shave her armpits. The subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical eflornithine and to apply the Compound to the affected area of the axilla for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical Vaniqa (eflornithine hydrochloride 13.9%) is started and she is evaluated after three weeks.

Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of axillary skin); thickness of fibers; length of hair fibers. The treated area of affected axillary skin is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g., G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

The subject is treated with 10 cycles of the protocol: alternating topical Compound (1 week) with eflornithine foam treatment (3 weeks). Response to therapy is measured by the methods described above.

6.14 INTERMITTENT TREATMENT OF AXILLARY HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL EFLORNITHINE

A Caucasian female human subject (with Fitzgerald Type II skin, wherein Type I is the lightest and Type VI is the darkest), 30 years old, complains of axillary hair growth and irritation from using razors to shave her armpits. The subject is administered a fractional and non-ablative laser therapy using an Erbium-YAG laser with an emission at 1540-1550 nm (set to 50-70 J/cm², treatment level of 8-10, and 8 passes) and the subject is provided with a
topical preparation of a Compound provided herein and instructed to discontinue topical
eflornithine and to apply the Compound to the treated area of the axilla for one week (to
synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the
Compound is discontinued and treatment with topical Vaniqa (eflornithine hydrochloride
13.9%) is started and she is evaluated after three weeks.

Response to therapy is determined by measuring new hair growth (increased
number of fibers in an affected area of axillary skin); thickness of fibers; length of hair fibers.
The treated area of affected axillary skin is biopsied and studied for distribution of follicles in
various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various
stages of cell cycle (e.g., G2, M, etc.); new follicle growth, bifurcating follicles; follicles
undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

The subject is optionally treated with 10 more cycles, e.g., to decrease hair
density, for example: topical Compound (1 week) followed by eflornithine foam treatment (3
weeks), with or without laser treatment. Response to therapy is measured by the methods
described above.

6.15 **INTERMITTENT TREATMENT OF BACK HAIR**

**USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL EFLORNITHINE**

A male human subject, 30 years old, complains of excess back hair growth.
Repeated waxings have had short term effects and are painful. The subject is provided with a
topical preparation of a Compound provided herein and instructed apply it to affected area of
the skin for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one
week, treatment with the Compound is discontinued and treatment with topical Vaniqa
(eflornithine hydrochloride 13.9%) is started and he is evaluated after three weeks.

Response to therapy is determined by measuring new hair growth (increased
number of fibers in an affected area of back skin); thickness of fibers; length of hair fibers.
The treated area of affected back skin is biopsied and studied for distribution of follicles in
various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various
stages of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles
undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

The subject is treated with 10 cycles of the protocol: alternating topical
Compound (1 week) with eflornithine foam treatment (3 weeks). Response to therapy is
measured by the methods described above.
6.16 INTERMITTENT TREATMENT OF BACK HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL EFLORNITHINE

[00709] A Caucasian male human subject (with Fitzgerald Type II skin, wherein Type I is the lightest and Type VI is the darkest), 30 years old, complains of excess back hair growth. Repeated waxings have had short term effects and are painful. The subject is administered a fractional and non-ablative laser therapy using an Erbium-YAG laser with an emission at 1540-1550 nm (set to 50-70 J/cm², treatment level of 8-10, and 8 passes) and the subject is provided with a topical preparation of a Compound provided herein and instructed apply it to the treated area of the skin for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical Vaniqa (eflornithine hydrochloride 13.9%) is started and he is evaluated after three weeks.

[00710] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of back skin); thickness of fibers; length of hair fibers. The treated area of affected back skin is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g., G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

[00711] The subject is optionally treated with 10 more cycles, e.g., to decrease hair density, for example: topical Compound (1 week) followed by eflornithine foam treatment (3 weeks), with or without laser treatment. Response to therapy is measured by the methods described above.

6.17 INTERMITTENT TREATMENT OF EAR HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL EFLORNITHINE

[00712] A male human subject, 55 years old, complains of ear (auricle) hair growth. The subject is provided with a topical preparation of a Compound provided herein and instructed discontinue topical eflornithine and to apply the Compound to affected area of the ear for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical eflornithine is re-started and he is evaluated after three weeks.

[00713] Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of ear); thickness of fibers; length of hair fibers. The
treated area of affected ear is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

The subject is treated with 10 cycles of the protocol: alternating topical Compound (1 week) with eflornithine foam treatment (3 weeks). Response to therapy is measured by the methods described above.

6.18 INTERMITTENT TREATMENT OF EAR HAIR USING A COMPOUND PROVIDED HEREIN ALTERNATING WITH TOPICAL EFLORNITHINE

A Caucasian male human subject (with Fitzgerald Type II skin, wherein Type I is the lightest and Type VI is the darkest), 55 years old, complains of ear (auricle) hair growth. The subject is administered a fractional and non-ablative laser therapy using an Erbium-YAG laser with an emission at 1540-1550 nm (set to 50-70 J/cm², treatment level of 8-10, and 8 passes) and the subject is provided with a topical preparation of a Compound provided herein and instructed to discontinue topical eflornithine and to apply the Compound to the treated area of the ear for one week (to synchronize the hair follicle cells in G2/M phase arrest). After one week, treatment with the Compound is discontinued and treatment with topical eflornithine is re-started and he is evaluated after three weeks.

Response to therapy is determined by measuring new hair growth (increased number of fibers in an affected area of ear); thickness of fibers; length of hair fibers. The treated area of affected ear is biopsied and studied for distribution of follicles in various stages of Follicle Cycle (anagen, catagen, etc.); distribution of follicle cells in various stages of cell cycle (e.g. G2, M, etc.); new follicle growth, bifurcating follicles; follicles undergoing follicle division; follicles growing new hair fibers, follicles with no hair fibers.

The subject is optionally treated with 10 more cycles, e.g., to decrease hair density, for example: topical Compound (1 week) followed by eflornithine foam treatment (3 weeks), with or without laser treatment. Response to therapy is measured by the methods described above.
7. **EXAMPLE 2: PROTOCOLS FOR WOUND AND SCAR TREATMENT**

7.1 **TREATMENT OF RADIATION SCAR**

[00718] A female human subject, 75 years old, underwent treatment of a basal cell carcinoma of the nose five years prior to presentation. The resulting scar is atrophic, hypo-pigmented and lacking normal pore pattern. The scar is mechanically disrupted by excision, dermatome planing, dermabrasion, laser ablation, or fractional laser ablation (*e.g.*, Fraxel), and treatment with topical Compound is initiated.

[00719] Response to treatment is determined by measuring skin thickness, return of pigmentation and re-establishment of adnexal structures.

7.2 **TREATMENT OF ACNE SCARS**

[00720] A male human subject, 28 years old, presents with extensive, broad, shallow, acne scars. The scars are atrophic, hypo-pigmented and lack normal pore pattern. The scars are dermabraded using a moderate grit diamond fraise and treatment with topical Compound is initiated.

[00721] Response to treatment is determined by measuring skin thickness of the scar, return of pigmentation and re-establishment of adnexal structures.

7.3 **TREATMENT OF CURETTAGE SCAR**

[00722] A female human subject, 45 years old, was treated for a basal cell carcinoma of the forehead by electrodessication and curettage. The resulting scar was depressed, atrophic, hypo-pigmented and lacked normal pore pattern. The scar is mechanically disrupted by excision, dermabrasion, laser ablation, or fractional laser (*e.g.*, Fraxel), and treatment with topical Compound is initiated.

[00723] Response to treatment is determined by measuring skin thickness, return of pigmentation and re-establishment of adnexal structures.

7.4 **TREATMENT OF A SPLIT THICKNESS SKIN GRAFT**

[00724] A male human subject, 75 years old, underwent excision of a large malignant melanoma of the forehead with subsequent reconstruction using a split-thickness skin graft. After one year, the resulting graft demonstrates depression, skin atrophy, hyper-pigmentation and loss of normal pore pattern. The graft is mechanically disrupted by excision, dermabrasion, laser ablation, or fractional laser (*e.g.*, Fraxel), and treatment with topical Compound is initiated.
Response to treatment is determined by measuring skin thickness, establishment of normal pigmentation and re-establishment of adnexal structures.

7.5 TREATMENT OF A SPREAD SCAR

A female human subject, 30 years old, undergoes excision of a large congenital nevus of the cheek. Due to the tension of closure, the incision line spreads and widens over time resulting in a scar that is atrophic, hypo-pigmented and lacking normal pore pattern. The scar is mechanically disrupted by excision, dermabrasion, laser ablation, or fractional laser (e.g., Fraxel), and treatment with topical Compound is initiated.

Response to treatment is determined by measuring skin thickness, return of pigmentation and re-establishment of adnexal structures.

7.6 TREATMENT OF FLAP NECROSIS

A female human subject, 65 years old, underwent a face-lift procedure. A portion of the cheek flap subsequently became necrotic and healed by secondary intent. The resulting scar was atrophic, hypo-pigmented and lacked normal pore pattern. The scar is mechanically disrupted by excision, dermabrasion, laser ablation, or fractional laser (e.g., Fraxel), and treatment with topical Compound is initiated.

Response to treatment is determined by measuring skin thickness, return of pigmentation and re-establishment of adnexal structures.

7.7 TREATMENT OF SCARRING FOLLOWING INFECTION

A female human subject, 37 years old, underwent a phenol chemical peel of her lips to decrease rhytides. During the healing phase, the patient developed a staphylococcal (herpetic) infection of the treated area. The infected area healed with an atrophic, hypo-pigmented scar that lacked the normal pore pattern. The scar is mechanically disrupted by excision, dermabrasion, laser ablation, or fractional laser (e.g., Fraxel), and treatment with topical Compound is initiated.

Response to treatment is determined by measuring skin thickness, return of pigmentation and re-establishment of adnexal structures.
7.8 TREATMENT OF LEG ULCERS

[00732] A male human subject, 70 years old, developed non-healing leg ulcers involving the tibial aspects of both legs. The affected area was pre-treated with topical Compound and subsequently grafted using small pinch grafts harvested from the thighs.

[00733] Response to treatment is determined by measuring skin thickness and re-establishment of adnexal structures over the grafted area following graft take.

7.9 TREATMENT OF SPLIT THICKNESS GRAFT DONOR SITES

[00734] A female human subject, 40 years old, underwent split thickness grafting to reconstruct a facial defect following excision of a squamous cell carcinoma. The graft donor site healed with a hypo-pigmented scar that lacked the normal pore pattern. The scar is mechanically disrupted by excision, dermabrasion, laser ablation, or fractional laser (e.g., Fraxel), and treatment with topical Compound is initiated.

[00735] Response to treatment is determined by measuring skin thickness, return of pigmentation, and re-establishment of adnexal structures.

7.10 TREATMENT OF A BURN SCAR

[00736] A male human subject, 30 years old, suffered a burn of the left cheek that healed with a contracted, hypo-pigmented scar that lacked normal pore pattern. The scar is mechanically disrupted by excision, dermabrasion, laser ablation, or fractional laser (e.g., Fraxel), and treatment with topical Compound is initiated.

[00737] Response to treatment is determined by measuring skin thickness, return of pigmentation, and re-establishment of adnexal structures.

7.11 IMMEDIATE TREATMENT FOLLOWING CURETTAGE

[00738] A 76 year old fair skin male with a history of multiple basal cell carcinomas presents with 2 new pigmented nodular pearly lesions of 1 cm on his left (A) and right (B) scapula. Shave biopsies reveal both lesions to be nodular BCCs. Lesion (A) is treated with aggressive 3 pass curettage then application of aluminum chloride. Lesion (B) is treated with aggressive 3 pass curettage then application of aluminum chloride. Lesion B is post-treated with topical Compound one time weekly post-procedure (in addition to routine wound care). In 3 and 6 week follow-up, the lesions are compared with respect to skin thickness, return of pigmentation, re-establishment of adnexal structures and global assessment the physician and
patient's satisfaction regarding the cosmetic result of the 2 distinct lesions using a Visual Analogue Scale (VAS).

7.12 TREATMENT FOLLOWING SURGICAL EXCISION

[00739] A 76 year old fair skin male with a history of multiple basal cell carcinomas presents with 2 biopsy proven BCC lesions of 1 cm on his left scapula (A) and 1 cm on his right scapula(B). Conservative surgical excision is performed on both lesions. Lesion (A) is treated with then treated with routine wound care. Lesion (B) is treated with pre-treated with topical Compound once prior to surgery followed by application one time weekly post-procedure (in addition to routine wound care). In 3 and 6 week follow-up, the lesions are compared with respect to skin thickness, return of pigmentation, re-establishment of adnexal structures and global assessment the physician and patient's satisfaction regarding the cosmetic result of the 2 distinct lesions using a Visual Analogue Scale (VAS).

7.13 TREATMENT FOLLOWING STERNOTOMY SCAR

[00740] A 74 year old black male with history CAD undergoes CABG surgery requiring median sternotomy. Following the procedure the scar is found to measure 14 cm in diameter. Starting Day 1, he applies topical Compound to the 7 cm superior portion of the scar (in addition to routine wound care) one time per week. In 3 and 6 week follow-up, the superior and inferior aspects of the sternotomy scar are compared with respect to skin thickness, return of pigmentation, re-establishment of adnexal structures and global assessment the physician and patient's satisfaction regarding the cosmetic result of the 2 distinct lesions using a Visual Analogue Scale (VAS).

7.14 TREATMENT FOLLOWING FOLLICULAR UNIT TRANSPLANTATION

[00741] A 51 year old white male with history Androgenic Alopecia undergoes Hair Transplant with the "Strip Harvesting Method" with donor area located at the posterior scalp along the occipital protuberance. Surgery requires a long scar on the posterior scalp measuring 28 cm. Starting Day 1, topical Compound is applied only to the 14 cm "left" portion of the scar (in addition to routine wound care), one time per week. In 3 and 6 week follow-up, the right and left aspects of the scar are compared with respect to skin thickness, return of pigmentation, re-establishment of adnexal structures and global assessment the
physician and patient's satisfaction regarding the cosmetic result of the 2 distinct lesions using a Visual Analogue Scale (VAS).

[00742] In a variation, hair that is regenerated in the treated donor area may-be used as a source of future, repeated hair transplants in accordance with the foregoing method.

7.15 TREATMENT FOLLOWING FOLLICULAR UNIT EXTRACTION

[00743] A 51 year old white male with history Androgenic Alopecia undergoes Hair Transplant with the "Follicular Unit Extraction" with donor area located at the posterior scalp above and below the occipital protuberance. In total 1000 punch graft were taken from the posterior scalp and left to heal with secondary intention. Starting Day 1, topical Compound is applied only to the "left" portion of the donor area (in addition to routine wound care), for one time per week. In 3 and 6 week follow-up, the right and left aspects of the scar are compared with respect to skin thickness, return of pigmentation, re-establishment of adnexai structures and global assessment the physician and patient's satisfaction regarding the cosmetic result of the 2 distinct lesions using a Visual Analogue Scale (VAS).

[00744] In a variation, hair that is regenerated in the treated donor area may be used as a source of future, repeated hair transplants in accordance with the foregoing method.

7.16 TREATMENT FOLLOWING CESAREAN SECTION

[00745] A 27 year old G1P1 white female, undergoes Cesarean section surgery secondary to failure for labor to progress. Surgery requires a long scar on the lower abdomen measuring 24 cm (patient has decided not to breast feed post-delivery due to prior breast augmentation surgery). Starting Day 1, topical Compound is applied only to the 12 cm "left" portion of the scar (in addition to routine wound care), one time per week. In 3 and 6 week follow-up, the right and left aspects of the scar are compared with respect to skin thickness, return of pigmentation, re-establishment of adnexai structures and global assessment the physician and patient's satisfaction regarding the cosmetic result of the 2 distinct lesions using a Visual Analogue Scale (VAS).

7.17 CLINICAL PROTOCOL FOR TESTING EFFICACY OF COMBINATION COMPOUND AND LASER TREATMENT OF SURGICAL SCARS

[00746] A patient has a 5 cm scar resulting from surgery. Two to three months after surgery, the scar is treated with fractional laser. Half the scar is treated with Compound and the whole scar is treated with laser. In another variation, a patient has two surgical scars, one
of which is treated with the Compound and laser combination and the other of which is treated with laser alone.

8. **EXAMPLE 3: LASER TREATMENT VARIATIONS**

[00747] The treatments presented in the examples in Sections 6 and 7 may alternatively be accomplished by applying one of the following laser treatments, which include ablative laser treatments. In such ablative laser treatments, the application of Compound is sterile and, optionally, the treatment area is covered by a bandage. For example, ablative laser treatment may accomplished using an Erbium-YAG laser at 2940 nm or a CO₂ laser at 10,600 nm.

8.1 **FRACTIONAL, NON-ABLATIVE LASER TREATMENT**

[00748] The subject is administered a fractional and non-ablative laser therapy using an Erbium-YAG laser with an emission at 1540-1550 nm (set to 50-70 J/cm², treatment level of 8-10 (density of the "dots"), and 8 passes) and the subject is provided with a topical preparation of Compound and instructed to apply the Compound to the treated area for one week. After one week, treatment with Compound is discontinued and he is evaluated after three weeks.

8.2 **ULTRAPULSE CO₂ FRACTIONAL LASER**

[00749] After shaving/clipping of the existing hair in the area to be treated, and followed by cleaning with antiseptic, Lidocaine HCL 2% with Epinephrine 1:100,000 are injected to anesthetize the surface of the area to be treated. An Ultrapulse (fractional mode) CO₂ laser is used to disrupt the epidermis and dermis to approximately 100 to 500 µM in depth. The Ultrapulse laser produces an effect that is similar to that of dermabrasion yet the disruption produced delivers a greater amount of energy deeper into the skin in a non-scarring fractional ablation. The treated area is a 1.5 cm x 1.5 cm square. The Ultrapulse is set to deliver up to 350 mJ, up to 52.5 Watts, using pattern size #8, density #4, and fill the square treatment site with up to 5 passes.

8.3 **ULTRAPULSE CO₂ ABLATION LASER**

[00750] After shaving/clipping of the existing hair in the area to be treated, and followed by cleaning with antiseptic, Lidocaine HCL 2% with Epinephrine 1:100,000 are injected to anesthetize the surface of the area to be treated. An Ultrapulse CO₂ laser (ablative mode) is used to disrupt the epidermis and dermis to approximately 100 to 500 µM in depth. The
Ultrapulse laser produces an effect that is similar to that of dermabrasion yet the disruption produced delivers a greater amount of energy deeper into the skin in a non-scarring ablation that resembles the dermabrasion. The treated area is a 1.5 cm x 1.5 cm square. The Ultrapulse is set to deliver up to 500 mJ in 1 msec, 1 Watts, using a spot size of 3 mm at 2 Hz to fill the square treatment site, which may require up to 15 passes.

8.4 CANDELA SMOOTH PEEL FULL-ABLATION ERBIUM LASER

After shaving/clipping of the existing hair in the area to be treated, and followed by cleaning with antiseptic, Lidocaine HCL 2% with Epinephrine 1:100,000 are injected to anesthetize the surface of the area to be treated. The ablative erbium laser is set to deliver up to 5 Joules 240 msec in of energy at level 3 so that in up to 15 passes it will produce a disruption up to 500 μM deep. The treated area is a 1.5 cm x 1.5 cm square.

9. EXAMPLE 4: DERMABRASION TREATMENT VARIATIONS

The treatments presented in the examples in foregoing Sections 6 and 7 may alternatively be accomplished by applying the following dermabrasion treatment in place of the laser treatment.

After shaving/clipping of the existing hair in the area to be treated, followed by cleaning with antiseptic, Lidocaine HCL 2% with Epinephrine 1:100,000 is injected to anesthetize the surface of the area to be treated. Standard dermabrasion, using the Aseptico Econo-Dermabrader from Tiemann and Company, is performed to a depth of approximately 150 μM, that includes removal the entire epidermis and disruption of the papillary dermis (detectable by a shiny, whitish appearance) inducing the formation of small pools of blood in the treated area. Each dermabraded area is a 1.5 cm x 1.5 cm square. In an alternative example, a Bell Hand dermabrasion device may be used.

Variations of this protocol are found in the example of Sections 10, 16, and 24, which present mouse studies using dermabrasion, and the protocols for use in humans in the examples of Section 17-19. In the mouse studies, dermabrasion was carried out using a microdermabrasion device. While dermabrasion in humans may also be carried out using a microdermabrasion device, where sterile conditions are preferential, a dermabrasion device is preferably used.
10. EXAMPLE 5: HUMAN SKIN XENOGRAFT ANIMAL MODEL FOR ASSESSING OR CONFIRMING EFFICACY OF COMPOUND TREATMENT REGIMEN

[00755] This protocol is adapted from the IACUC VA protocol. Specifically, 4 week old male SCID mice are obtained from Charles River and allowed to acclimate for at least 1 week. In preparation for surgery, mice are anesthetized with ketamine (80 mg/kg)/xylazine (20 mg/kg) delivered i.p. in a volume < 100 μl, and monitored by toe pinch to determine the surgical plane of anesthesia. Full thickness adult human skin (measuring approximately 1.5 cm x 2 cm; removed during surgical procedures from the CHTN, NDRI or cadaver scalp skin from ABS) is sutured into a full thickness skin excision site on the dorsal surface of the mouse. The grafts are bandaged and allowed to heal for at least 5 weeks. After healing and successful "take," prior to wounding, the human skin is analyzed using in vivo confocal microscopy, histology and/or photography to determine the "control" or "pre-wounded" state of the skin graft. Prior to wounding, mice are anesthetized with ketamine (80 mg/kg)/xylazine (20 mg/kg) delivered i.p. in a volume of < 100 μl, and monitored by toe pinch to determine the surgical plane of anesthesia. The epidermis of the human skin is removed using a microdermabrasion device to dermabrade as described above. (Experiments with dermabrasion on ex vivo human abdominal skin have established the initial parameters for removal of epidermis, however some testing in mice may be required to confirm and/or optimize these settings for human scalp xenografts. Additionally, some mice may be required to test the differences between full thickness and split thickness human scalp xenografts. Furthermore, reducing the overall thickness of the human skin may improve the "take" rate of the grafts, which is approximately 50%). The wounds are allowed scab and heal naturally. The mice are observed and photographed daily in order to monitor the formation of the scab and the timing of its detachment (scab detachment should occur within 2 weeks of wounding). As soon as the scab detaches, mice receive vehicle alone or the Compound, delivered systemically or topically, or neither vehicle nor Compound, for 5 consecutive days, (the Compound composition chosen is the one determined to be most efficacious in the C57BL/6J model, with efficacy determined to be increased number and/or size of neogenic hair follicles). One dose of the Compound is delivered, using the most efficacious dose as described above, systemically and, in a separate experiment, a dose is delivered topically. Additionally, in vivo confocal microscopy, histology and/or photography is performed daily (until the end of the experiment) following scab detachment in order to monitor hair follicle neogenesis (confocal microscopy is non-invasive but does require
Anesthesia). An additional set of mice are treated with the Compound or vehicle or neither, with the exception that the xenografted mice are not wounded, in order to assess the effect of the Compound in the absence of wounding. At approximately 2 weeks post-scab detachment, all mice are anesthetized with ketamine (80 mg/kg)/xylazine (20 mg/kg) delivered i.p. in a volume of < 100 µl, and monitored by toe pinch to determine the surgical plane of anesthesia. Subsequently, they have a terminal blood draw (to detect drug in the plasma), and are euthanized. The wound is then removed, which is trisected with one-third taken for biochemistry, one third for determination of Compound levels in the skin using mass spectrometry, and one third for histology/immunohistochemistry. For the experimental design in which the human (xenograft) skin is wounded, there are 3 treatment groups (Compound, vehicle, no Compound or vehicle) with 2 different delivery methods (IP and topical). With 10 mice per group, this requires 50 mice (only 1 group of "no Compound or vehicle"). The most efficacious combination of Compound and mode of delivery is repeated in 3 more independent experiments (with only Compound-treated and vehicle groups), thus adding 60 more mice, giving a total of 110 mice. An identical experiment is carried out, but without dermabrasion wounding (epidermal removal), requiring an additional 110 mice. This yields a total of 220 mice for the wounding and Compound portion of this experiment. An additional 20 mice are needed for the optimization of microdermabrasion settings and split thickness versus full thickness xenografts. Considering that the "take" rate of human skin xenografts is approximately 50%, the total number of mice to optimally receive human skin grafts is approximately 500.

11. **EXAMPLE 6: PHYSICOCHEMICAL PROPERTIES OF CHIR99021**

11.1 **SOLUBILITY**

CHIR99021 was purchased from Stemgent Corporation, MA. The solubility of CHIR99021 was determined in topical excipients. The results are provided in Table 2.

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.30</td>
</tr>
<tr>
<td>Propylene Gycol</td>
<td>3.56</td>
</tr>
<tr>
<td>PEG 300</td>
<td>10.21</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1.34</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.56</td>
</tr>
<tr>
<td>Glycerol</td>
<td>0.32</td>
</tr>
<tr>
<td>1:10 Water:HPCD</td>
<td>0.29</td>
</tr>
</tbody>
</table>
Solubility of CHIR99021 was determined as a function of pH. In this experiment, 10 mg of compound was dissolved in citrate buffer at pH 3, citrate buffer at pH 5, phosphate buffer at pH 7, and phosphate buffer at pH 9. The results are shown in Table 3.

**Table 3. Solubility of CHIR99021 as a function of PH**

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer at pH 3</td>
<td>3.1</td>
</tr>
<tr>
<td>Buffer at pH 5</td>
<td>1.1</td>
</tr>
<tr>
<td>Buffer at pH 7</td>
<td>0.5</td>
</tr>
<tr>
<td>Buffer at pH 9</td>
<td>0.2</td>
</tr>
</tbody>
</table>

11.2 **STABILITY**

Stability of CHIR99021 in IN HCl, IN NaOH and 1M hydrogen peroxide was determined. A 1 mg/ml solution of CHIR99021 was prepared in PEG300, with heating to 40 °C. The solution was then diluted to a concentration of 0.5 mg/ml with 1N HCl, IN NaOH and 1M hydrogen peroxide. The solution was incubated at 40 °C for a week and analyzed by HPLC.

11.3 **PARTITION COEFFICIENT**

The partition coefficient (Log P) of CHIR99021 was determined to be 4.5, using the shake-flask method. In this method, a pre-determined amount of the compound was shaken in an equivolume mixture of n-octanol and water for 24 hours at 37 °C. The mixture was then separated into two layers using a separatory funnel. Each of the layers was analyzed for dissolved compound using high performance chromatography (HPLC). Log P was determined by:
12. **EXAMPLE 7: A DERMAL FORMULATION FOR CHIR99021**

A stable oil-in-oil emulsion formulation was developed for CHIR99021, at a maximum concentration of 10 mg/ml. The formulation was developed for animal studies to assess *in vivo* pharmacokinetics of the formulation and hair follicle neogenesis when applied topically to wounded murine skin.

The formulation prepared was developed to be easily applicable to a wound via a blunt-tipped applicator attached to a 1 ml syringe. The formulation extruded as a flowable cream, spreading over the wound as the formulation warmed up to body temperature. The formulation was viscous with no-run off, after application to the skin.

The formulation was tested for physical and chemical stability for 6 weeks at 40 °C. The formulation was physically and chemical stable for this period of time.

Briefly, CHIR99021 was dissolved first in neat diethylene glycol-monomethyl ether. A solution was prepared that contained menthol, propyl paraben, methyl paraben, PEG300 and PEG 1000, which was added to lanolin alcohol, melted at 70 °C, and mixed (the "lanolin alcohol composition"). The solution containing CHIR99021 was added to the lanolin alcohol/menthol/PEG300/paraben composition and the resulting thick mixture was rolled overnight at room temperature. Compositions comprising compound at concentrations from 0.00001 mg/ml to 10 mg/ml were generated.

The composition of the formulation was as follows: 15% lanolin alcohol, 25% diethylene glycol monomethyl ether, 15% PEG300, 33.70% PEG1000, 0.1% propylparaben, 0.1% methylparaben, 0.1% menthol.

13. **EXAMPLE 8: IN VITRO RELEASE PROFILE OF CHIR99021 FROM A SKIN-COMPATIBLE TOPICAL FORMULATION.**

The following study was designed to determine the release of CHIR99021 from a topical formulation, using flow-through cells to simulate release of CHIR99021 *in vivo*. The topical formulation of CHIR99021 (10 mg/ml) was tested in in-line cells (PermeGear, Inc., Hellertown, PA) (Figure 6) fitted with either a polysulfone membrane (0.45 micron, Tuffryn, Pall® or a dialysis membrane (MWCO 25,000 Dalton; Spectrum Spectra/Por® lot 324263). The membranes were allowed to equilibrate with the receiver solution for approximately 30 minutes prior to the application of the test products. The receptor compartment consisted of a
solution of 50/50 PEG300/water, a solution which provides adequate sink conditions in the receptor fluid. Automated sampling of the receptor was performed every hour for the first five hours and every four hours thereafter for 12 hours.

[00766] The formulation contained 0.1% CHIR99021, 15% lanolin alcohol, 15% benzyl alcohol, 44.70% PEG 1000, 0.1% propyl paraben, 0.1% methyl paraben and 0.1% menthol.

[00767] The receptor solutions at each time point were analyzed by HPLC for CHIR99021, and the release profiles are shown in Table 4 as percent cumulative release vs. time in hours. These data show that CHIR99021 is released at a sustained rate.

**Table 4**

*In Vitro* Release of CHIR99021 from a hydrophobic matrix

<table>
<thead>
<tr>
<th>Time-Point in hours</th>
<th>Percent drug released</th>
</tr>
</thead>
<tbody>
<tr>
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<tr>
<td>26</td>
<td>22.9</td>
</tr>
</tbody>
</table>

14. **EXAMPLE 9: IN VITRO RELEASE OF CHIR99021 FROM A HYDROPHILIC MATRIX**

[00768] An aqueous solution of 0.1 mg/ml CHIR99021 was prepared in an aqueous formulation that contained 10% PEG300, 3% chitosan gluconate and water. The formulation was clear and extrudable. The experiment was performed in a similar manner as in the example in Section 13. Figure 7 shows the release kinetics of CHIR99021 from this matrix. The release experiment was carried out to 100% cumulative drug released.
15. **EXAMPLE 10: IN VITRO RELEASE OF CHIR99021 FROM AN ORGANOGEL MATRIX**

[00769] Figure 8 shows the in vitro cumulative release of CHIR99021 from a liquid-crystalline lecithin/PEO-PPO-PEO/Lanolin Alcohol organogel using a Franz Cell-type set-up, with a cellulose membrane. Due to low saturated solubility of CHIR99021 in water, the receptor chamber contained 50% PEG300. Analysis was performed by RP-HPLC.

16. **EXAMPLE 11: MOUSE MODEL OF COMPOUND TREATMENT FOLLOWING INTEGUMENTAL PERTURBATION USING FULL THICKNESS EXCISION OR DERMABRASION**

[00770] The example in this section provides exemplary protocols for integumental perturbation by full thickness excision (FTE) and dermabrasion in mice and the results of studies conducted using these protocols that demonstrate the efficacy of combination therapies comprising Compound and integumental perturbation in inducing hair growth.

[00771] Specifically, this example provides protocols using two different combinations of Compound and integumental perturbation to induce hair follicle neogenesis: 1) full thickness excision + topically delivered Compound; and 2) dermabrasion + topically delivered Compound.

16.1 **FULL THICKNESS EXCISION PROTOCOL**

[00772] 1. Twelve (12)-day old C57BL6/J mice pups are used. They are fed high fat food from the day they arrive to the day of surgery (10 days).

[00773] 2. When the mice are 21 days old, full thickness excision (FTE) surgery is carried out.

[00774] 3. Mothers, domes, and high fat food is removed from the cages. Food is replaced with normal food.

[00775] 4. All pups for the experiment are placed into a large container to randomize.

[00776] 5. The pups are weighed one at a time. If under 7 g, they are placed into a separate "runts" cage and FTE is not performed on these mice. If weight is made, mice are injected with calculated buprenorphine ("BUP"; 0.05 mg/kg). A stock BUP solution may be used that works out to 0.009 mg/ml, so 50 µl per 9 g mouse is injected. Place 6 mice per cage until all mice are weighed and given buprenorphine. During the administration of the anesthesia and for the duration of the time that the mice were anesthetized, the cages are placed on heating pads which are set to low heat.
a. ALTERNATE: If working with many mice (50+), it may be preferable to stagger the BUP dosing in order to avoid the effect of the analgesic wearing off before surgery. It is preferable to stay as close as possible to BUP administration at 1-2 hours pre-surgery.

6. One hour after giving the analgesic, the mice from one cage are injected with ketamine (70 mg/kg) / xylazine (8 mg/kg). Again, a stock that works out to 50 μl per 9 g mouse may be used.

a. ALTERNATE: Giving an additional 10 μl KX above the calculated dose (based on weight) seems to put the mice out to toe pinch quicker without increased fatality. However, it is only suggested to do this if few mice are not fully going out to toe pinch.

7. Once mice are anesthetized, the proper number is ear-punched, their weight recorded, their back hair shaved with clippers, and a 1.5 cm x 1.5 cm box is marked on the rear dorsum.

8. Eye ointment is applied to the mice, to keep their eyes from drying out during their immobilization, and the cages are pre-warmed on low-setting heating pads.

10. The surgery site on the rear dorsum is sprayed/wiped with alcohol (70% ethanol) to prevent infections.

11. A full thickness excision (FTE) (1.5 cm²) along the marked lines is cut out from the skin (cut inside the vertical lines and directly on the horizontal lines) using a pair of blunt-tip scissors and curved-tip forceps.

a. ALTERNATE: If letting mice live past day-5 post scab detachment, India ink should be used to mark the corners and sides of the wound (8 "dots").

12. Finished mice are placed back in the pre-warmed cages and the cages are left on the pads until all mice awake. Eye ointment is reapplied if/when necessary.

13. Each cage is supplied with a dish of wet food (moistened regular chow), regular dry chow, water, a dish of flavored JELL-O®, and a water bottle with flavored Prang (bio-serv.com, F2351-S; also avail. From Fisher) mixed in to aid in hydration. Saline is administered to mice that exhibit signs of pain, dehydration, malnourishment, or stress. Optionally, all cages can be provided with flavored Prang for the duration of the experiment in addition to their water supply. The mice may also be given flavored JELL-O® for the 2 days post surgery (JELL-O® when introduced in earlier experiments for rehydration purposes had a positive effect - mice eagerly ate the JELL-O® and looked healthier on days following the procedure).
14. The mice are monitored and weighed over the next two days, as well as 2-3 times per week thereafter. The FTE wounds are allowed to heal by secondary intention. Dose mice AM and PM (approximately 10 AM and 5 PM) with BUP on day 1 after FTE. Dose AM and if necessary in PM on day 2. Replace JELL-O® or water as needed.

15. Mice are monitored daily for scab detachment (occurs 11-18 days post FTE).

16. On the day of scab detachment, mice are placed into treatment groups as follows: (Optionally include no treatment with mock handling [mock handling = Pick up and handle mice as if being dosed. An empty capillary tube is used to mock spread out drug onto their back under the bandage. This may be done to ensure all mice undergo the same stresses during dosing]); Placebo (n=15); 0.01 µg Compound (e.g., CHIR99021) (n=15); 0.1 µg Compound (n=16); 1 µg Compound (n=16); 10 µg Compound (n=15); 100 µg Compound (n=15) (numbers correspond to the mice in the actual experiment summarized in Sections 16.3 and 16.4, in which 90 mice remained alive at the time of scab detachment).

16.3 Bandages (to prevent mice from licking off the drug) are applied as follows: On the day of scab detachment, the mice are administered 75% of a normal dose of ketamine-xylazine according to weight. While the mice are anesthetized, the posterior dorsal, posterior lateral, and posterior ventral sides are shaved and treated with Nair. Care is taken to ensure that Nair did not cover the wounded area. Mice are then wrapped in Tegaderm & Telfa pad bandages. The Tegaderm is tightly wrapped around the mouse with the Telfa pad fixed over the wound / treatment area. The first treatment is administered once the bandages are in place. The mice are placed back into their cages before the anesthesia wears off. If at any point during the treatment period the mice escape from their bandage, the bandage is replaced. Mice are checked for bandages at every dosing interval.

16.4 Dosing is as follows: The day of scab detachment is denoted as Scab Detachment Day 0 (SD0). Mice in treatment groups receive one dose on SD0 and another dose 2 days later (SD2; i.e., 48 h after the first dose) (2 total doses). Drumphond wiretrols and accompanying 100µl capillary tubes are used to dispense 100µl onto the wound site of each mouse. The tube is then used to spread out the drug to encompass the entire wound. One capillary tube is used per mouse. Drug treatment vials are replaced when necessary.

17. All mice are harvested on SD5, including mice used for determination of "peak" and "trough" Compound levels (see below). The mice are not treated after SD2, nor on their respective harvest day (SD5), unless, optionally, that mouse is used for a peak dosage sample, i.e., selected as a peak value point for testing Compound levels in blood and skin. Some mice from each group were selected for evaluation of Compound levels in the skin and
blood. Per group, three mice are used for "peak" levels and three mice for "trough" levels. Mice selected for the peak levels have blood drawn 1 hour post AM dosing on SD5 (9 total doses). Mice selected for "trough" levels have blood drawn 18-24 hours post SD4 PM dose (i.e., prior to harvest without treatment). On day 5 post scab detachment, approximately 0.5 mL of blood is collected per mouse, using the cheek lancet technique, and the mice are sacrificed. Wound skin is then analyzed by confocal microscopy and collected as described below.

[00793] 19. Processing of mice for data collection: In preparation for in vivo scanning laser microscopy, the hair surrounding the healed wound is trimmed to reveal the wound surface. The surface is imaged with a confocal microscope; this includes 1-3 Vivablocks (8 mm x 8 mm) at depths between 40-100 µm, and 1-3 Vivastacks (500 µm x 500 µm) of areas with many neogenic hair follicles (NHF's) or interesting features. The mice are sacrificed, and the entire wound is excised (excluding normal surrounding tissue as much as possible), and tissue allocated in a procedure that lasts ~ 3-5 minutes per mouse after the time of death. Following excision, the wound is bisected. Half of the wound is placed on a section of a 3x5 inch index card and stored in cold 4% Paraformaldehyde for histology. The other half is frozen on dry ice for biochemical assays, including Compound levels. The frozen samples are placed on dry ice for the duration of the harvest period and then transferred to a -80°C Celsius freezer for long term storage. The histology samples are taken out of the 4% Paraformaldehyde solution following overnight fixation and moved to 30% sucrose/1 X PBS. Within 24 hours the samples are taken from the sucrose solution and dabbed dry, then embedded in OCT. The OCT is frozen in a slurry of crushed dry ice and 2-O-methyl-butane. Cryosections are generated for histology. In the actual experiment the results of which are summarized in Sections 16.3 and 16.4, for quantification of histology, approximately 10 slides were generated from the cut edge (midline) of the sample, and the first slide was stained with hematoxylin and eosin and was used for quantification. In the minority of cases, the tissue sections on the first slide were damaged, which then necessitated use of the next slide in the sequence. Only samples in which the wound was clearly demarcated from the adjacent normal were used for quantification of neogenic hair follicles.

[00794] Sections 16.3 and 16.4 provide the results of an experiment in which the foregoing FTE protocol was used.
16.2 DERMABRASION PROTOCOL

In the following protocol, a microdermabrasion device is used to perform dermabrasion.

1. Mice are 10 weeks old on the day of procedure.

2. Mice are weighed and treated with buprenorphine ("BUP"; 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 dermabrasion (two doses, ~ 8 hours apart), and as needed the second day after dermabrasion.

3. After one hour has passed mice are re-weighed (because they are not ear punched before ketamine/xylazine dose), anesthetized with ketamine (80 mg/kg) / xylazine (8 mg/kg), and ear punched for identification. Mice are given an eye ointment to keep their eyes from drying out during their immobilization.

4. Once the mice have ceased being mobile both the left and right sides of the dorsal rear back skin are clipped.

a. Nair® is applied for 1 minute to the right and left flank, the hair wiped off with a wet paper towel, and dried with paper towel.

5. The mice are dermabraded once they do not react to a toe-pinich.

6. After dermabrasion, the 4 corners and the midpoints along the edge of the wound are tattooed with an injection of India ink (using a tuberculin syringe); 8 total tattoo marks made.

7. Prior to waking up, the mice are bandaged as in the FTE protocol, and dosing initiated (day 0) as follows: Animals are assigned to groups as follows: (Optionally include no treatment with mock handling [mock handling = Pick up and handle mice as if being dosed. An empty capillary tube is used to mock spread out drug onto their back under the bandage. This may be done to ensure all mice undergo the same stresses during dosing]); Placebo (n=15 in Placebo A experiment, which was contaminated with CHIR99021, and
n=20 in Placebo B experiment, in which the Placebo A experiment was repeated without contaminating CHIR99021; it is noted that no significant effect on the overall results were observed comparing Placebo A and Placebo B; 0.01 µg Compound (e.g., CHIR99021) (n=15); 0.1 µg Compound (n=15); 1 µg Compound (n=15); 10 µg Compound (n=15); 100 µg Compound (n=15) (numbers correspond to the mice in the actual experiment using CHIR99021 summarized in Sections 16.3 and 16.5, in which approximately 90 mice remained alive at the time of scab detachment).

8. The mice are 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 wake up.

9. The mice are dosed again on day 2 post-DA.

10. During the dosing period, weights and observations of mice are recorded daily. The time of delivery of dose is recorded.

11. Tissue is harvested from 5 mice of each group (8 mice for the Placebo B experiment) on day 5 post-DA for i) bioassay of target, ii) assay for concentration of Compound in skin, and iii) histology.

a. This is done by resecting the skin from the right side and observing the ink spots from the dermal side of the skin. The wound area is cut out and divided into three pieces for assays as indicated above.

12. Blood samples are also collected into potassium-EDTA vacutainers (2 ml capacity, lavender cap) from these same animals via cheek puncture lancets. The blood is centrifuged (maximum speed in non-refrigerated microfuge), the supernatant (plasma) is removed to a separate microfuge tube, labeled, and then frozen (along with the RBC cell pellet) for storage and shipment. Blood is taken within 1-2 hours of the last dose to determine peak levels of Compound in skin, or alternatively prior to harvest without dosing if trough levels are desired.

13. Remaining animals (10 per group; for Placebo B = 12 mice) are allowed to survive until ~21 days post dermabrasion at which time they were clipped and razored (disposable razor) on both the wound and non-wounded skin. A full thickness excision of skin from both treatment sites is excised between 21 and 25 days post-DA. The skin samples are separately placed on 3x5 note cards and the surface of the excised skin (wound - right side; non-wounded - left side) analyzed by confocal microscopy in order to quantify the thickness of the hair shafts, density of hair shafts, the density of hair pores, and the number of shafts per pore.
14. Following confocal microscopy, the skin is divided into three pieces as above.

a. For biochemical assays, samples are immediately placed in Eppendorf tubes and frozen on dry ice, after which they are transferred to -80 °C freezer.

b. For histology, a piece of tissue is immersed in 4% PFA/PBS and stored at 4 °C until processing for paraffin histology; or for cryosectioning, then changed to 30% sucrose/PBS (after overnight fixation) for between 12-24 hours. Samples are then embedded in optimal cutting temperature (OCT) and stored at -80 °C.

Sections 16.3 and 16.5 provide the results of an experiment in which the foregoing DA protocol was used.

16.3 SUMMARY: PHARMACOKINETIC PROFILES OF A TOPICAL FORMULATION OF CHIR99021 IN TWO WOUNDING MODELS (DERMABRASION AND FULL THICKNESS EXCISION)

An experiment to assess pharmacokinetics of topically administered CHIR99021 in mice was undertaken using the protocols described in Section 16.1 and 16.2 supra, as follows.

16.3.1 INTRODUCTION/STUDY GOALS

CHIR99021 is a small molecule GSK-3P inhibitor. It is a cell permeable compound and does not exhibit cross-reactivity against cyclin-dependent kinases (CDKs). It has a 350-fold selectivity toward GSK-3P compared to CDKs with a K, of <10 nM in vitro. In vivo, the concentration for GSK-3P inhibition was estimated to be approximately 100 times great, for example, in a cell-based assay using CHO-IR cells, the EC50 was measured to be 763 nM. See, Ring et al, 2003, Diabetes 52:588-595, the contents of which are incorporated herein by reference in its entirety.

The pharmacokinetics studies presented in this example were geared toward a time-course characterization of skin and blood levels of CHIR99021 obtained with doses administered twice - once on the 1st day and once on the 3rd day. The dose range and regimen tested in this example was used in the corresponding hair follicle neogenesis studies (HFN) presented in Sections 16.4 and 16.5. Furthermore, the concentration as a function of dose level of CHIR99021 was compared in the extravascular (skin) compartment and vascular compartment. General assessments of single dose versus multiple dosing regimens were performed.
16.3.2 PHYSICOCHEMICAL PROPERTIES OF CHIR99021

[00822] CHIR99021 (C_{22}H_{28}Cl_{2}N_{8})_{6}-(2-((4-(2, 4-dichlorocyclohexyl)-5-(4-methyl-1H-imidazol-2-yl) pyrimidin-2-yl)arnino)ethy)arnino)nicotinonitrile) is a highly hydrophobic molecule, of molecular weight 465.34 g/mol.

[00823] CHIR99021 is soluble to 10 mg/mL in PEG 300, to 25 mg/mL in Benzyl Alcohol and to 15 mg/mL in diethylene glycol-monomethyl ether. Solubility in aqueous solutions is < 100 µg/mL.

[00824] The log P of CHIR99021 was determined to be 4.5.

[00825] Protein binding of CHIR99021 is unknown.

16.3.3 FORMULATION DEVELOPMENT CRITERIA

[00826] The formulations for the PK/PD experiments had the following acceptance criteria: (a) solubility to 10 mg/mL, in order to achieve the entire range of doses planned (0.0001 mg/mL to 10 mg/mL), (b) no run-off of the formulation when applied to the wound, and (c) stability at 40 °C/75% RH for 6 weeks.

[00827] One of the formulations was selected for release studies through regenerated cellulose membranes mounted on Franz Diffusion chambers. Specifically, 0.1 g of the formulation was measured in the donor chamber of the diffusion cell. The receptor chamber contained 10% PEG 100 to allow "sink" conditions to be maintained. The solubility of CHIR99021 in water is 100 µg/mL, so phosphate buffer as the receptor solution results in rapid saturation with drug.

16.3.4 FORMULATION COMPOSITIONS

[00828] The formulations for the PK/PD studies had the following compositions, as shown in Table 5. Briefly, CHIR99021 was dissolved first in neat Diethylene Glycol-monomethyl ether by heating the suspension in a 30 °C bath. Another solution containing menthol, propyl paraben, methyl paraben, PEG300 and PEG1000, was added to lanolin alcohol melted at 70
°C and mixed by vortexing. Lastly, the CHIR99021/PEG300 was added to the lanolin alcohol composition. The resultant thick mixture was rolled overnight at room temperature.

Table 5. Formulation Compositions

<table>
<thead>
<tr>
<th>Number</th>
<th>[CHIR99021], mg/mL</th>
<th>Lanolin Alcohol (%)</th>
<th>Diethylene Glycol-monomethyl ether, (%)</th>
<th>PEG 300 (%)</th>
<th>PEG 1000 (%)</th>
<th>Menthol (%)</th>
<th>Propyl Paraben (%)</th>
<th>Methyl Paraben (%)</th>
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16.3.5 METHODS

(00829) The formulations prepared as described in Table 5 were analyzed by RP-HPLC, for CHIR99021 content. The column conditions were as follows: Column Temperature: 30 °C; Detector Settings: 245 nm and 274 nm; Flow Rate: Gradient (See Table 6), 1 mL/min.

Table 6. Time Table for CHIR99021 HPLC gradient

<table>
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<tr>
<th>Time (Minutes)</th>
<th>% Mobile Phase A</th>
<th>% Mobile Phase B</th>
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16.3.6 ANIMALS/STUDY DESIGN/DOSE ADMINISTRATION

[00830] The study was conducted to IACUC regulations. All animals were wounded by either FTE or DA, at approximately n=16 per group. The study designs for both FTE and DA pharmacokinetics are provided in Figures 9 and 10. Briefly, the pharmacokinetic (PK) profile of CHIR99021 in the vascular and extravascular compartments (skin) was assessed by administering the drug formulation on two different wound models: Dermabrasion (DA) and Full-thickness Excision (FTE). The CHIR99021 concentrations tested in the pharmacokinetic
experiments were 0.0001 mg/mL, 0.001 mg/mL, 0.01 mg/mL, 0.1 mg/mL, 1 mg/mL and 10 mg/mL.

[00831] In the DA model, the 0.1 mL of the CHIR99021 formulation was delivered via syringe in the 1st (T=0) and 3rd day (T=48 hours) after wounding. In the FTE model, the formulations were delivered on the 1st day (T=0) and 3rd day (T=48 hours) after scab detachment (-10 days after wounding). The formulations were prepared at CHIR99021 concentrations of 0.001 mg/mL, 0.01 mg/mL, 0.1 mg/mL, 1 mg/mL and 10 mg/mL. Absolute doses administered to the mice were 0.1 µg, 1 µg, 10 µg, 100 µg and 1000 µg, per mouse, per dose. The pharmacokinetic experiments were carried out as PK Exp #1 and Exp #2 in both models, to assess the PK profiles of the full dose and time range. The time-points for skin and blood sampling were counted in hours after the first dose for each model. For PK Experiment #1, the time-points were 1 hour, 4 hours, 24 hours, 48 hours, 49 hours, 52 hours, 72 hours and 96 hours, with a dose range between 0.1-1000 µg. For PK experiment #2, the dose range for DA was between 0.001 - 1 µg.

[00832] For the FTE model, the dose range for PK Experiment #1 was between 0.1-1000 µg, with skin and blood sampling time-points of 1 hour, 4 hours, 24 hours, 48 hours, 49 hours, 52 hours, 72 hours and 96 hours. PK Experiment #2 (FTE) assessed the PK profile in skin and blood for a single dose (100 µg) to a duration of 14 days. PK Experiment #3 (FTE) assessed the PK profile of CHIR99021 in skin and blood compartments in the dose range 0.1 -0.01 µg. The tissue and blood samples were measured using a qualified LC/MS/MS technique.

16.3.7 RESULTS AND DISCUSSION

[00833] The formulations were stable at 25 °C and 40 °C for six weeks. The formulations adhered well to tissue, with no run-off post application. The formulations were smooth to the touch and easily administered to the wound. All excipients were GRAS and in accordance to levels specified by the FDA IIG Database. Briefly, all formulations contained lanolin alcohol, diethylene glycol -monomethyl ether, PEG 300, PEG 1000, menthol, propyl paraben and methyl paraben. The concentration of CHIR99021 was varied, with slight changes in the PEG300 concentration accordingly (Table 5).

[00834] In the full-thickness excision model, higher levels of CHIR99021 were measured at all sampling time-points in both the skin and vascular compartments with increases in absolute dose levels between 0.1 and 1000 µg. At 5 days, the lowest dose of 0.01 µg produced an average skin concentration of ~51 nM and the highest dose of 1000 µg produced
an average skin concentration of 17,687.5 nM CHIR99021 (See Figure 11). In contrast, concentrations in the vascular compartment were markedly lower. At the highest dose level of 1000 μg, the concentration of CHIR99021 in whole blood at 96 hours (5 days) was ~20 nM; the blood concentration of dose levels ≤ 1 μg were less than 1 nM (Figure 12). The skin levels did not markedly increase with the second dose, indicating that the transport pathway for the CHIR99021 may be saturated with hydrophobic formulation excipients still at the target site. Furthermore, a sustained presence of CHIR99021 in the skin compartment was noted at all doses (See Figures 12 and 13). In contrast, blood levels at all doses declined rapidly, indicating more rapid clearance from the vascular compartment as opposed to the extravascular compartment, which is the skin, in this case. There are several plausible causes for this effect. Topical hydrophobic drugs that bind to tissue proteins may demonstrate a slower clearance; binding to proteins also results in a higher volume of distribution. However, protein binding effects are typically observed in both vascular and extravascular compartments. Saturation of the skin with dose 1 of the formulation may also result in a modest increase in skin levels if there is high residual drug and/or excipients still at the tissue site. A rapidly clearing drug typically has a marked increase at the second dose, unlike CHIR99021, which has a slow rate of clearance of not just the active agent, but also the formulation carrier depot. It is suggested that the depot properties of the formulation excipients may have contributed to the sustained presence of active agent in skin.

A similar pattern was noted in the dermabrasion cohort. In animals that received dermabrasion, CHIR99021 levels in skin and blood increased with increasing dose levels (0.001-1000 μg) (Figure 14). At 96 hours (5 days), the lowest dose of 0.001 μg produced an average skin concentration of 0.22 nM and the highest dose of 1000 μg produced an average skin concentration of 339,996 nM of CHIR99021. As in the FTE model, CHIR99021 concentrations in the vascular compartment were orders of magnitude lower, with even the highest dose of 1000 μg producing drug concentrations of ~15 nM at 96 hours, much lower than levels that may cause systemic toxicity (Figure 14). The 96th hour time-point was taken as the equilibrium time-point for the CHIR99021 to have distributed in its vascular and extravascular compartments. As in the FTE cohort, the animals that received DA demonstrated sustained skin concentrations of the CHIR99021 at all doses. The concentration of CHIR99021 in the vascular compartment declined at a faster rate. These data suggest that the sustained presence of CHIR99021 in the skin (in contrast to blood) is due to the depot properties imparted by the formulation components. In summary, Figure 14 shows that: (i) dose response achieved at all time-points in both skin and blood; (ii) dose response is
maintained 48 hrs after last dose administration; (iii) blood levels are many-fold lower than
skin levels; and (iv) blood levels at dose levels 0.01 and 0.001 µg are below detectable by
LC/MS/MS.

[00836] The skin concentrations achieved in the PK experiment spanned the in vivo
estimated level of GSK-3P inhibition (-700 nM).

[00837] Based on the foregoing results, dose concentrations that were selected for the hair
follicle neogenesis experiment were 0 (placebo), 0.01 µg, 0.10 µg, 1.00 µg, 10 µg and 1000
µg. There was good agreement in skin and blood concentrations achieved for the
pharmacokinetics and neogenesis experiments (Figure 15) in both wounding models.

. 16.4 HAIR FOLLICLE NEOGENESIS AND
WOUND COVERAGE IN FTE EXPERIMENT

[00838] A pharmacodynamic study with topical CHIR99021 was conducted using the full
thickness excision (FTE) model as described in Section 16.1. Figures 16 and 17 show a trend
toward an increased number of germs and increased area of germ forming region,
respectively, in the 10 µg CHIR99021 treatment group. As shown in Figures 18 and 19, the
germ density in the germ forming area and the total wound area, respectively, are not affected
by the CHIR99021 at the doses tested. Notably, the experiments revealed an upward trend
for the 1 µg and 10 µg CHIR99021 treatment groups, and statistically significant increase of
the 10 µg CHIR99021 treatment group over the lowest dose and placebo groups, with regard
to germ density in the total wound area and wound coverage (germ forming region/total
wound area), as shown in Figures 20 and 21, respectively. Representative and highest
responding mice confocal images are shown in Figures 22 and 23, respectively.

[00839] In conclusion, and without being bound by any theory, increased wound coverage
appears to be mostly due to an increased area of the germ forming region and an increased
number of germs. Wound shrinkage does not appear to be involved in the CHIR99021-
induced increased coverage observed.

. 16.5 HAIR FOLLICLE NEOGENESIS AND
WOUND COVERAGE IN DA EXPERIMENT

[00840] A pharmacodynamic study with topical CHIR99021 was conducted using the
dermabrasion (DA) model as described in Section 16.2. Figures 24-27 show that, at all doses
tested, CHIR99021 administration had no statistically significant effect on hair shaft
diameter, shafts/pore, shaft density, or pore density.
17. **EXAMPLE 12: CLINICAL EVALUATION OF NEOFOLLICULAR DEVELOPMENT RESULTING FROM APPLICATION OF A COMPOUND PROVIDED HEREIN IN COMBINATION WITH DERMABRASION WITH OPTIONAL REPROGRAMMING**

[00841] The following example provides a protocol for demonstrating the importance of timing of a Compound treatment for the optimization of follicular neogenesis after epidermal disruption. In this protocol, patients are treated with a topical preparation of a Compound provided herein in combination with dermabrasion, optionally also in combination with an additional treatment to enhance hair growth. As controls, patients may be treated intermittently with the Compound (as described *infra*), dermabrasion alone (or with a vehicle, *e.g.*, petrolatum), or may not receive any treatment.

[00842] Although any patient population may be treated, preferred patients are Caucasian males 20-50 years of age, have androgenetic alopecia with the presence of a transition zone defined as an area possessing both terminal and miniaturized hairs, have a Fitzpatrick skin type 1-4 (higher Fitzpatrick skin type ratings are not preferred due to the increased risk of keloid formation and hypopigmentation in these subjects). Patients for whom treatment may be contraindicated (particularly at the clinical trial stage) are those who are currently participating in or have participated in any clinical study with an investigational drug within the thirty (30) days immediately preceding treatment, with current or recent use (<1 y) of isotretinoin (Accutane), currently taking hormone therapy, or steroids or other immunomodulators or have taken these medications within the past thirty (30) days (inhaled steroids are acceptable), currently using Rogaine or Propecia or used them in the past forty-five (45) days, immune compromised or undergoing therapy to treat an immune disorder, have a clinically significant medical condition that may interfere with the protocol described herein, have other active skin diseases (such as actinic keratosis or psoriasis) or skin infections (bacterial, fungal or viral, esp. HSV infection) in the area to be treated, have a history of keloids or hypertrophic scarring, hypersensitivity to lidocaine, poor wound healing, diabetes, or coagulopathy, undergoing current drug or alcohol abuse, psychiatric dysfunction, or other factors that would limit compliance, have sunburned skin, or who are currently taking anti-platelet agents other than aspirin.

[00843] Dermabrasion using alumina particles is performed on Day 0. Dermabrasion is performed to a depth of approximately 100 µM, which includes removal of the entire epidermis and disruption of the papillary dermis (detectable by a shiny, whitish appearance) inducing the formation of small pinpoints of blood in the treated area. Dermabrasion is
performed in two sites of the scalp skin corresponding to transitional areas (or advancing margin) of balding in the vertex region. The area is then allowed to heal without manipulation. A 4 mm punch biopsy is performed on days 11 and 14, and the presence of new hair follicles is examined in these subjects based on histological assessment. A third biopsy is optionally performed on Day 14 on an untreated area 1 cm away from the treated area to serve as histologic control. In the event that limited follicle neogenesis is observed on day 14, another biopsy may be performed on day 17. Subjects scheduled for day 11 biopsies for whom the scab in the wound detaches before day 8, will have the biopsy rescheduled for 3 days afterwards. Conversely, subjects for whom the scab has not detached by day 10 will have the biopsy visit rescheduled for 3 days after the scab detaches. It is expected that the scab will detach around days 6-10. Subjects return on day 46-50 for follow up photography of the treated area.

[00844] The protocol may be amended in accordance with the findings. For example, if dermabrasion causes presence of neogenic hairs in a 4 mm punch biopsy in, for example, at least three of the first 15 patients, then additional patients will be treated in two sites: one site corresponding to the area of greatest balding on the vertex and one site corresponding to a transitional area (or advancing margin) of balding in the vertex region.

17.1 DETERMINATION OF TREATMENT SITES

[00845] Two sites on each subject's scalp are identified for treatment, both corresponding to transitional areas (or advancing margins) of balding in the vertex region. Some patients may be treated in a site of greatest balding on the vertex region.

17.2 SCANNING LASER CONFOCAL MICROSCOPY

[00846] Scanning laser confocal microscopy (CM) is a non-invasive imaging technique that uses laser light to visualize the skin in vivo. Progress of treatment, starting at day 0, may be monitored using CM alone or in combination with photography. This fluid immersion microscope requires oil / water immersion to measure changes in the index of refraction within the tissue as detected by the reflected laser light. Real-time noninvasive confocal infrared imaging of the epidermis, papillary dermis, and superficial reticular dermis to a maximum depth of 350 µm is possible with resolution comparable to conventional light microscopy. Skin can be imaged in its native state without the fixing, sectioning, and staining necessary for histology. As such, dynamic processes can be noninvasively observed over an extended period of time. As a research tool, RCM has been reported to facilitate the in vivo

Confocal microscopy is performed using a device (Vivascope; Lucid, Inc.) used for human studies. Subjects are positioned in an inclined or sitting position to allow visualization of the treated area of the scalp. Each subject remains still in the imaging position for a minimum of 15 minutes of imaging per subject. A medical grade adhesive secures the fluid immersion ring to the surface of the skin. The ring remains attached throughout the imaging session and a new ring is applied on each test site. CM is performed on baseline Day 0, 7, and 14 (11 and 17 if punch biopsy was not taken on Day 14).

17.3 DERMABRASION

The procedure begins with shaving/clipping of the existing hair in the area to be treated followed by a thorough cleaning with antiseptic cleansing agent. Numbing agents, such as lidocaine HCL 2% and Epinephrine 1:100,000, are injected to anesthetize the surface to be treated. Standard dermabrasion is performed to a depth of approximately 100 μM, which includes removal the entire epidermis and disruption of the papillary dermis (detectable by a shiny, whitish appearance) inducing the formation of small pinpoints of blood in the treated area. Each dermabraded area is approximately a 1.5 cm x 1.5 cm square. Suitable dermabrasion devices are the ASEPTICO ECONO-DERMABRADER from Tiemann and Company, the DX system from Advanced Microderm (see, e.g., http://www.advancedmicroderm.com/products/tech_specs.html), or the M2-T system from Genesis Biosystems. Adhesive ocular shields are worn by the patient during the procedure to avoid complications due to aluminum crystals entering the eye (chemosis, photophobia,
punctuate keratitis) and the doctor should wear safety goggles. The dermabrasion tool is carefully maneuvered over the area to carefully remove layers of skin until the desired level is reached. The procedure usually takes only a few minutes.

Pre-dermabrasion, patients should be asked to: not wear contact lenses during the procedure, discontinue use of over the counter exfoliation products such as Retinol, Glycolic or other hydroxy acids. Salicylic acid, Beta hydroxyl acids 3 days prior to treatment, discontinue use of retinoids 30 days prior to treatment, not receive Botox or collagen injections for 2 weeks prior to treatment.

Following the procedure the treated skin will be red, swollen and tender, and the wound should be cared for as follows until new skin starts to grow; this usually takes 7-10 days: 1) Keep the area clean and dry for today. Do not cover, bandage, or otherwise manipulate the treated area; 2) Avoid touching the area when washing hair; 3) Pat the area dry. Do not cover, bandage, or otherwise manipulate the treated area.

The treated are may itch as the new skin grows and may be slightly swollen, sensitive, and bright pink for several weeks after dermabrasion.

The following measures are taken to prevent any complications.

Inform your doctor of any yellow crusting or scabs - this may be the start of an infection.

Swelling and redness should subside after a few days to a month. Persistent redness of an area could be the sign of a scar forming so contact your doctor immediately.

No swimming is permitted for the first 7 days following dermabrasion.

To avoid pigmentation, once the new skin is healed, keep out of the sun and apply a broad spectrum sunscreen daily for at least 3 months after microdermabrasion. Even the sun through window-glass can promote unwanted pigmentation.

17.4 PUNCH BIOPSY

The procedure begins with thoroughly cleaning the area to be biopsied with antiseptic cleansing agent. Lidocaine HCL 2% and Epinephrine 1:100,000 (approximately 0.5 cc to each site) are injected to anesthetize the site that will be biopsied. 4 mm punch biopsy is performed. Biopsied site is closed with 2 4.0 Ethalon sutures. Vaseline and band-aid are applied. Tissue samples are stored in formalin for histological analysis.
17.5 PRIMARY ENDPOINTS

[00858] Histologic analysis of hair follicle neogenesis following dermabrasion. The null hypothesis is that no (0) neogenic follicles will form, since that is the current dogma in humans. A positive response to treatment is characterized as the appearance of 3 or more neogenic follicles in a 4 mm punch biopsy.

[00859] Among the factors to be evaluated when determining success of treatment are: crusting/scabbing; comedones; infection; pigmented changes (e.g., absent, hypopigmentation (mild, moderate or severe), or hyperpigmentation (mild, moderate or severe)); scarring; re-epithelialization; or presence of hair follicles by gross observation.

17.6 SECONDARY ENDPOINTS

[00860] 1) To determine which day after epithelial disruption is follicle neogenesis most active.

[00861] 2) To quantify the number and morphological developmental stage of follicles in each biopsy.

[00862] 3) Clinical characteristics of dermabraded areas.

18. EXAMPLE 13: PERTURBATION/GSK-3 INHIBITOR/MINOXIDIL ALOPECIA CLINICAL STUDY

[00863] The example in this section provides a protocol for controlled cutaneous perturbation with dermabrasion and pharmacologic modulation with a topical GSK-3 inhibitor and topical Minoxidil (ROGAINE® FOAM) for inducing hair growth in male or female subjects with androgenetic alopecia.

[00864] Active treatment is compared to control treatment (see Figure 28). Active treatment includes the following steps: controlled cutaneous perturbation using dermabrasion (Day 0), followed by topical GSK-3 inhibitor twice daily for 1 week (Day 0 through Day 6), and then no topical therapy for the next 3 weeks (Day 7 through Day 27); and then starting 4 weeks after dermabrasion, topical minoxidil 5% foam (ROGAINE FOAM) applied twice daily for 5 months (Day 28 through Day 167). The control treatment includes the following steps: controlled cutaneous perturbation using dermabrasion (Day 0), followed by topical placebo twice daily for 1 week (Day 0 through Day 6), and then no topical therapy for the next 3 weeks (Day 7 through Day 27); and then starting 4 weeks after dermabrasion, topical minoxidil 5% foam (ROGAINE FOAM) applied twice daily for 5 months (Day 28 through Day 167).
This is a double blind, placebo-controlled, protocol with each subject receiving two treatment regimens randomized to different sites in the scalp. Once eligibility is confirmed (Day -6 / 0), subjects are subjected to Baseline photography that includes a pinpoint tattoo and hair dye. Two sites are assigned, each measuring 1.5 cm x 1.5 cm and designated right (R) and left (L) with a minimal distance of 2 cm between the two sites, identified in transitional areas of the balding vertex scalp, which has a very low density of terminal hairs. The two sites are selected such that the hair density at these two sites are as similar to each other as possible.

Subjects for this protocol are Caucasian males 20-65 years of age, providing written informed consent, who have androgenetic alopecia with the presence of a vertex transition zone defined as an area possessing both terminal and miniaturized hairs, Hamilton-Norwood type 3V, 4, 5, 5A, or 5V, with a vertex area large enough to accommodate both treatment sites, and Fitzpatrick skin type 1-4. Subjects taking 5 alpha reductase inhibitors, such as finasteride (PROPECIA), will be allowed to participate in this study, provided that this has been maintained at constant dose and dosing regimen beforehand, and the subject intends to continue on this stable regimen throughout the duration of the study.

Active treatment site: For each subject, on Day 0 dermabrasion of one of the two sites per subject is carried out using a hand-held dermabrader with a standard grit diamond fraise to achieve pinpoint capillary bleeding (estimated depth 100 microns, and therefore not anticipated to cause scarring). After the procedure, GSK-3 inhibitor is applied to the dermabraded site twice daily from Day 0 through Day 6. From Day 7 through Day 27, there is no topical therapy. Then from Day 28 through Day 167, minoxidil 5% foam is applied to the healed dermabraded site twice daily.

Control monotherapy treatment site: For each subject, the other site serves as control. This control site is treated exactly the same as the active site, except that after the dermabrasion, placebo gel (instead of GSK-3 inhibitor) is applied to the dermabraded site twice daily from Day 0 through Day 6.

In addition to the 2 specifically-targeted 1.5 cm x 1.5 cm areas described above, during the ROGAINE treatment period from Day 28 through Day 167, minoxidil 5% foam is applied twice daily to the other balding areas of his scalp according to the manufacturer's instructions for ROGAINE FOAM.

Subjects are subjected to photography and clinical evaluation after 3 months (Day 84) and 6 months (Day 168). After completion of topical treatment and the photography 'visit' on Day 168, the subject has a safety follow-up 2 weeks later (Day 182).
The number of photographically detectable hairs is determined prior to treatment, at Day 84 and at Day 168. Further, changes from pre-treatment to Day 84 and from pre-treatment to Day 169 in the hair shaft thickness of photographically detectable hairs is determined. The determined values are compared between the active treatment and the control treatment.

Safety and tolerability is monitored during the clinical protocol by targeted examination of the treated scalp sites and the reporting of adverse events (AEs).

The primary efficacy analysis is performed for the Full Analysis Set (all subjects who were enrolled and who received dermabrasion and at least one dose of study drug). Descriptive statistics is tabulated for each of the efficacy and safety endpoints.

19. **EXAMPLE 14: PROTOCOL FOR TOPICAL GSK-3 INHIBITOR FOR CICATRICAL ALOPECIA**

The following example describes a phase 2, open-label, single-arm study to explore the effect of controlled cutaneous perturbation and topical pharmacologic modulation for inducing hair growth in patients with primary scarring alopecia.

The study is designed to demonstrate, in areas of hair loss in patients with primary scarring alopecia, that removal of affected scalp tissue with fractional ablative laser treatment followed by treatment with topical GSK-3 inhibitor, such as a Compound, induces new follicles that grow hair shafts that can be detected and quantified by macrophotography.

19.1 **OBJECTIVES**

19.1.1 **EFFICACY OBJECTIVES**

Primary: Compare changes from Baseline to Day 84 in the number of photographically detected hairs.

Secondary:

i) Compare changes from Baseline to Day 168 in the number of photographically detected hairs.

ii) Evaluate skin biopsies taken on Day 168 for hair follicle structures and scar attributes
19.1.2 SAFETY OBJECTIVES

Safety objectives are to assess the safety and tolerability of topical Compound applied in combination with controlled cutaneous perturbation with fractional ablative laser treatment.

19.2 STUDY DESIGN AND PROCEDURES

This is an open-label, single-arm study. Once eligibility is confirmed, subjects undergo baseline photography, which includes a pin-point tattoo and hair dye. On Day 0, controlled perturbation is carried out using a fractional ablative laser. Following laser treatment, a formulation comprising a Compound is applied topically twice daily for 14 days from Day 0 through the end of Day 13. Subjects return after 3 months (Day 84) and 6 months (Day 168) for repeat photographic and clinical evaluations. On Day 168 a skin punch biopsy is performed in the treated area. The biopsy site is closed by sutures. The duration of the study per subject is 196 days, comprising a 14-day screening period, and 182-day treatment and follow-up period. The total duration of the study is approximately 12 months.

19.3 STUDY DRUG TREATMENT REGIMEN

The following timeline shows the study drug treatment regimen.

19.4 ENDPOINTS

Efficacy is evaluated as follows:
[00890] Primary endpoint: The change from Baseline to Day 84 in the number of hairs captured by photography

[00891] Secondary endpoints:

[00892] i) The change from Baseline to Day 168 in the number of hairs captured by photography

[00893] ii) Histological characteristics in a skin punch biopsy on Day 168

[00894] The safety and tolerability of controlled cutaneous perturbation by laser combined with topical application of Compound is evaluated and monitored through the collection of data from targeted examination of the treated scalp sites and the reporting of adverse events (AEs)

19.5 PATIENT POPULATION AND SAMPLE SIZE

[00895] The study enrolls eight patients (male or female), 18-65 years of age, providing written informed consent, who have lichen planopilaris or frontal fibrosing alopecia. Eligible subjects have one or more patches of alopecia that measure at least 1.5 cm x 1.5 cm in size and that are clinically quiescent with respect to inflammatory activity.

19.6 DATA ANALYSIS / STATISTICS

[00896] The primary efficacy analysis is performed for the Full Analysis Set (all subjects who were enrolled and who received laser treatment and at least one dose of study drug). Descriptive statistics are tabulated for each of the efficacy and safety endpoints.

20. EXAMPLE 15: SOLID-LIQUID IN-SITU CROSS-LINKING SPRAY ON A WOUND

[00897] Certain issues exist when preparing formulations that release sustained concentrations of GSK-3 inhibitor, 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. One way to slow down release of GSK-3 inhibitor 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 GSK-3 inhibitor. 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 GSK-3 inhibitor.

Microsphere encapsulating drugs can be used as ways to sustain release of a molecule. GSK-3 inhibitor can be encapsulated in poly (lactide-co-glycolide) (PLG) microspheres and thus modulating the release of GSK-3 inhibitor. The rate of release of GSK-3 inhibitor 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 GSK-3 inhibitor) will be sequestered in the hydrogel, releasing GSK-3 inhibitor 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 GSK-3 inhibitor-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(amiadimeine) (PAMAM) or poly(ethyleneimine)(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.
[00902] The GSK-3 inhibitor can be dissolved directly in the hydrogel components prior to
formation of the cross-linked hydrogel. Dissolution of the GSK-3 inhibitor directly in the pre-
hydrogel components may not result in sustained release.
[00903] 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 (+GSK-3 inhibitor-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 GSK-3 inhibitor) 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.

20.1 TWO-CHAMBER SPRAYER WITH A LYOPHILIZED SOLID IN ONE
CHAMBER AND A LIQUID IN THE OTHER CHAMBER

[00904] 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.
[00905] 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.

[00906] 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.

[00907] Considering which chamber the GSK-3 inhibitor-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 GSK-3 inhibitor 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.

[00908] 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.
20.1.1 REACTION OF POLYETHYLENE GLYCOL 4-ARMED AMINE (PEG-AM) WITH POLYETHYLENE GLYCOL 4-ARMED SUCCINIMIDYL ESTER (PEG-NHS)

[00909] 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 at the site.

[00910] 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 44.

[00911] Polymer 1: Polyethylene Glycol 4-Armed Succinimidyl Ester (PEG-NHS) was purchased from Nanocs, Catalog # 4APN05122010, Molecular weight 10,000 Daltons

[00912] 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 DVS1 with temperature/ RPM control
- Accument pH meter 25
- Water bath

**Procedures**

[00913] (1) **Preparation of Phosphate Buffer**

[00914] 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 1) 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 7.

<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>

[00915] (2) **Preparation of Phosphate Buffered Saline (PBS)**

[00916] The 1xPBS was prepared by dissolving 8.0 g of Sodium Chloride, 0.2 g of Potassium Chloride, 1.44 g of Sodium Phosphate Dibasic, and 0.24 g of Potassium Phosphate Monobasic in 1.0 L of water.

[00917] (3) **Preparation and Characterization of PEG-AM/PEG-NHS Hydrogels**

[00918] (a) **Preparation of PEG-AM/PEG-NHS Hydrogels**

[00919] 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)**

[00920] Two stock solutions of 5% w/w PEG-NHS and 5% w/w PEG-AM were prepared by dissolution of 50.0 mg of each polymer in 1.0 mL 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 8, the gel time increases with increased storage time in water, indicating hydrolytic instability of PEG-NHS.

Table 8: 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

Because of instability, PEG-NHS cannot be stored in an aqueous solution at ambient temperatures and should be stored in a solid form.

(b) Mixing of Solid PEG-NHS with PEG-AM Solution.

**TWO STEPHYDROGEL PREPARATION**

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).

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 9.
Table 9. 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

[00925] 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 9 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).

[00926] 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**

[00927] 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).

[00928] 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 10). 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 10. 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>

[00929] 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 10).

[00930] 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.

[00931] (4) Determination of Gel Time, As a Measure of Cross-linking Kinetics

[00932] 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.
(A) **EFFECT OF PH ON GEL TIME**

[00933] The effect of pH on gel times was investigated in the experiments (Experiment 08-06-mix, Table 11), 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.

[00934] 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 11 and Figure 45, 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.

**Table 11.** Effect of pH on Gel Time (0.1M Phosphate buffer concentration, PEG-NHS and PEG-AM concentration 5%)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS mg</th>
<th>PEG-AM μl</th>
<th>pH</th>
<th>Gel time</th>
</tr>
</thead>
<tbody>
<tr>
<td>08-06-mix1a</td>
<td>11.5</td>
<td>230</td>
<td>7</td>
<td>7'32&quot;</td>
</tr>
<tr>
<td>08-06-mix1b</td>
<td>11.1</td>
<td>222</td>
<td>7</td>
<td>7'28&quot;</td>
</tr>
<tr>
<td>08-06-mix1c</td>
<td>10.2</td>
<td>222</td>
<td>7</td>
<td>7'20&quot;</td>
</tr>
<tr>
<td>08-06-mix2a</td>
<td>11.3</td>
<td>226</td>
<td>7.5</td>
<td>3'50&quot;</td>
</tr>
<tr>
<td>08-06-mix2b</td>
<td>11.7</td>
<td>234</td>
<td>7.5</td>
<td>3'52&quot;</td>
</tr>
<tr>
<td>08-06-mix2c</td>
<td>11.7</td>
<td>234</td>
<td>7.5</td>
<td>3'37&quot;</td>
</tr>
<tr>
<td>08-06-mix3a</td>
<td>11.4</td>
<td>228</td>
<td>8</td>
<td>2'13&quot;</td>
</tr>
<tr>
<td>08-06-mix3b</td>
<td>12.1</td>
<td>242</td>
<td>8</td>
<td>2'17&quot;</td>
</tr>
<tr>
<td>08-06-mix3c</td>
<td>10.2</td>
<td>204</td>
<td>8</td>
<td>2'17&quot;</td>
</tr>
</tbody>
</table>

[00935] As can be seen in Figure 45, the increasing of pH from 7 to 8 decreases the gel time from 7'32" to 2'13".

(B) **EFFECT OF PEG CONCENTRATION ON GEL TIME**

[00936] The effect of pH on gel times was investigated in the experiments (Experiment 08-23-mix, Table 12), 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 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 12. 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>

[00937] As can be seen in Table 12 and Figures 46 and 4, 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.

20.1.2 PEG-NHS/PEI HYDROGELS

[00938] 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. Polyethylenimine (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.

[00939] In the examples below, the gels were prepared by the addition of PEI solutions in phosphate buffer to solid PEG-NHS. Polyethylenimine (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, Catalogs 485595, Lor# 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.

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 13, 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.

At 0.5% w/w PEI concentration, phosphate buffer capacity was not enough to maintain pH of its solution at 7.0, which, as can be seen in Table 13 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 48 gel time decreased when PEI concentration increased for all three phosphate concentrations.

The concentration of phosphate appeared to have an opposite effect on gel time—gel time increased when the phosphate concentration was increased (Figure 48). In the extreme case, 0.5M phosphate at 0.125% PEI failed to produce a gel (08-13-mix9, Table 13 (0.125% PEI in 0.5M Phosphate)).

Table 13. 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.

20.1.3 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 0.1 M 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 0.1 M 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 14). The experimental conditions, final reagent concentrations, and gel times for PEG-NHS/PEG-AM/chitosan hydrogels are shown in Table 14.

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 14, 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 14. 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>

[00948] 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.

[00949] (5) Determination of Equilibrium Swelling

[00950] 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.

[00951] 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.

[00952] 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:
Percentage Swell = ((Weight after swelling - Weight before swelling) / Weight before swelling) x 100

[00953] As outlined in Table 15, 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.

[00954] 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%.

[00955] 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.

[00956] 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.

[00957] d. Gel plugs were fabricated to test equilibrium swelling at room temperature.

Table 15. 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></td>
<td></td>
<td></td>
<td></td>
<td></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></td>
<td></td>
</tr>
<tr>
<td>Experiment</td>
<td>PEG-NHS</td>
<td>PEI</td>
<td>PEG-NHS</td>
<td>PEI</td>
</tr>
<tr>
<td></td>
<td>mg</td>
<td>µl</td>
<td>Conc., %</td>
<td>Conc., %</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></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG-NHS/PEG-AM/Chitosan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment</td>
<td>PEG-NHS</td>
<td>PEG-AM</td>
<td>Chitosan</td>
<td>PEG-NHS</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>

[00958] 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 16 and Figure 49. 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 16 and Figure 49). 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 49). 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 16 and Figure 49. 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 16, Fig. 49). 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 49). 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 16. 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>08-25-mix1a 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></td>
<td>08-25-mix1b 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></td>
<td>08-25-mix1c 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></td>
<td>08-25-mix2a 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></td>
<td>08-25-mix2b 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></td>
<td>08-25-mix2c 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></td>
<td>08-25-mix3a 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></td>
<td>08-25-mix3b 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></td>
<td>08-25-mix3c 7.5</td>
<td>7.5</td>
<td>15.0</td>
<td>102.3</td>
<td>245.1</td>
<td>139.6</td>
</tr>
</tbody>
</table>

PEG-NHS/PEI

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS Conc., %</th>
<th>PEI 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/PEI</td>
<td>08-25-mix4a 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></td>
<td>08-25-mix4b 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></td>
<td>08-25-mix4c 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></td>
<td>08-25-mix5a 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></td>
<td>08-25-mix5b 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></td>
<td>08-25-mix5c 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></td>
<td>08-25-mix6a 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></td>
<td>08-25-mix6b 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></td>
<td>08-25-mix6c 5.0</td>
<td>0.500</td>
<td>5.0</td>
<td>n.d</td>
<td>n.d</td>
<td>n.d</td>
</tr>
</tbody>
</table>

PEG-NHS/PEG-AM/Chitosan

<table>
<thead>
<tr>
<th>Experiment</th>
<th>PEG-NHS Conc., %</th>
<th>PEG-AM Conc., %</th>
<th>Chitosan 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/Chitosan</td>
<td>08-25-mix7a 4.5</td>
<td>4.5</td>
<td>0.23</td>
<td>9</td>
<td>101.1</td>
<td>280.8</td>
<td>177.7</td>
</tr>
<tr>
<td></td>
<td>08-25-mix7b 4.5</td>
<td>4.5</td>
<td>0.23</td>
<td>9</td>
<td>98.4</td>
<td>279.9</td>
<td>184.5</td>
</tr>
<tr>
<td></td>
<td>08-25-mix7c 4.5</td>
<td>4.5</td>
<td>0.23</td>
<td>9</td>
<td>100.9</td>
<td>301.6</td>
<td>198.9</td>
</tr>
</tbody>
</table>

* Weight b.s. - weight of gel plugs before swelling; Weight a.s. - weight of gel plugs after swelling

20.2 HYDROGEL TESTING ON SKIN

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 17). 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/PE1 Hydrogel: 0.5% PE1 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/PE1 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 17. Preparation of Hydrogels for Testing on Skin

<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>08-27-mix1</td>
<td>25.9</td>
<td>500</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>PEG-NHS/PE1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>08-27-mix4</td>
<td>25.7</td>
<td>500</td>
<td>5.0</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>08-27-mix3</td>
<td>24.0</td>
<td>500</td>
<td>49.6</td>
<td>4.4</td>
</tr>
</tbody>
</table>

21. **EXAMPLE 16: SOLID-LIQUID IN-SITU CROSS-LINKING SPRAY ON A BIODEGRADABLE SCAFFOLD THAT RELEASES A DRUG**

Example 15 is directed to sustained release of drug 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, GSK-3 inhibitor uptake into the dermis may become limited as the skin heals and slowly re-establishes its barrier function. Thus, sustained transport of GSK-3 inhibitor into the dermis may diminish as a thick, fibrous scab is formed on the wound. Accordingly, drug will be released from the microspheres sequestered in the hydrogel, but transport of the drug through the scab will likely be limited.

When the problem is transport of drug, such as a GSK-3 inhibitor, 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 drug, such as a GSK-3 inhibitor, will be from the scab into the healing dermis. Thus, the scab becomes part of the delivery system.

[00967] 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.

[00968] In the present example, the sprayer contains only the in-situ cross-linking polymer components. The drug is incorporated in the biodegradable scaffold.

[00969] This concept is advantageous and important implications for wound healing. The scaffold allows close contact of the drug system with the wound. The fibrin-incorporated drug delivery system is an excellent "scaffold" for cells to attach to.

21.1 DESCRIPTION OF THE EXAMPLE

[00970] 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.

[00971] 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. GSK-3 inhibitor will be incorporated in the biodegradable scaffold.

[00972] 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."

21.2 METHOD OF USE

[00973] 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.
As described in Example 15, 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.

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 15.

21.3 THE EXAMPLE

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. In this example, the drug was lithium gluconate. Varying the polymer composition of the scaffold matrix can modulate Li+ release rates from 3 days to 14 days.

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.

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 1g 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.
[00979] Scanning electron micrographs (SEM) were taken of the patches (see Figure 43). 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).

21.4 RESULTS

[00980] SEM. Scanning electron micrographs of 100/0 PLA and 0/100 PLG are shown in Figure 43. The micrographs demonstrate a fibrous texture.

[00981] 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.

[00982] Release Rates. As shown in Figure 43, 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.

[00983] 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.

[00984] 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.

In this example, the experiments were performed with lithium gluconate as the incorporated drug. 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.

EXAMPLE 17: 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.

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 GSK-3 inhibitor 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 GSK-3 inhibitor suspended in a FDA-approved liquid excipient. Chamber 2 could contain dissolved GSK-3 inhibitor in an aqueous sprayable gel. Co-spraying both forms of GSK-3 inhibitor provides instantly-bioavailable, GSK-3 inhibitor and a sustained form GSK-3 inhibitor made available as the micronized GSK-3 inhibitor dissolves.

[00989] 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 GSK-3 inhibitor can be combined with administration of a GSK-3 inhibitor-containing gel to the target site.

[00990] 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 15 and 16, 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 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."

[00991] 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 GSK-
3 inhibitor 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.

[00992] Provided below are some examples of the two-chamber liquid-liquid drug delivery system.

22.1 DELIVERY OF IONIZED LI+ AND MICRONIZED LITHIUM CARBONATE PEO-PPO-PEO FORMULATIONS FROM A TWO CHAMBER LIQUID-LIQUID SPRAY DELIVERY SYSTEM

[00993] In this example, ionized lithium and micronized lithium carbonate were delivered 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.

[00994] 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 Brookfield 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.

[00995] 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.

22.2 DETERMINATION OF THE VISCOSITY THAT CAN BE SPRAYED FROM A SPRAYER

[0096] 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

• Polyvinyl Alcohol (PVA); 5, 10, 15, 20, 25, 30% solutions - Lot #D14T017, Alfa Aesar
• Distilled Water
• Brookfield Rheometer, DV III

Methods

[0097] a. Six solutions of PVA were prepared by weighing out the appropriate amounts of PVA solid and dissolving it into distilled water.
[0098] b. To measure viscosity, a Brookfield DV-111 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.
[0099] 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

[00100] Table I 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 18. 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>

[001001] Consistent with the properties of Newtonian fluids, PVA lessens in viscosity as temperature increases, as shown in Figure 50. Table 18 includes the actual viscosity values obtained and the flow properties were recorded qualitatively by observing the flow of solution down their vials.

[001002] 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 18, (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.

23. EXAMPLE 18: CO-DELIVERY OF GSK-3 INHIBITOR FOR CLEANSING AND SUBSEQUENT TREATMENT OF INFECTIONS IN WOUNDS

[001003] 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.

[001004] An example of a complete spray delivery system delivers both a cleansing agent (to clean and debride the wound) and an in-situ "gelling" gel comprising a GSK-3 inhibitor. The gel can also include an antimicrobial agent. 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 drug optionally with an antimicrobial agent, 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 drug-containing (with optional antimicrobial) gel.

[001005] The spraying mechanism for step 2 involves the 2nd chamber. The solution contained in chamber 2 would contain micronized GSK-3 inhibitor.

[001006] High-energy spraying the contents of chamber 1 (containing a formulation of the surfactant and dissolved GSK-3 inhibitor) on the wound cleanses and debrides the wound, while eliminating/killing surface bacteria from the wound.

[001007] After spraying the contents of chamber 2 (which contains a formulation of micronized GSK-3 inhibitor) on the wound, the dissolution of micronized GSK-3 inhibitor is triggered by the higher temperature of the wound releasing a sustained stream of GSK-3 inhibitor. The micronized GSK-3 inhibitor 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 to be held at the site of administration.

[001008] This method provides a complete treatment of the wound at the clinic, by debridement/cleansing followed by administration of a sustained release GSK-3 inhibitor 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 GSK-3 inhibitor hydrogel, which the person will apply twice daily after 2 days as a maintenance regimen.

[001009] An alcoholic solution ($\pm$drug) may be used to first "prepare" the wound by thorough cleansing, followed by spraying the micronized GSK-3 inhibitor formulation for sustained release. The spraying mechanism may be at high energy or low energy, depending upon the application.
23.1 DELIVERY OF IONIZED Li+ AND MICRONIZED LITHIUM CARBONATE FORMULATIONS FROM A TWO CHAMBER LIQUID-LIQUID SPRAY DELIVERY SYSTEM

[001010] 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 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. In this example, 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. A high energy spray of a sterile, cleansing liquid prepares the wound tissue, for delivery of the antimicrobial gel.

[001011] Materials:
[001012] Pharmaceutical grade Lithium Carbonate from Spectrum chemicals, Inc.
[001013] Micronized lithium carbonate (particle size 1-3 microns) from FMC Corporation, Inc.
[001014] Cetyl Pyridinium chloride from Sigma Aldrich.
[001015] PEO-PPO-PEO polymer (F127) from BASF Corporation. 12,600 daltons in molecular weight, with each polymer segment approximately 4000 daltons.
[001016] Benzyl alcohol, used as a preservative.
[001017] Allantoin and sodium alginate, used as wound healing aids.
[001018] Lithium is contained in both the cleansing solution and the antimicrobial sustained release system.
[001019] 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.
[001020] 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.

23.1.1 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 described 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 described above for the two chamber system.

After the wound is cleansed, a sterile film containing micronized lithium carbonate will be applied to the wound.

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. Carrageenan 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.

PVA/films 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 carrageenan 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.

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 19-22. 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 19-22 below and the percentages of each excipient in the film after dry are also listed in the tables. Tables 19-22 show Film formulations with lot # TH-001-051, TH-001-052a, TH-001-052b, TH-001-052c (30g batch).

Table 19: 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 20: 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>
**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 51.

**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-001-051 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-001-052c 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-001-052b of high molecular weight (88~97k) PVA and formulations Lot# TH-001-53a, TH-001-053b, TH-001-054 with Carrageenan and low molecular weight of PVA survived in dissolution bath for more than three days in water.

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### Table 21: 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><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>100</strong></td>
<td><strong>30.01</strong></td>
<td><strong>100.00</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>

### Table 22: 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><strong>Total</strong></td>
<td><strong>30</strong></td>
<td><strong>100</strong></td>
<td><strong>30.21</strong></td>
<td><strong>100.00</strong></td>
<td><strong>100.00</strong></td>
</tr>
</tbody>
</table>
24. **EXAMPLE 19: MOUSE MODEL OF COMPOUND 41 TREATMENT FOLLOWING INTEGUMENTAL PERTURBATION USING FULL THICKNESS EXCISION OR DERMABRASION**

[001029] The example in this section provides exemplary protocols for integumental perturbation by full thickness excision (FTE) and dermabrasion in mice.

[001030] Specifically, this example provides protocols using two different combinations of Compound (e.g., compound 41) and integumental perturbation to induce hair follicle neogenesis: 1) full thickness excision + topically delivered Compound; and 2) dermabrasion + topically delivered Compound.

### 24.1 FULL THICKNESS EXCISION PROTOCOL

[001031] 1. Twelve (12)-day old C57BL6/J mice pups are used. They are fed high fat food from the day they arrive to the day of surgery (10 days).

[001032] 2. When the mice are 21 days old, full thickness excision (FTE) surgery is carried out.

[001033] 3. Mothers, domes, and high fat food is removed from the cages. Food is replaced with normal food.

[001034] 4. All pups for the experiment are placed into a large container to randomize.

[001035] 5. The pups are weighed one at a time. If under 7 g, they are placed into a separate "runts" cage and FTE is not performed on these mice. If weight is made, mice are injected with calculated buprenorphine ("BUP"; 0.05 mg/kg). A stock BUP solution may be used that works out to 0.009 mg/ml, so 50 µl per 9 g mouse is injected. Place 6 mice per cage until all mice are weighed and given buprenorphine. During the administration of the anesthesia and for the duration of the time that the mice were anesthetized, the cages are placed on heating pads which are set to low heat.

[001036] a. ALTERNATE: If working with many mice (50+), it may be preferable to stagger the BUP dosing in order to avoid the effect of the analgesic wearing off before surgery. It is preferable to stay as close as possible to BUP administration at 1-2 hours pre-surgery.

[001037] 6. One hour after giving the analgesic, the mice from one cage are injected with ketamine (70 mg/kg) / xylazine (8 mg/kg). Again, a stock that works out to 50 µl per 9 g mouse may be used.
a. ALTERNATE: Giving an additional 10 µl KX above the calculated dose (based on weight) seems to put the mice out to toe pinch quicker without increased fatality. However, it is only suggested to do this if few mice are not fully going out to toe pinch.

[001039] 7. Once mice are anesthetized, the proper number is ear-punched, their weight recorded, their back hair shaved with clippers, and a 1.5 cm x 1.5 cm box is marked on the rear dorsum.

[001040] 8. Eye ointment is applied to the mice, to keep their eyes from drying out during their immobilization, and the cages are pre-warmed on low-setting heating pads.

[001041] 10. The surgery site on the rear dorsum is sprayed/wiped with alcohol (70% ethanol) to prevent infections.

[001042] 11. A full thickness excision (FTE) (1.5 cm²) along the marked lines is cut out from the skin (cut inside the vertical lines and directly on the horizontal lines) using a pair of blunt-tip scissors and curved-tip forceps.

[001043] a. ALTERNATE: If letting mice live past day-5 post scab detachment, India ink should be used to mark the corners and sides of the wound (8 "dots").

[001044] 12. Finished mice are placed back in the pre-warmed cages and the cages are left on the pads until all mice awake. Eye ointment is reapplied if/when necessary.

[001045] 13. Each cage is supplied with a dish of wet food (moistened regular chow), regular dry chow, water, a dish of flavored JELL-O®, and a water bottle with flavored Prang (bio-serv.com, F2351-S; also avail. From Fisher) mixed in to aid in hydration. Saline is administered to mice that exhibit signs of pain, dehydration, malnourishment, or stress. Optionally, all cages can be provided with flavored Prang for the duration of the experiment in addition to their water supply. The mice may also be given flavored JELL-O® for the 2 days post surgery (JELL-O® when introduced in earlier experiments for rehydration purposes had a positive effect - mice eagerly ate the JELL-O® and looked healthier on days following the procedure).

[001046] 14. The mice are monitored and weighed over the next two days, as well as 2-3 times per week thereafter. The FTE wounds are allowed to heal by secondary intention. Dose mice AM and PM (approximately 10 AM and 5 PM) with BUP on day 1 after FTE. Dose AM and if necessary in PM on day 2. Replace JELL-O® or water as needed.

[001047] 15. Mice are monitored daily for scab detachment (occurs 11-18 days post FTE).

[001048] 16. On the day of scab detachment, mice are placed into treatment groups as follows: (Optionally include no treatment with mock handling [mock handling = Pick up and handle mice as if being dosed. An empty capillary tube is used to mock spread out drug onto
their back under the bandage. This may be done to ensure all mice undergo the same stresses during dosing); Placebo (e.g., n=15); 0.01 μg Compound (e.g., compound 41) (e.g., n=15); 0.1 μg Compound (e.g., n=15); 1 μg Compound (e.g., n=15); 10 μg Compound (e.g., n=15); 100 μg Compound (e.g., n=15).

Bandages (to prevent mice from licking off the drug) are applied as follows: On the day of scab detachment, the mice are administered 75% of a normal dose of ketamine-xylazine according to weight. While the mice are anesthetized, the posterior dorsal, posterior lateral, and posterior ventral sides are shaved and treated with Nair. Care is taken to ensure that Nair did not cover the wounded area. Mice are then wrapped in Tegaderm & Telfa pad bandages. The Tegaderm is tightly wrapped around the mouse with the Telfa pad fixed over the wound I treatment area. The first treatment is administered once the bandages are in place. The mice are placed back into their cages before the anesthesia wears off. If at any point during the treatment period the mice escape from their bandage, the bandage is replaced. Mice are checked for bandages at every dosing interval.

Dosing is as follows: The day of scab detachment is denoted as Scab Detachment Day 0 (SD0). Mice in treatment groups receive one dose on SD0 and another dose 2 days later (SD2; i.e., 48 h after the first dose) (2 total doses). Drummond wiretrols and accompanying 100μl capillary tubes are used to dispense 100μl onto the wound site of each mouse. The tube is then used to spread out the drug to encompass the entire wound. One capillary tube is used per mouse. Drug treatment vials are replaced when necessary.

All mice are harvested on SD5, including mice used for determination of "peak" and "trough" Compound levels. The mice are not treated after SD2, nor on their respective harvest day (SD5), unless, optionally, that mouse is used for a peak dosage sample, i.e., selected as a peak value point for testing Compound levels in blood and skin. Some mice from each group are selected for evaluation of Compound levels in the skin and blood. Per group, three mice are used for "peak" levels and three mice for "trough" levels. Mice selected for the peak levels have blood drawn 1 hour post AM dosing on SD5 (9 total doses). Mice selected for "trough" levels have blood drawn 18-24 hours post SD4 PM dose (i.e., prior to harvest without treatment). On day 5 post scab detachment, approximately 0.5 ml of blood is collected per mouse, using the cheek lancet technique, and the mice are sacrificed. Wound skin is then analyzed by confocal microscopy.

Processing of mice for data collection: In preparation for in vivo scanning laser microscopy, the hair surrounding the healed wound is trimmed to reveal the wound surface. The surface is imaged with a confocal microscope; this includes 1-3 Vivablocks (8
mm x 8 mm) at depths between 40-100 µπ, and 1-3 Vivastacks (500 µm x 500 µη) of areas with many neogenic hair follicles (NHF's) or interesting features. The mice are sacrificed, and the entire wound is excised (excluding normal surrounding tissue as much as possible), and tissue allocated in a procedure that lasts ~ 3-5 minutes per mouse after the time of death. Following excision, the wound is bisected. Half of the wound is placed on a section of a 3x5 inch index card and stored in cold 4% Paraformaldehyde for histology. The other half is frozen on dry ice for biochemical assays, including Compound levels. The frozen samples are placed on dry ice for the duration of the harvest period and then transferred to a -80°C Celsius freezer for long term storage. The histology samples are taken out of the 4% Para-Formaldehyde solution following overnight fixation and moved to 30% sucrose/1 X PBS. Within 24 hours the samples are taken from the sucrose solution and dabbed dry, then embedded in OCT. The OCT is frozen in a slurry of crushed dry ice and 2-O-methyl-butane. Cryosections are generated for histology.

24.2 **DERMABRASION PROTOCOL**

[001053] In the following protocol, a microdermabrasion device is used to perform dermabrasion.

[001054] 1. Mice are 10 weeks old on the day of procedure.

[001055] 2. Mice are weighed and treated with buprenorphine ("BUP"; 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 dermabrasion (two doses, ~ 8 hours apart), and as needed the second day after dermabrasion.

[001056] 3. After one hour has passed mice are re-weighed (because they are not ear punched before ketamine/xylazine dose), anesthetized with ketamine (80 mg/kg) / xylazine (8 mg/kg), and ear punched for identification. Mice are given an eye ointment to keep their eyes from drying out during their immobilization.

[001057] 4. Once the mice have ceased being mobile both the left and right sides of the dorsal rear back skin are clipped.

[001058] a. Nair® is applied for 1 minute to the right and left flank, the hair wiped off with a wet paper towel, and dried with paper towel.

[001059] 5. The mice are dermabraded once they do not react to a toe-pinchn.

[001060] a. The microdermabrasion device settings (Advanced Microderm, DX model) are set to max vacuum, large tip, and max mixture.

[001061] b. aluminum oxide crystals are used for dermabrasion.
c. 10 passes on the right dorsal rear flank are carried out (each pass is a single movement from cranial to caudal direction; skin is held taught).

d. The left side of the body is not dermabraded (left flank is considered the nonwounded internal control).

6. After dermabrasion, the 4 corners and the midpoints along the edge of the wound are tattooed with an injection of India ink (using a tuberculin syringe); 8 total tattoo marks made.

7. Prior to waking up, the mice are bandaged as in the FTE protocol, and dosing initiated (day 0) as follows: Animals are assigned to groups as follows: (Optionally include no treatment with mock handling [mock handling = Pick up and handle mice as if being dosed. An empty capillary tube is used to mock spread out drug onto their back under the bandage. This may be done to ensure all mice undergo the same stresses during dosing]); Placebo (e.g., n=15 or 20); 0.01 µg Compound (e.g., compound 41) (e.g., n=15); 0.1 µg Compound (e.g., n=15); 1 µg Compound (e.g., n=15); 10 µg Compound (e.g., n=15); 100 µg Compound (e.g., n=15).

8. The mice are 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 wake up.

9. The mice are dosed again on day 2 post-DA.

10. During the dosing period, weights and observations of mice are recorded daily. The time of delivery of dose is recorded.

11. Tissue is harvested from 5 mice of each group on day 5 post-DA for i) bioassay of target, ii) assay for concentration of Compound in skin, and iii) histology.

a. This is done by resecting the skin from the right side and observing the ink spots from the dermal side of the skin. The wound area is cut out and divided into three pieces for assays as indicated above.

12. Blood samples are also collected into potassium-EDTA vacutainers (2 ml capacity, lavender cap) from these same animals via cheek puncture lancets. The blood is centrifuged (maximum speed in non-refrigerated microfuge), the supernatant (plasma) is removed to a separate microfuge tube, labeled, and then frozen (along with the RBC cell pellet) for storage and shipment. Blood is taken within 1-2 hours of the last dose to determine peak levels of Compound in skin, or alternatively prior to harvest without dosing if trough levels are desired.
13. Remaining animals (e.g., 10 per group) are allowed to survive until 21 days post dermabrasion at which time they were clipped and razored (disposable razor) on both the wound and non-wounded skin. A full thickness excision of skin from both treatment sites is excised between 21 and 25 days post-DA. The skin samples are separately placed on 3x5 note cards and the surface of the excised skin (wound - right side; non-wounded - left side) analyzed by confocal microscopy in order to quantify the thickness of the hair shafts, density of hair shafts, the density of hair pores, and the number of shafts per pore.

14. Following confocal microscopy, the skin is divided into three pieces as above.

a. For biochemical assays, samples are immediately placed in Eppendorf tubes and frozen on dry ice, after which they are transferred to -80 °C freezer.

b. For histology, a piece of tissue is immersed in 4% PFA/PBS and stored at 4 °C until processing for paraffin histology, or for cryosectioning, then changed to 30% sucrose/PBS (after overnight fixation) for between 12-24 hours. Samples are then embedded in optimal cutting temperature (OCT) and stored at -80 °C.

25. **EXAMPLE 20: MOUSE MODEL OF CHIR99021 TREATMENT FOLLOWING INTEGUMENTAL PERTURBATION BY FULL THICKNESS EXCISION WOUNDING**

In this example, mice were wounded by full thickness excision (FTE) and then treated with CHIR99021, essentially as described in Section 16.1. Mice were divided into six groups (~15 mice per group) for the following treatments: Placebo; 0.01 µg CHIR99021 (0.0001 mg/ml x 100 µl per dose = 0.01 µg x 2 doses = 0.02 µg total dosed); 0.1 µg CHIR99021 (0.001 mg/ml x 100 µl per dose = 0.1 µg x 2 doses = 0.2 µg total dosed); 1 µg CHIR99021 (0.1 mg/ml x 100 µl per dose = 1 µg x 2 doses = 2 µg total dosed); 10 µg CHIR99021 (0.1 mg/ml x 100 µl per dose = 10 µg x 2 doses = 20 µg total dosed); and 100 µg (1 ml/ml x 100 µl per dose = 100 µg x 2 doses = 200 µg total dosed).

Twenty-one day old mice (male and female) were FTE wounded on rear dorsum. On the day of scab detachment, mice were assigned to the treatment groups described in the preceding paragraph, bandaged, and dosed once daily (SID) topically with CHIR99021 on two non-consecutive days: the day of scab detachment (day 0) and on the second day post-FTE wounding (day 2). On day 5 post-FTE wounding, skin was analyzed with *in vivo* confocal microscopy, followed by harvest of skin and blood.

**Tissue harvesting and sectioning.** Skin was harvested at five days post scab detachment (following FTE wound) from mouse dorsum, including normal tissue
surrounding scar and regenerating zone. Harvested tissue was bisected along the anterior-posterior (A-P) axis, through the middle of scar. One half of the bisected tissue was placed in 4% paraformaldehyde for 24 hours, then transferred to 30% sucrose in 1x PBS for 24 hours. The tissue was removed from sucrose and embedded in Optimal Cutting Medium (OCT) so that sections could be collected from the bisected edge. The OCT section was frozen with a bath made from crushed dry ice and 2-O-methylbutane. Cryoblocks were then sectioned with a cryostat. The cryoblock was sectioned (8 μm per section) until tissue was fully faced. A total of 10-15 slides (2 sections per slide) were collected. The first slide was stained with Hematoxylin & Eosin (H&E); if a section was damaged or unreadable, the most intact section was used, and an attempt was made to use sections closest to the midline of the scar (i.e., closest to slide 1), which correspond to the regenerating zone (see Figure 52A).

**[001079] Hair follicle counting and classification.** One tissue section was counted per animal. All hairs contained within the wound were counted. Follicles with pigmented shafts within regenerated zone were not counted (see Figure 52B), and scars were absent from the samples. Follicles were classified according to the classification scheme of Paus, R et al., "A comprehensive guide for the recognition and classification of distinct stages of hair follicle morphogenesis," *J Invest Dermatol*. 1999 Oct;113(4):523-32. See Figure 53A to Figure 53H. Follicles were staged to the latest identifiable stage within each section; some germs unable to be classified due to problems with sections Folding/tearing obscuring germ characteristics.

**[001080] Results.** Representative images are shown in Figure 54A to Figure 54F. As shown in Figure 55A, the 1 μg CHIR99021 treatment group had an increased number of hair follicles at or above stage 5 and, as shown in Figure 55B, the 1 μg treatment group had the fewest numbers of hair follicles at stage 4 or below. As shown in Figure 55C, the 1 μg treatment group had an increased "average germ stage" (average follicle maturity).

**[001081]** All references cited herein are incorporated herein by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

**[001082]** The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing
description and accompanying figures. Such modifications are intended to fall within the 
scope of the illustrative embodiments and/or appended claims.

26. **ILLUSTRATIVE EMBODIMENTS**

[001083] The invention can be illustrated by the non-limiting, embodiments set forth in the following paragraphs.

1. A method for modulating hair growth in a human subject, comprising 
administering to a human in need thereof a composition comprising an effective amount of a 
compound having Formula (I):

```
\[
\begin{array}{c}
\text{A} \\
\text{R}_1 \text{R}_2 \text{R}_3 \text{R}_4 \\
\text{N} \text{N} \\
\text{Y} \\
\text{R}_5 \text{R}_6 \text{R}_7
\end{array}
\]
```

Formula I

or pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

\( Y \) is N or CH;

\( R_1, R_2, R_3, \) and \( R_4 \) are independently hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, cycloalkyl, 
aryl, heteroaryl, heterocyclyl, or alkoxy;

\( R_5 \) and \( R_7 \) are independently hydrogen or halo or optionally substituted alkyl, cycloalkyl, aryl, 
aminoalkyl, aminoaralkyl, aminocycloalkylaryl, arylcarboxamidocycloalkylarylalkyl, 
arylcarboxamidocycloalkylarylalkyl, arylcarboxamidocycloalkylarylalkyl, arylcarboxamidoaryl, 
arylcarboxamidoalkyl, arylcarboxamidoaralkyl, arylcarboxamidoalkoxyalkyl, 
aminoalkoxyalkyl, or arylsulfonamidoaralkyl;

\( R_6 \) is hydrogen, carboxyl, nitro, amino, cyano, or an optionally substituted alkyl, aryl, aralkyl, 
aminoalkyl, aminoaralkyl, aminoalkoxyalkyl, arylaminoalkyl, arylaminoaryl, 
arylaminaralkyl, arylalkylamino, arylalkylaminooalkyl, arylalkylaminooalkyl, 
carboxycycloamido, acylxoyalkyl, acyloxyaryl, acyloxyaralkyl, acyloxyalkylcycloalkyl, 
acyloxyalkylaminooalkyl, sulfonylalkyl, carbamylalkyl, carbamylaryl, carbamylaralkyl, 
carbamylalkylamino, carbamylalkylaminooalkyl, carbamylalkylaminooalkyl, or 
carbamylalkylaminooalkyl; and

\( A \) is pyridyl, pyrimidiny, thiazolyl, indolyl, imidazolyl, oxadiazolyl, tetrazolyl, pyrazinyl, 
triazolyl, thiophenyl, furanyl, quinolinyl, purinyl, naphthyl, benzoazolyl, benzopyridyl, or
benzimidazolyl, which can be optionally substituted with from 0 to 3 of the following substitution groups: nitro, amino, cyano, halo, thioamido, amidino, oxamidino, alkoxyamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, loweralkyl, haloloweralkyl, loweralkoxy, haloloweralkoxy, loweralkoxyalkyl, loweralkylamino loweralkoxy, loweralkylcarbonyl, loweralkylcarbonyl, lowerheteroaralkylcarbonyl, alkylthio, aminoalkyl or cyanoalkyl.

2. The method of paragraph 1 in which the compound having Formula (I) is administered by topical administration to the skin.

3. The method of paragraph 1 in which the compound having Formula (I) is administered to the skin surface, transdermally, or intradermally.

4. The method of paragraph 1 in which the compound having Formula (I) is administered by subcutaneous, parenteral or oral administration.

5. The method of paragraph 1 in which the $R_1$, $R_2$, $R_3$, and $R_4$ are hydrogen.

6. The method of paragraph 1 in which the $Y$ is N.

7. The method of paragraph 1 in which the compound having Formula (I) in the composition is encapsulated in microspheres.

8. The method of paragraph 7, wherein the compound having Formula (I) in the composition is encapsulated in microspheres of sizes between 0.10 microns and 200 microns.

9. The method of paragraph 8, wherein microspheres are between 0.20 microns and 50 microns.

10. The method of paragraph 7, wherein the compound having Formula (I) in the composition is encapsulated in liposomes of sizes between 10 nm and 50 microns.

11. The method of paragraph 10, wherein the liposomes are between 500 nm and 20 microns.

12. The method of paragraph 1 in which the composition is administered as a cold liquid, which gels at a temperature of 32 °C - 37 °C.
13. The method of paragraph 1 in which the composition is administered as a liquid, which then hardens into a depot that delivers the compound over time.

14. The method of paragraph 1 in which the composition is administered as a hydrogel.

15. The method of paragraph 1 in which the composition comprises one or more excipients that complex to the compound.

16. The method of paragraph 15, wherein the excipient comprises hyaluronic acid, polyacrylic acid or alginic acid.

17. The method of paragraph 1 in which the composition comprises one or more permeation enhancing agents or carriers that solubilize the compound in skin.

18. The method of paragraph 1 in which the composition comprises propylene glycol, polyethylene glycol or ethanol.

19. The method of paragraph 1 in which the treatment is a single dose of the composition administered over a period of 1 day to 1 month.

20. The method of paragraph 1 in which the treatment is intermittent such that it consists of multiple courses of treatments with the compound interrupted by compound treatment holidays.

21. The method of paragraph 20 in which a second treatment is administered during the compound treatment holidays.

22. The method of paragraph 21 in which the duration of the second treatment is similar to the duration of the compound treatment.

23. The method of paragraph 21 in which the duration of the second treatment is different from the duration of the compound treatment.

24. The method of paragraph 1 in which a second treatment is administered to the human subject receiving the compound treatment.

25. The method of paragraph 24 in which the duration of the second treatment is identical to the duration of the compound treatment.
26. The method of paragraph 24 in which the duration of the second treatment is different from the duration of the compound treatment.

27. The method of paragraph 24 in which the second treatment is administered concurrently with the compound treatment.

28. The method of paragraph 24 in which the second treatment is administered before the compound treatment.

29. The method of paragraph 24 in which the second treatment is administered after the compound treatment.

30. The method of paragraph 21 or 24 in which the second treatment is integumental perturbation.

31. The method of paragraph 30 in which the integumental perturbation removes the epidermis partially or completely.

32. The method of paragraph 30 in which the integumental perturbation removes all of the epidermis and part of the dermis.

33. The method of paragraph 30 in which the integumental perturbation does not remove the epidermis.

34. The method of paragraph 30 in which the integumental perturbation is accomplished by laser, light, heat, microneedle rollers, ultrasound, iontophoresis, electrophoresis, or radiation treatment, or a combination thereof.

35. The method of paragraph 30 in which the integumental perturbation is accomplished by laser.

36. The method of paragraph 35 wherein the integumental perturbation by laser is fractional and non-ablative.

37. The method of paragraph 36, wherein the fractional, non-ablative integumental perturbation by laser is performed by use of an Erbium-YAG laser at 1500-1590 nm.

38. The method of paragraph 35 wherein the integumental perturbation by laser is fractional and ablative.
39. The method of paragraph 38, wherein the fractional, ablative integumental perturbation results in fractional ablation of the skin at a depth between 100 microns and 4000 microns into the skin.

40. The method of paragraph 38, wherein the fractional, ablative integumental perturbation results in fractional ablation of the skin at a depth approximating the depth of a full-thickness excision wound.

41. The method of paragraph 38, wherein the fractional, ablative integumental perturbation results in fractional ablation of the skin over an area of 1.5 cm x 1.5 cm to 15 cm x 15 cm.

42. The method of paragraph 38, wherein the fractional, ablative integumental perturbation results in fractional ablation of the skin at a depth density of the micro-thermal zones of the fractional ablation approximates that of a full bulk ablation of the entire area of treatment.

43. The method of paragraph 38, wherein the fractional, ablative integumental perturbation is by full bulk ablation, wherein the tissue of the entire area of treatment is ablated.

44. The method of paragraph 43, wherein the fractional, ablative integumental perturbation by bulk ablation is over an area of 1.5 cm x 1.5 cm to 15 cm x 15 cm.

45. The method of paragraph 43, wherein the fractional, ablative integumental perturbation by bulk ablation is accomplished at 10,600 nm using a carbon dioxide laser.

46. The method of paragraph 43, wherein the fractional, ablative integumental perturbation by bulk ablation is accomplished at 2940 nm using a Erbium-YAG laser.

47. The method of paragraph 35 wherein the integumental perturbation by laser is non-fractional and ablative.

48. The method of paragraph 47, wherein the non-fractional, ablative integumental perturbation is by full bulk ablation, wherein the tissue of the entire area of treatment is ablated.
49. The method of paragraph 47, wherein the non-fractional, ablative integumental perturbation by bulk ablation is over an area of 1.5 cm x 1.5 cm to 15 cm x 15 cm.

50. The method of paragraph 47, wherein the non-fractional, ablative integumental perturbation by bulk ablation is accomplished at 10,600 nm using a carbon dioxide laser.

51. The method of paragraph 47, wherein the non-fractional, ablative integumental perturbation by bulk ablation is accomplished at 2940 nm using an Erbium-YAG laser.

52. The method of paragraph 30 in which the integumental perturbation is accomplished using a microneedle array.

53. The method of paragraph 52, wherein the microneedle array is in the form of a roller or flat plate.

54. The method of paragraph 52, wherein the microneedle array can disrupt a skin area of 1.5 cm x 1.5 cm to 15 cm x 15 cm.

55. The method of paragraph 52, wherein the microneedle array can disrupt skin at a depth of 100 microns to 4000 microns.

56. The method of paragraph 52, wherein the microneedle array has hollow needles.

57. The method of paragraph 52, wherein the microneedle array top has a luer-lock fitting that can accommodate a syringe to deliver drug.

58. The method of paragraph 57, wherein the volume of the syringe is 1 ml to 3 ml.

59. The method of paragraph 30 in which the integumental perturbation is accomplished by injury.

60. The method of paragraph 59, wherein the mode of injury is mechanical.

61. The method of paragraph 60, wherein the mechanical injury is accomplished by microdermabrasion, dermabrasion, tape stripping, full thickness excision of tissue.

62. The method of paragraph 59, wherein the mode of injury is thermal injury.

63. The method of paragraph 60, wherein the thermal injury is accomplished by laser.
64. The method of paragraph 30 in which the integumental perturbation is accomplished by inducing inflammation.

65. The method of paragraph 64 in which the method of inducing inflammation is by application of an adjuvant.

66. The method of paragraph 65, wherein the adjuvant is selected from the group of sodium dodecyl sulfate, aluminum salts, monophosphoryl lipid A, and cetyl trimmonium bromide (CTAB).

67. The method of paragraph 64 in which the method of inducing inflammation is injury to tissue.

68. The method of paragraph 64 in which the method of inducing inflammation is by application of a cytokine (e.g., IL-1beta).

69. The method of paragraph 64 in which of inducing inflammation is by application of an antigen (e.g., tetanus toxoid).

70. The method of paragraph 30 in which the method of integumental perturbation is accompanied by stem cell mobilization (i.e., G-CSF).

71. The method of paragraph 30 in which a third treatment is administered to the human subject receiving the compound treatment and integumental perturbation.

72. The method of paragraph 71 in which the third treatment stimulates hair growth.

73. The method of paragraph 72 in which the third treatment is the administration of minoxidil.

74. The method of paragraph 72 in which the third treatment is the administration of a 5-a reductase inhibitor.

75. The method of paragraph 72 in which the third treatment is the administration of minoxidil and a 5-a reductase inhibitor.

76. The method of paragraph 74 in which the 5-a reductase inhibitor is finasteride.

77. The method of paragraph 75 in which the 5-a reductase inhibitor is finasteride.
78. The method of paragraph 72 in which the third treatment is the administration of bimatoprost.

79. The method of paragraph 72 in which the third treatment is the administration of an anti-senescence agent.

80. The method of paragraph 72 in which the third treatment is a surgical transplantation of hair follicles.

81. The method of paragraph 72 in which the third treatment is the administration of an anti-senescence agent.

82. The method of paragraph 71 in which the third treatment inhibits hair growth.

83. The method of paragraph 82 in which the third treatment is the administration of efomithine.

84. The method of paragraph 82 in which the third treatment is the administration of a cytotoxic drug.

85. The method of paragraph 82 in which the third treatment is the administration of efomithine and a cytotoxic drug.

86. The method of paragraph 84 in which the cytotoxic drug is 5-fluorouracil.

87. The method of paragraph 85 in which the cytotoxic drug is 5-fluorouracil.

88. The method of paragraph 82 in which the inhibition of hair growth is on the head, chest, breast, abdomen, neck, back, arms, armpits, legs, hands, feet, buttocks, genitals, or a wounded or scarred area of the skin of the subject.

89. The method of paragraph 21 or 24 in which the second treatment stimulates hair growth.

90. The method of paragraph 89 in which the second treatment is by low intensity laser.
91. The method of paragraph 90 in which the low intensity laser is used either concurrently or intermittently with the compound treatment.

92. The method of paragraph 90 in which the low intensity laser is the HairMax Laser comb.

93. The method of paragraph 89 in which the second treatment is the administration of minoxidil.

94. The method of paragraph 89 in which the second treatment is the administration of a 5-a reductase inhibitor.

95. The method of paragraph 89 in which the second treatment is the administration of minoxidil and a 5-a reductase inhibitor.

96. The method of paragraph 94 in which the 5-a reductase inhibitor is finasteride.

97. The method of paragraph 95 in which the 5-a reductase inhibitor is finasteride.

98. The method of paragraph 89 in which the second treatment is the administration of bimatoprost.

99. The method of paragraph 89 in which the second treatment is the administration of an arti-senescence agent.

100. The method of paragraph 89 in which the second treatment is a surgical transplantation of hair follicles.

101. The method of paragraph 89 in which the stimulation of hair growth is on the scalp or face, chest, abdomen, arms, armpits, legs, or genitals, or a wounded or scarred area of the skin of the subject.

102. The method of paragraph 21 or 24 in which the second treatment inhibits hair growth.

103. The method of paragraph 102 in which the second treatment is the administration of eflornithine.
104. The method of paragraph 102 in which the second treatment is the administration of a cytotoxic drug.

105. The method of paragraph 102 in which the second treatment is the administration of eflozithine and a cytotoxic drug.

106. The method of paragraph 104 in which the cytotoxic drug is 5-fluorouracil.

107. The method of paragraph 105 in which the cytotoxic drug is 5-fluorouracil.

108. The method of paragraph 102 in which the inhibition of hair growth is on the head, chest, breast, abdomen, neck, back, arms, armpits, legs, hands, feet, buttocks, genitals, or a wounded or scarred area of the skin of the subject.

109. The method of paragraph 1, 5, or 6, wherein the Compound inhibits the activity of GSK-3beta in a cell-based assay.

110. The method of paragraph 1, 5, or 6, wherein the Compound modulates hair growth in an animal model system.

111. The method of paragraph 110, wherein the Compound, alone or in combination with a therapy described in any one of paragraphs 72-81 or 89-101, stimulates hair growth in an animal model system.

112. The method of paragraph 110, wherein the Compound, alone or in combination with a therapy described in any one of paragraphs 82-88 or 102-108, reduces hair growth in an animal model system.

113. The method of paragraph 1, 5, or 6, wherein the Compound enhances wound healing in an animal model system.

114. A method for healing a wound with reduced scar formation in a human subject, comprising administering to a human in need thereof a composition comprising an effective amount of a compound having Formula (I):
or pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

- Y is N or CH;
- R₁, R₂, R₃, and R₄ are independently hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, or alkoxy;
- R₅ and R₇ are independently hydrogen or halo or optionally substituted alkyl, cycloalkyl, aryl, aminoalkyl, aminoaralkyl, aminocycloalkylaryl, arylcarboxamidocycloalkylaralkyl, arylcarboxamidocycloalkylaryl, arylcarboxamidoalkylcycloalkyl, arylcarboxamidoaryl, arylcarboxamidoaralkyl, arylcarboxamidoalkoxyaryl, aminoalkoxyalkyl, or arylsulfonamidoaralkyl;
- R₆ is hydrogen, carboxyl, nitro, amino, cyano, or an optionally substituted alkyl, aryl, aralkyl, aminoalkyl, aminoaryl, aminoaralkyl, aminoalkoxyalkyl, arylaminoalkyl, arylaminoaryl, arylaminoaralkyl, arylalkylamino, arylalkylaminooaryl, arylalkylaminooaryl, carboxycyamoimo, acyloxyalkyl, acyloxyaryl, acyloxyaralkyl, acyloxyalkylcycloalkyl, acyloxyalkylaminooalkyl, sulfonylalkyl, carbamylalkyl, carbamylaryl, carbamylaralkyl, carbamylaminooalkyl, carbamylalkylaminooaryl, or carbamylalkylaminoaralkyl; and
- A is pyridyl, pyrimidinyl, thiazolyl, indolyl, imidazolyl, oxadiazolyl, tetrazolyl, pyrazinyl, triazolyl, thiophenyl, furanyl, quinolinyl, purinyl, naphthyl, benzothiazolyl, benzopyridyl, or benzimidazolyl, which can be optionally substituted with from 0 to 3 of the following substitution groups: nitro, amino, cyano, halo, thioamido, amidino, oxamidino, alkoxamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, loweralkyl, haloloweralkyl, loweralkoxy, haloloweralkoxy, loweralkoxyaryl, loweralkylaminooalkyl, loweralkoxy, loweralkylcarbonyl, loweralkylcarbonyl, lowerheteroaralkylcarbonyl, alkylthio, aminoalkyl or cyanoalkyl.

115. A method for revising a scar in a human subject, comprising administering to a human in need thereof a composition comprising (i) an effective amount of a compound having Formula (I):
or pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

Y is N or CH;

R₁, R₂, R₃, and R₄ are independently hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, or alkoxy;

R₅ and R₇ are independently hydrogen or halo or optionally substituted alkyl, cycloalkyl, aryl, aminoalkyl, aminoaralkyl, aminocycloalkylaryl, arylcarboxamidocycloalkylaralkyl, arylcarboxamidocycloalkylaryl, arylcarboxamidoalkylcycloalkyl, arylcarboxamidoaryl, arylcarboxamidoaralkyl, arylcarboxamidooalkoxyalkyl, aminoalkoxyalkyl, or arylsulfonamidoaralkyl;

R₆ is hydrogen, carboxyl, nitro, amino, cyano, or an optionally substituted alkyl, aryl, aralkyl, aminoalkyl, aminoaryl, aminoaralkyl, aminoalkoxyalkyl, arylaminoalkyl, arylaminoaryl, arylaminoaralkyl, arylalkylamino, arylalkylaminoalkyl, arylalkylaminoaralkyl, carboxycycloamido, acyloxyalkyl, acyloxyaryl, acyloxyaralkyl, acyloxyalkycycloalkyl, acyloxyalkylaminoalkyl, sulfonylalkyl, carbamylalkyl, carbamylaryl, carbamylaralkyl, carbamylalkylamino, carbamylalkylaminoalkyl, carbamylalkylaminoaralkyl, or carbamylalkylaminoaralkyl; and

A is pyridyl, pyrimidinyl, thiazolyl, indolyl, imidazolyl, oxadiazolyl, tetrazolyl, pyrazinyl, triazolyl, thiophenyl, furanyl, quinolinyl, purinyl, naphthyl, benzothiazolyl, benzopyridyl, or benzimidazolyl, which can be optionally substituted with from 0 to 3 of the following substitution groups: nitro, amino, cyano, halo, thioamido, amidino, oxamidino, alkoxyamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, loweralkyl, haloloweralkyl, loweralkoxy, haloloweralkoxy, loweralkoxyalkyl, loweralkylamino loweralkoxy, loweralkylcarbonyl, loweralkylcarbonyl, lowerheteroaralkylcarbonyl, alkylthio, aminoalkyl or cyanoalkyl,

(ii) in combination with integumental perturbation.
A method for treating a wound in a human subject, comprising administering to a human in need thereof a composition comprising an effective amount of a compound having Formula (I):

\[
\begin{align*}
\text{Formula I}
\end{align*}
\]

or pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

Y is N or CH;

R1, R2, R3, and R4 are independently hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, cycloalkyi, aryl, heteroaryl, heterocycl, or alkoxy;

R5 and R7 are independently hydrogen or halo or optionally substituted alkyl, cycloalkyi, aryl, aminoalkyi, aminoaralkyi, aminocycloalkylaryl, ary1carboxamidocycloalkylarylalkyi, ary1carboxamidocycloalkylaryl, ary1carboxamidoaryl, ary1carboxamidoalkyi, ary1carboxamidoaralkyi, ary1carboxamidoalkoxyalkyi, aminoalkoxyalkyi, or ariysulfonamidoaralkyi;

R6 is hydrogen, carboxyi, nitro, amino, cyano, or an optionally substituted alkyl, aryl, alalkyi, aminoalkyi, aminoaralkyi, aminoalkoxyalkyi, ariylamin0alkyi, ariylaninoaryl, ary1aminoaralkyi, aryalkylamin0alkyi, ariylalkylamin0aralkyi, carboxycycloamido, ariyloxalkyi, acyloxyaryl, acyloxyaralkyi, acyloxyalkylcycloalkyi, acyloxyalkylamin0alkyi, sulfonlayalkyi, carbamylalkyi, carbamylaryl, carbamylaralkyi, carbamylalkylamin0alkyi, carbamylalkylamin0aralkyi, or carbamylalkylamin0aralkyi; and

A is pyridyi, pyrimidinyl, thiaz0yl, ind0lyl, imidaz0yl, oxadiaz0yl, tetraz0yl, pyrazinyl, triaz0yl, thiophenyl, furanyl, quinolinyl, purinyl, naphthyl, benzothiaz0yl, benzopyridyi, or benzimidaz0yl, which can be optionally substituted with from 0 to 3 of the following substitution groups: nitro, amino, cyano, halo, thioamido, amidino, oxamidino, alkxyamidino, imidino, guanidino, sulfonamido, carboxyi, formyi, loweralkyi, haloloweralkyi, loweralkoxy, haloloweralkoxy, loweralkoxyalkyi, loweralkylamin0 loweralkoxy, loweralkylcarbonyl, loweralkylcarbonyl, lowerheteroalkylcarbonyl, alkylthio, aminoalkyi or cyanoalkyi.
117. The method of paragraph 114, 115, or 116 in which the R₁, R₂, R₃, and R₄ are hydrogen.

118. The method of paragraph 114, 115, or 116 in which the Y is N.

119. The method of paragraph 114, 115, or 116, wherein the scar revision or wound heals by primary intention.

120. The method of paragraph 114, 115, or 116, wherein the scar revision or wound heals by secondary intention.

121. The method of paragraph 114, 115, or 116, wherein the scar or wound is caused by a burn.

122. The method of paragraph 114, 115, or 116, wherein the scar or wound is caused by trauma.

123. The method of paragraph 114, 115, or 116, wherein the scar or wound is caused by acne.

124. The method of paragraph 114 or 116, wherein the human subject has wound dehiscence.

125. The method of paragraph 114 or 116, wherein the wound is an ulcer.

126. The method of paragraph 114 or 115, wherein the treatment improves the appearance of the scar or restores function of the scarred tissue.

127. The method of paragraph 114 or 115, wherein the treatment has one or more of the following effects: no or reduced scarring or improves the function of the wounded tissue.

128. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is added to freshly wounded skin.

129. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is added to the skin around a wound.

130. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is administered by a laser delivery device.
131. The method of paragraph 130, wherein the laser is a Smoothpeel laser, set at high, 20 passes.

132. The method of paragraph 130, wherein the laser is an ultrapulse laser, set at 350 mJ, 1.8 mm spot size, density 9, 2 passes.

133. The method of paragraph 130, wherein the laser is a Mixto laser, set at 84.8 J/cm, W=12, index=4, 4 passes.

134. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is administered by topical administration to the skin.

135. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is administered to the skin surface, transdermally, or intradermally.

136. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is administered as part of a wound dressing.

137. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is administered as part of a wound irrigation solution.

138. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is administered via a scaffold that is applied to the skin.

139. The method of paragraph 138, wherein the scaffold is in the form of a gel, a spray, a dressing, or a wrap.

140. The method of paragraph 138, wherein the scaffold comprises a PLA:PLG scaffold.

141. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is administered by subcutaneous, parenteral or oral administration.

142. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is CHIR99021.

143. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is encapsulated in microspheres.
144. The method of paragraph 143, wherein the microspheres are of sizes between 0.10 microns and 200 microns.

145. The method of paragraph 143, wherein the microspheres are of sizes between 0.20 microns and 50 microns.

146. The method of paragraph 14, 15, or 16, in which the compound having Formula (I) is encapsulated in liposomes of sizes between 10 nm and 50 microns.

147. The method of paragraph 146, wherein the liposomes are between 500 nm and 20 microns.

148. The method of paragraph 14, 15, or 16, in which the compound having Formula (I) is administered as a cold liquid, which gels at a temperature of 32 °C - 37 °C.

149. The method of paragraph 14, 15, or 16, in which the compound having Formula (I) is administered as a liquid, which then hardens into a depot that delivers compound over time.

150. The method of paragraph 14, 15, or 16, in which the compound having Formula (I) is administered as a hydrogel.

151. The method of paragraph 14, 15, or 16, in which the composition comprising the compound having Formula (I) also comprises one or more excipients that complex to the compound having Formula (I).

152. The method of paragraph 151, wherein the excipient comprises hyaluronic acid, polyacrylic acid or alginic acid.

153. The method of paragraph 14, 15, or 16, in which the composition comprising the compound having Formula (I) also comprises one or more permeation enhancing agents or carriers that solubilize the compound having Formula (I) in skin.

154. The method of paragraph 14, 15, or 16, in which the composition comprising the compound having Formula (I) also comprises propylene glycol, polyethylene glycol or ethanol.
155. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is administered in a pulse treatment of a single dose of the compound having Formula (I) administered over a period of 1 day to 1 month.

156. The method of paragraph 115 in which the integumental perturbation treatments are administered during holidays between treatments with the compound having Formula (I).

157. The method of paragraph 115 in which the treatments are multiple courses of the compound having Formula (I) and integumental perturbation treatment interrupted by treatment holidays.

158. The method of paragraph 114, 115, or 116, in which the compound having Formula (I) is administered as multiples courses of treatment interrupted by treatment holidays.

159. The method of paragraph 114, 115, or 116, in which an additional treatment is administered.

160. The method of paragraph 159 in which the additional treatment is administered during a treatment holiday.

161. The method of paragraph 159 or 160, in which the additional treatment is integumental perturbation.

162. The method of paragraph 115 or 161 in which the compound having Formula (I) is administered before the integumental perturbation treatment.

163. The method of paragraph 115 or 161 in which the compound having Formula (I) is administered concurrently with the integumental perturbation treatment.

164. The method of paragraph 115 or 161 in which the compound having Formula (I) is administered after the integumental perturbation treatment.

165. The method of paragraph 114 or 116, wherein the treatment with the compound having Formula (I) is begun before the skin has been wounded.

166. The method of paragraph 114 or 116, wherein the treatment with the compound having Formula (I) is begun concurrently with wounding.
167. The method of paragraph 114 or 116, wherein the treatment with the compound having Formula (I) is begun after skin has been wounded.

168. The method of paragraph 115 or 161 in which the integumental perturbation removes the epidermis partially or completely.

169. The method of paragraph 115 or 161 in which the integumental perturbation removes all of the epidermis and part of the dermis.

170. The method of paragraph 115 or 161 in which the integumental perturbation does not remove the epidermis.

171. The method of paragraph 115 or 161 in which the integumental perturbation comprises surgical excision of skin, serial expansion of skin, a skin graft, or combination thereof.

172. The method of paragraph 115 or 161 in which the integumental perturbation is accomplished by laser, light, heat, microneedle rollers, a felt wheel, ultrasound, iontophoresis, electrophoresis, dermabrasion with diamond fraise, or radiation treatment, or a combination thereof.

173. The method of paragraph 115 or 161 in which the integumental perturbation is accomplished by laser.

174. The method of paragraph 173 wherein the laser is a pulsed dye laser.

175. The method of paragraph 173 wherein the integumental perturbation by laser is fractional and non-ablative.

176. The method of paragraph 175, wherein the fractional, non-ablative integumental perturbation by laser is performed by use of an Erbium-YAG laser at 1500-1590 nm.

177. The method of paragraph 173 wherein the integumental perturbation by laser is fractional and ablative.

178. The method of paragraph 177, wherein the fractional, ablative laser integumental perturbation results in fractional ablation of the skin at a depth between 100 microns and 4000 microns into the skin.
179. The method of paragraph 177, wherein the fractional, ablative laser integumental perturbation results in fractional ablation of the skin at a depth approximating the depth of a full-thickness excision wound.

180. The method of paragraph 177, wherein the fractional, ablative laser integumental perturbation results in fractional ablation of the skin over an area of 1.5 cm x 1.5 cm to 15 cm x 15 cm.

181. The method of paragraph 177, wherein the fractional, ablative laser integumental perturbation results in fractional ablation of the skin at a depth density of the micro-thermal zones of the fractional ablation approximates that of a full bulk ablation of the entire area of treatment.

182. The method of paragraph 177, wherein the fractional, ablative laser integumental perturbation is by full bulk ablation, wherein the tissue of the entire area of treatment is ablated.

183. The method of paragraph 182, wherein the fractional, ablative laser integumental perturbation by bulk ablation is over an area of 1.5 cm x 1.5 cm to 15 cm x 15 cm.

184. The method of paragraph 182, wherein the fractional, ablative laser integumental perturbation by bulk ablation is accomplished at 10,600 nm using a carbon dioxide laser.

185. The method of paragraph 182, wherein the fractional, ablative laser integumental perturbation by bulk ablation is accomplished at 2940 nm using a Erbium-YAG laser.

186. The method of paragraph 173 wherein the integumental perturbation by laser is non-fractional and ablative.

187. The method of paragraph 186, wherein the non-fractional, ablative laser integumental perturbation is by full bulk ablation, wherein the tissue of the entire area of treatment is ablated.

188. The method of paragraph 186, wherein the non-fractional, ablative laser integumental perturbation by bulk ablation is over an area of 1.5 cm x 1.5 cm to 15 cm x 15 cm.
189. The method of paragraph 186, wherein the non-fractional, ablative laser integumental perturbation by bulk ablation is accomplished at 10,600 nm using a carbon dioxide laser.

190. The method of paragraph 186, wherein the non-fractional, ablative laser integumental perturbation by bulk ablation is accomplished at 2940 nm using a Erbium-YAG laser.

191. The method of paragraph 115 or 161 in which the integumental perturbation is accomplished using a microneedle array.

192. The method of paragraph 191, wherein the microneedle array is in the form of a roller or flat plate.

193. The method of paragraph 191, wherein the microneedle array can disrupt a skin area of 1.5 cm x 1.5 cm to 15 cm x 15 cm.

194. The method of paragraph 191, wherein the microneedle array can disrupt skin at a depth of 100 microns to 4000 microns.

195. The method of paragraph 191, wherein the microneedle array has hollow needles.

196. The method of paragraph 191, wherein the microneedle array top has a luer-lock fitting that can accommodate a syringe to deliver drug.

197. The method of paragraph 196, wherein the volume of the syringe is 1 ml to 3 ml.

198. The method of paragraph 115 or 161 in which the integumental perturbation is accomplished by injury.

199. The method of paragraph 198, wherein the mode of injury is mechanical.

200. The method of paragraph 199, wherein the mechanical injury is accomplished by microdermabrasion, dermabrasion, tape stripping, or full thickness excision of tissue.

201. The method of paragraph 198, wherein the mode of injury is thermal injury.

202. The method of paragraph 201, wherein the thermal injury is accomplished by laser.
203. The method of paragraph 115 or 161 in which the integumental perturbation is accomplished by inducing inflammation.

204. The method of paragraph 203 in which the method of inducing inflammation is by application of an adjuvant.

205. The method of paragraph 204, wherein the adjuvant is selected from the group of sodium dodecyl sulfate, aluminum salts, monophosphoryl lipid A, and cetyl trimmonium bromide (CTAB).

206. The method of paragraph 203 in which the method of inducing inflammation is injury to tissue.

207. The method of paragraph 203 in which the method of inducing inflammation is by application of an cytokine (e.g., IL-1 beta).

208. The method of paragraph 203 in which the method of inducing inflammation is by application of an antigen (e.g., tetanus toxoid).

209. The method of paragraph 115 or 161 in which the method of integumental perturbation is accompanied by stem cell mobilization (i.e., G-CSF).

210. The method of paragraph 159 or 160 in which the additional treatment stimulates hair growth.

211. The method of paragraph 159 or 160 in which the additional treatment is the administration of an anti-senescence agent.

212. The method of paragraph 159 or 160 in which the additional treatment is a surgical transplantation of hair follicles.

213. The method of paragraph 159 or 160 in which the additional treatment inhibits hair growth.

214. The method of paragraph 159 or 160, wherein the additional treatment is with an agent that modulates wound healing.

215. The method of paragraph 214, wherein the agent is an agent that enhances wound healing.
216. The method of paragraph 214, wherein the agent is an agent that slows wound healing.

217. The method of paragraph 216, wherein the agent that slows wound healing is rapamycin, a corticosteroid, a prostaglandin inhibitor, an inhibitor of fibrin, an inhibitor of collagen, an inhibitor of myofibroblast activity, an anti-inflammatory agent.

218. The method of paragraph 214, wherein the agent is an agent that reduces scarring.

219. The method of paragraph 217, wherein the anti-inflammatory agent is an NSAID or an antagonist of TNFa, TGFp, NFkB, IL-1, IL-6, IL-8, IL-10, or IL-18.

220. A method for inducing hair growth on the scalp of a human subject with androgenetic alopecia, wherein the method comprises (a) performing dermabrasion with a depth of about 100 microns at Day 0; (b) commencing at Day 0, topical administration of a GSK-3 inhibitor twice daily for about 7 days followed by a period without topical treatment of about 21 days; and (c) administering topically 5% minoxidil foam for at least 5 months.

221. The method of paragraph 220, wherein the GSK-3 inhibitor is a compound having Formula (I):

```
\[
\begin{align*}
A & \quad N \\
R_1 & \quad R_2 \\
R_3 & \quad Y \\
R_4 & \quad R_5 \\
R_6 & \quad R_7
\end{align*}
\]
```

Formula I

or pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein, Y is N or CH;

Ri, R2, R3, and R4 are independently hydrogen, hydroxyl, alkyl, alkenyl, aikynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, or alkoxy;

R5 and R7 are independently hydrogen or halo or optionally substituted alkyl, cycloalkyl, aryl, aminoalkyl, aminoaralkyl, aminocycloalkylaryl, arylcarboxamidocycloalkylarylalkyl, arylcarboxamidocycloalkylaryl, arylcarboxamidoalkylcycloalkyl, arylcarboxamidoaryl, arylcarboxamidoalkyl, arylcarboxamidoaralkyl, arylcarboxamidoalkoxyalkyl, aminoalkoxyalkyl, or arylsulfonamidoaralkyl;

R6 is hydrogen, carboxyl, nitro, amino, cyano, or an optionally substituted alkyl, aryl, aralkyl,
aminoalkyl, aminoaryl, aminoaralkyl, aminoalkoxyalkyl, arylaminoalkyl, arylaminoaryl, arylalkylamino, arylalkylaminoalkyl, arylalkylaminoaralkyl, carboxycycloamido, acyloxyalkyl, acyloxyaryl, acyloxyaralkyl, acyloxyalkylcycloalkyl, acyloxyalkylaminoalkyl, sulfonylalkyl, carbamylalkyl, carbamylaryl, carbamylaralkyl, carbamylalkylamino, carbamylalkylaminoalkyl, carbamylalkylaminoaryl, or carbamylalkylaminoaralkyl; and

A is pyridyl, pyrimidinyl, thiazolyl, indolyl, imidazolyl, oxadiazolyl, pyrazinyl, triazolyl, thiophenyl, furanyl, quinolinyl, purinyl, naphthyl, benzothiazolyl, benzopyridyl, or benzimidazolyl, which can be optionally substituted with from 0 to 3 of the following substitution groups: nitro, amino, cyano, halo, thioamido, amidino, oxamidino, alkoxyamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, loweralkyl, haloloweralkyl, haloloweralkoxy, haloloweralkoxyl, loweralkylamino, loweralkylamidino, loweralkylamidinyl, loweralkylaminoalkyl, loweralkylaminoaryl, loweralkylaminoaralkyl; and

222. The method of paragraph 220, wherein the method further comprises administering finasteride or dutasteride.

223. The method of paragraph 220, wherein the method further comprises administering a 5-alpha-reductase inhibitor.

224. A method for enhancing hair growth in a human subject having cicatricial alopecia, comprising administering a pulse treatment or intermittent treatments of a GSK-3 inhibitor formulation that delivers an effective amount of the GSK-3 inhibitor to a human subject in need thereof.

225. The method of paragraph 224, wherein the GSK-3 inhibitor is a compound having Formula (I):

```
\begin{align*}
    & A - N \\
    & \text{R}_1 \quad \text{R}_2 \\
    & \text{R}_3 \quad \text{R}_4 \\
    & \text{R}_5 \\
    & \text{Y} \\
    & \text{R}_6 \quad \text{R}_7 \\
\end{align*}
```

Formula (I)

or pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein, Y is N or CH;
Ri, R₂, R₃, and R₄ are independently hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, or alkoxy;
R₅ and R₇ are independently hydrogen or halo or optionally substituted alkyl, cycloalkyl, aryl, aminoalkyl, aminoaralkyl, aminocycloalkylaryl, arylcarboxamidocycloalkylaryl, arylcarboxamidocycloalkyl, arylcarboxamidoalkylcycloalkyl, arylcarboxamidoaryl, arylcarboxamidoaralkyl, arylcarboxamidoalkoxyalkyl, aminoalkoxyalkyl, or arylsulfonamidoaralkyl;
R₁₅ is hydrogen, carboxyl, nitro, amino, cyano, or an optionally substituted alkyl, aryl, aralkyl, aminoalkyl, aminoaryl, aminoaralkyl, aminoalkoxyalkyl, arylaminoalkyl, arylaminoaryl, arylaminoaralkyl, arylalkylamino, arylalkylaminoalkyl, arylalkylaminoaralkyl, carboxycycloamido, acyloxyalkyl, acyloxyaryl, acyloxyaralkyl, acyloxyalkylcycloalkyl, acyloxyalkylaminoalkyl, sulfonylalkyl, carbamylalkyl, carbamylaryl, carbamylaralkyl, carbamylalkylamino, carbamylalkylaminoalkyl, carbamylalkylaminoaralkyl, or carbamylalkylaminoaralkyl; and
A is pyridyl, pyrimidinyl, thiazolyl, indolyl, imidazolyl, oxadiazolyl, tetrazolyl, pyrazinyl, triazolyl, thiophenyl, furanyl, quinolinyl, purinyl, naphthyl, benzothiazolyl, benzopyridyl, or benzimidazolyl, which can be optionally substituted with from 0 to 3 of the following substitution groups: nitro, amino, cyano, halo, thioamido, amidino, oxamidino, alkoxamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, loweralkyl, haloloweralkyl, loweralkoxy, haloloweralkoxy, loweralkoxyalkyl, loweralkylamino loweralkoxy, loweralkylcarbonyl, loweralkylcarbonyl, lowerheteroaralkylcarbonyl, alkylthio, aminoalkyl or cyanoalkyl.

226. The method of paragraph 224, wherein the human subject has primary cicatricial alopecia.

227. The method of paragraph 226, wherein the primary cicatricial alopecia is one or more of the following forms of primary cicatricial alopecia: lichen planopilaris, frontal fibrosing alopecia, central centrifugal cicatricial alopecia, pseudopelade, folliculitis decalvans, tufted folliculitis, dissecting cellulitis, or folliculitis keloidalis.

228. The method of any one of paragraphs 224-227, wherein the pulse or intermittent Compound treatment is administered following integumental perturbation.
229. The method of paragraph 228, wherein the integumental perturbation is accomplished by laser.

230. The method of paragraph 229, wherein the laser is a fractional, ablative laser.

231. The method of paragraph 230, wherein the integumental perturbation by fractional ablative laser is followed by twice daily topical administration of the GSK-3 inhibitor formulation for 14 days.

232. A drug delivery device as shown in any one of Figures 38 to 42, wherein the solid component 88 comprises a polymer macromonomer (Polymer 1) (a polymer that can further crosslink with another component) and microspheres containing a GSK-3 inhibitor and wherein the liquid component 86 comprises another polymer macromonomer (Polymer 2) that is capable of reacting with Polymer 1.

233. A method for treating a wound, said method comprising: applying a polymer to the wound using a drug delivery device as shown in any one of Figures 38 to 42, wherein the solid component 88 comprises a polymer macromonomer (Polymer 1) (a polymer that can further crosslink with another component) and the liquid component 86 comprises another polymer macromonomer (Polymer 2) that is capable of reacting with Polymer 1; and applying to the wound a biodegradable scaffold that comprises GSK-3 inhibitor.

234. A drug delivery device as shown in any one of Figures 34 to 37, wherein the first liquid component 62 is a GSK-3 inhibitor in a first formulation (e.g., micronized GSK-3 inhibitor) and wherein the second liquid component 64 is a GSK-3 inhibitor in a second formulation (e.g., dissolved GSK-3 inhibitor in an aqueous sprayable gel).

235. A method for inducing hair growth on the scalp of a human subject with androgenetic alopecia, wherein the method comprises (a) performing dermabrasion with a depth of about 100 microns at Day 0; (b) commencing at Day 0, topical administration of a GSK-3 inhibitor twice daily for about 7 days followed by a period without topical treatment of about 21 days; and (c) administering topically 5% minoxidil foam for at least 5 months.
236. The method of paragraph 235, wherein the GSK-3 inhibitor is a compound having Formula X:

![Formula X](image)

or pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

- X is , wherein A is O or S;
- Y is S, SO, or S0₂;
- n is 1 or 2; and
- Z is hydrogen, halo, COOH, COOR, CH₂COOH, or CH₂COOR, wherein R is alkyl.

237. The method of paragraph 235, wherein the method further comprises administering finasteride or dutasteride.

238. The method of paragraph 235, wherein the method further comprises administering a 5-alpha-reductase inhibitor.

239. A method for enhancing hair growth in a human subject having cicatricial alopecia, comprising administering a pulse treatment or intermittent treatments of a GSK-3 inhibitor formulation that delivers an effective amount of the GSK-3 inhibitor to a human subject in need thereof.

240. The method of paragraph 239, wherein the GSK-3 inhibitor is a compound having Formula X:

![Formula X](image)

or pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

- X is , wherein A is O or S;
- Y is S, SO, or S0₂;
n is 1 or 2; and
Z is hydrogen, halo, COOH, COOR, CH₂COOH, or CH₂COOR, wherein R is alkyl.

241. The method of paragraph 240, wherein the human subject has primary cicatricial alopecia.

242. The method of paragraph 241, wherein the primary cicatricial alopecia is one or more of the following forms of primary cicatricial alopecia: lichen planopilaris, frontal fibrosing alopecia, central centrifugal cicatricial alopecia, pseudopelade, folliculitis decalvans, tufted folliculitis, dissecting cellulitis, or folliculitis keloidalis.

243. The method of any one of paragraphs 239-242, wherein the pulse or intermittent Compound treatment is administered following integumental perturbation.

244. The method of paragraph 243, wherein the integumental perturbation is accomplished by laser.

245. The method of paragraph 244, wherein the laser is a fractional, ablative laser.

246. The method of paragraph 245, wherein the integumental perturbation by fractional ablative laser is followed by twice daily topical administration of the GSK-3 inhibitor formulation for 14 days.

247. The method of any one of paragraphs 235-238, wherein the GSK-3 inhibitor is

248. The method of any one of paragraphs 239-246, wherein the GSK-3 inhibitor is

249. The drug delivery device of paragraph 232, wherein the GSK-3 inhibitor is
250. The method of paragraph 233, wherein the GSK-3 inhibitor is

![Chemical structure](image1)

251. The drug delivery device of paragraph 234, wherein the GSK-3 inhibitor in the first formulation, the GSK-3 inhibitor in the second formulation, or both, is

![Chemical structure](image2)
WHAT IS CLAIMED IS:

1. A method for inducing hair growth on the scalp of a human subject, comprising performing integumental perturbation of the scalp of the subject, followed by administering to the subject a pharmaceutical composition of a Compound having a formula selected from:

   (i) Formula I:

   ![Formula 1]

   or a pharmaceutically salt, solvate, or prodrug thereof, wherein,

   \( Y \) is \( \text{N} \) or \( \text{CH} \);

   \( R_i, R_2, R_3 \), and \( R_4 \) are independently hydrogen, hydroxyl, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, heterocyclyl, or alkoxy;

   \( R_5 \) and \( R_7 \) are independently hydrogen or halo or optionally substituted alkyl, cycloalkyl, aryl, aminoalkyl, aminoaralkyl, aminocycloalkylaryl, arylcarboxamidocycloalkylaralkyl, arylcarboxamidoalkylaryl, arylcarboxamidoalkylcycloalkyl, arylcarboxamidoaryalkyl, arylcarboxamidoalkoxyalkyl, arylcarboxamidoalkoxyalkyl, aminoalkoxyalkyl, or arylsulfonamidoaralkyl;

   \( R_6 \) is hydrogen, carboxyl, nitro, amino, cyano, or an optionally substituted alkyl, aryl, aralkyl, aminoalkyl, aminoaryl, aminoaralkyl, aminoalkoxyalkyl, arylaminoalkyl, arylaminoaryl, arylaminoaralkyl, aminocycloalkylaryl, arylalkylaminooalkyl, arylalkylaminooaryl, arylalkylaminooaralkyl, carbocycloamido, acyloxyalkyl, acyloxyaryl, acyloxyaralkyl, acyloxyalkylcycloalkyl, acyloxyalkylaminooalkyl, sulfonylalkyl, carbamylalkyl, carbanylelalkyl, carbamylaralkyl, carbamylalkylaminooalkyl, carbamylalkylaminooaryl, or carbamylalkylaminooaralkyl; and

   \( A \) is pyridyl, pycrimidinidyl, thiazolyl, indolyl,imidazolyl, oxadiazolyl, tetrazolyl, pyrazinyl, triazolyl, thiophenyl, furanyl, quinolinyl, purinyl, naphthyl, benzothiazolyl, benzopyridyl, or benzimidazolyl, which can be optionally substituted with from 0 to 3 of the following substitution groups: nitro, amino, cyano, halo, thioamido, amidino, oxamidino, alkoxyamidino, imidino, guanidino, sulfonamido, carboxyl, formyl, loweralkyl, haloloweralkyl, loweralkoxy, haloloweralkoxy, loweralkoxyalkyl, loweralkylamino
loweralkoxy, loweralkylcarbonyl, loweralkylcarbonyl, lowerheteroaralkylcarbonyl, alkylthio, aminoalkyl or cyanoalkyl;

(ii) Formula II:

![Formula II](image)

or a pharmaceutically salt, solvate, or prodrug thereof, wherein,

R₅, R₆, and R₇ are as defined as for Formula I above; and

R₈ and R₉ are independently hydrogen, nitro, amino, cyano, halo, thioamido, sulfonamido, carboxyl, formyl, loweralkyl, haloloweralkyl, aminoloweralkyl, cyanoloweralkyl, loweralkoxy, haloloweralkoxy, loweralkoxyalkyl, loweralkylaminoloweralkoxy, alkylthio, aryl, and aralkyl;

(iii) Formula III:

![Formula III](image)

or a pharmaceutically salt, solvate, or prodrug thereof, wherein,

R₆, R₇, Rg, and R₉ are as defined for Formula II; and

R₁₀, R₁₁, R₁₂, R₁₃, and R₁₄ are independently hydrogen, nitro, amino, cyano, halo, thioamido, carboxyl, hydroxy, or optionally substituted loweralkyl, loweralkoxy, loweralkoxyalkyl, haloloweralkyl, haloloweralkoxy, aminoalkyl, alkylamino, alkylthio, alkylcarbamylamino, aralkylcarbamylamino, heteroaralkylcarbamylamino, arylcarbamylamino, heteroarylcarbamylamino, aminocarbonyl, loweralkylaminocarbonyl, aminoaralkyl, loweralkylaminoalkyl, aryl, heteroaryl, cycloalkyl, aralkyl, alkylcarbonyloxy, arylcarbonyloxy, aralkylcarbonyloxy, arylcarbonyloxyalkyl, alkxycarbonylalkyl, heteroarylcarbonyloxyalkyl, aralkylcarbonyloxyalkyl, or heteroaralkylcarbonyloxyalkyl;
or a pharmaceutically salt, solvate, or prodrug thereof;

(v) Formula V:

or a pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,
Z is H or Br;
X is H, F, Cl, Br, I, or CH=CH₂;
R is H or CH₃;
W is H, CH₃, Cl, NH₂, or NO₂;
L is H or Cl; and
Y is O, NOH, NOCH₃, or NOC(=0)CH₃;

(vi) Formula VI:

or a pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,
X and Y are independently O, S, NR₃, and CR₃R₂;
R¹ and R² are independently H, alkyl, cycloalkyl, haloalkyl, aryl, (Z)ₙ—aryl, heteroaryl, OR₃,
C(=0)R₃, C(=0)OR₃, (Z)ₙ—C(=0)OR₃, or together with NR₃ can form a fused aryl group;
Z is independently CR₃R₄, C(=0)−, C(=0)−, C(=NR₃)−, S(O)−, or NR₃;
n is 0, 1, or 2;
t is 0, 1, or 2;
R³ and R⁴ are independently H, alkyl, aryl, or heterocyclyl; and
R<sup>a</sup> and R<sup>b</sup> are independently H, alkyl, cycloalkyl, haloalkyl, aryl, (Z)—aryl, heteroaryl, OR<sup>3</sup>, C(=0)R<sup>3</sup>, C(=0)OR<sup>3</sup>, or (Z)<sub>n</sub>—C(=0)OR<sup>3</sup>;

(vii) Formula VII:

![Formula VII](image)

or a pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein
X is O, S, or NH (optionally substituted with a lower alkyl);
Y is O or NH (optionally substituted with a lower alkyl);
Z is N or CH (optionally substituted with a lower alkyl);
A is H, F, Cl, Br, CN, N<sub>2</sub>O, or lower alkyl; and
B is H, lower alkyl, lower alkoxy, or NH<sub>2</sub> (optionally substituted with one or two lower alkyl);

(viii) Formula VIII:

![Formula VIII](image)

wherein A and E are independently selected from the group consisting of a hydrogen substituted carbon atom and a nitrogen atom; Z is selected from O; alternatively, Z is selected from dihydro; wherein each hydrogen atom is attached by a single bond;

R<sub>4</sub> and R<sub>5</sub> are independently selected from C1-8 alkyl, C2-8 alkenyl and C2-8 alkynyl optionally substituted with oxo;

R<sub>2</sub> is selected from the group consisting of --C1-8 alkyl-, --C2-8 alkenyl-, --C2-8 alkynyl-, -0-(C1-8)alkyl-0-, -0-(C2-8)alkenyl-0-, -0-(C2-8)alkynyl-0-, -C(0)-(Cl-8)alkyl- C(0)- (wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are straight carbon chains optionally substituted with one to four substituents independently selected from the group consisting of C1-8 alkyl, C1-8 alkoxy, C1-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, -C(0)0-(Cl-8)alkyl, -Cl -8 alkyl-C(0)0-(Cl-8)alkyl, amino
(substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), halogen, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alkoxy, hydroxy, hydroxy(Cl-8)alkyl and oxo; and, wherein any of the foregoing alkyl, alkenyl and alkynyl linking groups are optionally substituted with one to two substituents independently selected from the group consisting of heterocyclyl, aryl, heteroaryl, heterocyclyl(Cl-8)alkyl, aryl(Cl-8)alkyl, heteroaryl(Cl-8)alkyl, spirocycloalkyl and spiroheterocyclyl (wherein any of the foregoing cycloalkyl, heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, Cl-8 alkoxy(Cl-8)alkyl, carboxyl, carboxylic(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), halogen, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alkoxy, hydroxy and hydroxy(Cl-8)alkyl; and, wherein any of the foregoing heterocyclyl substituents are optionally substituted with oxo)), cycloalkyl, heterocyclyl, aryl, heteroaryl (wherein cycloalkyl, heterocyclyl, aryl and heteroaryl are optionally substituted with one to four substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, Cl-8 alkoxy(Cl-8)alkyl, carboxyl, carboxylic(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), halogen, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alkoxy, hydroxy and hydroxy(Cl-8)alkyl; and, wherein heterocyclyl is optionally substituted with oxo), ~(0-(CH2)1-6)0-5 0- , -0-(CH2)l-6 0-0-(CH2)l-6 0-, -0-(CH2)l-6 0-0-(CH2)l-6 0-, ~(0-(CH2)l-6)0-5 0- , -0-(CH2)l-6 0-0-(CH2)l-6 0-, -0-(CH2)l-6 0-0-(CH2)l-6 0-, ~(0-(CH2)l-6)0-5 0- , -0-(CH2)l-6 0-0-(CH2)l-6 0-, -0-(CH2)l-6 0-0-(CH2)l-6 0-, ~(0-(CH2)l-6)0-5 0- , -0-(CH2)l-6 0-0-(CH2)l-6 0-, -0-(CH2)l-6 0-0-(CH2)l-6 0-, ~(0-(CH2)l-6)0-5 0- , -0-(CH2)l-6 0-0-(CH2)l-6 0-, -0-(CH2)l-6 0-0-(CH2)l-6 0-, ~(0-(CH2)l-6)0-5 0- , -0-(CH2)l-6 0-0-(CH2)l-6 0-, -0-(CH2)l-6 0-0-(CH2)l-6 0-, ~(0-(CH2)l-6)0-5 0- , -0-(CH2)l-6 0-0-(CH2)l-6 0-,
8)-alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and Cl-4 alkyl), hydroxy(Cl-8)alkyl, heterocyclyl(CI-8)alkyl, aryl(CI-8)alkyl and heteroaryl(CI-8)alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of CI-8 alkyl, CI-8 alkoxy, CI-8 alkoxy(CI-8)alkyl, carboxyl, carboxyl(CI-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), amino(CI-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), halogen, (halo)1-3 (CI-8)alkyl, (halo)1-3 (CI-8)alcohol, hydroxy and hydroxy(CI-8)alkyl; and, wherein heterocyclyl is optionally substituted with oxo));

with the proviso that, if A and E are selected from a hydrogen substituted carbon atom, then R₂ is selected from the group consisting of -C2-8 alkenyl-,-0-(Cl-8)alkyl-0—, -0—(C2-8)alkenyl-0—, -0—(C2-8)alkynyl-0—, -C(0)-(Cl-8)alkyl-C(0)— (wherein any of the foregoing alkyl, alkenyl and alkylnyl linking groups are straight carbon chains optionally substituted with one to four substituents independently selected from the group consisting of CI-8 alkyl, CI-8 alkoxy, CI-8 alkoxy(CI-8)alkyl, carboxyl, carboxyl(CI-8)alkyl, -C(0)0-(Cl-8)alkyl, --C1-8 alkyl-C(0)0—(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), amino(CI-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), halogen, (halo)1-3 (Cl-8)alkyl, (halo)1-3 (Cl-8)alcohol, hydroxy, hydroxy(CI-8)alkyl and oxo; and, wherein any of the foregoing alkyl, alkenyl and alkylnyl linking groups are optionally substituted with one to two substituents independently selected from the group consisting of heterocyclyl, aryl, heteroaryl, heterocyclyl(Cl-8)alkyl, aryl(Cl-8)alkyl, heteroaryl(Cl-8)alkyl, spirocycloalkyl and spiroheterocyclyl (wherein any of the foregoing cycloalkyl, heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of Cl-8 alkyl, CI-8 alkoxy, CI-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), halogen, (halo)1-3 (Cl-8)alkyl, (halo)1-3 (Cl-8)alcohol, hydroxy and hydroxy(Cl-8)alkyl; and, wherein any of the foregoing heterocyclyl substituents are optionally substituted with oxo)), cycloalkyl (wherein cycloalkyl is optionally substituted with one to four substituents independently selected from the group consisting of CI-8 alkyl,
CI-8 alkoxy, CI-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), halogen, (halo) 1-3 (CI-8)alkyl, (halo) 1-3 (CI-8)alkoxy, hydroxy and hydroxy(Cl-8)alkyl, -(0-(CH₂)l-6)-5 -O-, -(0-(CH₂)l-6 -0-(CH₂)l-6 -0-, -(0-(CH₂)l-6 -0-(CH₂)l-6 -0-, -(0-(CH₂)l-6)-5 -NR₆ -, -(0-(CH₂)l-6 -NR₆ -(CH₂)l-6 -0-, -(0-(CH₂)l-6 -0-(CH₂)l-6)0-5 -S-, -(0-(CH₂)l-6 -S-(CH₂)l-6 -0-, -(0-(CH₂)l-6 -0-(CH₂)l-6 -S-, -NR₆ ~NR₇ ~, ~NR₆ -(CH₂)l-6 ~NR₇ ~, ~NR₆ -(CH₂)l-6 ~NR₇ -(CH₂)l-6 -NR8 ~, ~NR9 -(C(0)-), ~C(0)-, -C(0)-NR₉ ~, ~C(0)-(CH₂)0-6 -NR6 -(CH₂)0-6 ~C(0)-, -NR₆ -(CH₂)0-6 -C(0)-(CH₂)l-6 -C(0)-(CH₂)0-6 -NR₇ ~, -NR₆ -(C(0)-(CH₂)0-6 ~C(0)-(CH₂)l-6 -NR₆ -(CH₂)0-6 ~C(0)-(CH₂)l-6 -NR₆ -(CH₂)l-6 -0-, -(CH₂)l-6 -NR₆ -(CH₂)l-6 -S-, and -NR₆ -(CH₂)l-6 -S-(CH₂)l-6 ~NR₇ ~ (wherein R₆, R₇ and R₉ are independently selected from the group consisting of hydrogen, CI-8 alkyl, CI-8 alkoxy(Cl-8)alkyl, carboxyl(Cl-8)alkyl, amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), hydroxy(Cl-8)alkyl, heterocyclyl(Cl-8)alkyl, aryl(Cl-8)alkyl and heteroaryl(Cl-8)alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of CI-8 alkyl, CI-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), halogen, (halo) 1-3 (CI-8)alkyl, (halo) 1-3 (CI-8)alkoxy, hydroxy and hydroxy(Cl-8)alkyl; and, wherein heterocyclyl is optionally substituted with oxo); and, wherein R₈ is selected from the group consisting of CI-8 alkyl, CI-8 alkoxy(Cl-8)alkyl, carboxyl(Cl-8)alkyl, amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), hydroxy(Cl-8)alkyl, heterocyclyl(Cl-8)alkyl, aryl(Cl-8)alkyl and heteroaryl(Cl-8)alkyl (wherein the foregoing heterocyclyl, aryl and heteroaryl substituents are optionally substituted with one to four substituents independently selected from the group consisting of CI-8 alkyl, CI-8 alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent
independently selected from the group consisting of hydrogen and CI-4 alkyl), halogen, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alkoxy, hydroxy and hydroxy(Cl-8)alkyl; and, wherein heterocyclyl is optionally substituted with o xo)); and, R1 and R3 are independently selected from the group consisting of hydrogen, CI-8 alkyl, C2-8 alkenyl, C2-8 alkynyl (wherein alkyl, alkenyl and alkynyl are optionally substituted with a substituent selected from the group consisting of CI-8 alkoxy, alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), amino(Cl-8)alkyl (wherein amino is substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), (halo) 1-3, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alkoxy, hydroxy, hydroxy(Cl-8)alkyl and o xo), CI-8 alkoxy, CI-8 alkoxy carbonyl, (halo) 1-3 (Cl-8)alkoxy, CI-8 alkylthio, aryl, heteroaryl (wherein aryl and heteroaryl are optionally substituted with a substituent selected from the group consisting of CI-8 alkyl, CI-8 alkoxy, alkoxy(Cl-8)alkyl, carboxyl, carboxyl(Cl-8)alkyl, amino (substituted with a substituent independently selected from the group consisting of hydrogen and CI-4 alkyl), cyano, halogen, hydroxy and nitro; or a pharmaceutically acceptable salt thereof;

(ix) Formula IX:

![Formula IX](attachment:image.png)

wherein Ri and R2 are independently selected from the group consisting of: hydrogen, CI-8 alkyl, C2-8 alkenyl, C2-8 alkynyl (wherein alkyl, alkenyl and alkynyl are optionally substituted with one to two substituents independently selected from the group consisting of -0-(Cl-8)alkyl, -0-(Cl-8)alkyl-OH, -0-(Cl-8)alkyl-0-(Cl-8)alkyl, -0-(Cl-8)alkyl- NH2, -0-(Cl-8)alkyl-NH-(Cl-8)alkyl, -0-(Cl-8)alkyl-N[(Cl-8)alkyl]2, -0-(Cl-8)alkyl-S-(Cl-8)alkyl, -0-(Cl-8)alkyl-S0 -(Cl-8)alkyl, -0-(Cl-8)alkyl-S02 -(Cl-8)alkyl, -0-(Cl-8)alkyl-S02 -N[(Cl-8)alkyl]2, -0-C(0)H, -O-...
C(0)-(Cl-8)alkyl, -0-C(0)-NH 2, -0-(Cl-8)alkyl-C(0)H, -0-(Cl-8)alkyl-C(0)-H, -0-(Cl-8)alkyl-C(0)-[Cl-8]alkyl, -0-(Cl-8)alkyl-C(0)-NH 2, -0-(Cl-8)alkyl-C(0)-NH--(Cl-8)alkyl, -0-(Cl-8)alkyl-C(0)-N[(Cl-8)alkyl] 2, -0-(Cl-8)alkyl-C(0)-NH--(Cl-8)alkyl, --C(0)-H, --C(0)-(Cl-8)alkyl, --C0 2 H, --C(0)-(Cl-8)alkyl, --C(0)-0-(Cl-8)alkyl, --C(0)-NH 2, -C(NH)-NH 2, -C(0)-NH-(Cl-8)alkyl, --C(0)-N[(Cl-8)alkyl] 2, -SH, -S--(Cl-8)alkyl, -S-(Cl-8)alkyl-S-(Cl-8)alkyl, --S-(Cl-8)alkyl-0-(Cl-8)alkyl, --S-(Cl-8)alkyl-0-(Cl-8)alkyl, S-(Cl-8)alkyl-0-(Cl-8)alkyl-0-(Cl-8)alkyl-NH 2, S-(Cl-8)alkyl-0-(Cl-8)alkyl-NH--(Cl-8)alkyl, --S-(Cl-8)alkyl-0-(Cl-8)alkyl-N[(Cl-8)alkyl] 2, --S-(Cl-8)alkyl-NH-(Cl-8)alkyl, --S0 2 --(Cl-8)alkyl, --S0 2 --NH--(Cl-8)alkyl, -S0 2 -NH--(Cl-8)alkyl 2, amino (substituted with two substituents independently selected from the group consisting of hydrogen, Cl-8 alkyl, C2-8 alkenyl, C2-8 alkynyl, -(Cl-8)alkyl-0-(Cl-8)alkyl, -0-(Cl-8)alkyl-0(C 1-8)alkyl-NH--(Cl-8)alkyl, -(C 1-8)alkyl-0-(Cl-8)alkyl-0-(Cl-8)alkyl-NH--(Cl-8)alkyl, -S-(Cl-1-8)alkyl-N[(Cl-8)alkyl] 2, -S-(Cl-8)alkyl-N[(Cl-8)alkyl], -C(0)-(Cl-8)alkyl, --C(0)-0-(Cl-8)alkyl, --C(0)-NH-(Cl-8)alkyl, --C(0)-N[(Cl-8)alkyl] 2, -C(0)-NH-(Cl-8)alkyl, --S0 2 --(Cl-8)alkyl, -S0 2 -NH--(Cl-8)alkyl, -S0 2 -NH-(Cl-8)alkyl, --S0 2 -N[(Cl-8)alkyl] 2, -C(N)-NH 2, aryl and aryl(Cl-8)alkyl (wherein aryl is optionally substituted with one to three substituents independently selected from the group consisting of halogen, Cl-8 alkyl, Cl-8 alkoxy, amino (substituted with two substituents selected from the group consisting of hydrogen and Cl-8 alkyl), cyano, halo, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alkoxy, hydroxy, hydroxy(Cl-8)alkyl and nitro), cyano, (halo) 1-3, hydroxy, nitro, oxo, heterocyclyl, aryl and heteroaryl (wherein heterocyclyl, aryl and heteroaryl are optionally substituted with one to three substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, amino (substituted with two substituents selected from the group consisting of hydroxyl and Cl-8 alkyl), cyano, halo, (halo) 1-3 (Cl-8)alkyl, (halo) 1-3 (Cl-8)alkoxy, hydroxy, hydroxy(Cl-8)alkyl and nitro), -C(0)-(Cl-8)alkyl, -C(0)-ary, -C(0)-0-(Cl-8)alkyl, -C(0)-0-ary, -C(0)-NH-(Cl-8)alkyl, -C(0)-NH 2, -C(0)-H 2, -C(0)-NH-(Cl-8)alkyl, -C(0)-N[(Cl-8)alkyl] 2, -S0 2 -(Cl-8)alkyl, --S0 2 -ary, aryl and heteroaryl (wherein aryl and heteroaryl are optionally substituted with one to three substituents independently selected from the group consisting of Cl-8 alkyl, Cl-8 alkoxy, -C(0)H, -C(0)-(Cl-8)alkyl, -C0 2 H, -C(0)-0-(Cl-8)alkyl, -C(0)-NH 2, -C(0)-NH--(Cl-8)alkyl, -C(0)-NH--(Cl-8)alkyl, -C(0)-N[(Cl-8)alkyl] 2, --SH, -S-(Cl-8)alkyl, --S0 2 -(Cl-8)alkyl, -S0 2 -NH--(Cl-8)alkyl, -S0 2 -NH--(Cl-8)alkyl, -S0 2 -N[(Cl-8)alkyl] 2, amino (substituted with two substituents independently selected from the group consisting of hydroxyl, Cl-8 alkyl, C2-8 alkenyl, C2-8 alkynyl, -(Cl-8)alkyl- NH 2, -C(0)-
(Cl-8)alkyl, -C(0)-0-(Cl-8)alkyl, ~C(0)- NH₂, --C(0)-NH-(Cl-8)alkyl, -C(0)-N[(Cl-8)alkyl]₂, -S₀₂- (Cl-8)alkyl, -- S₀₂- NH₂, - S₀₂--NH-(Cl-8)alkyl, - S₀₂ - N[(Cl-8)alkyl]₂ and -C(NH)~ NH₂, amino-(Cl-8)alkyl- (wherein amino is substituted with two substituents independently selected from the group consisting of hydrogen, Cl-8 alkyl, C-2-8 alkenyl, C-2-8 alkynyl, -(Cl-8)alkyl- NH₂, -C(0)-(Cl-8)alkyl, -C(0)-0-(Cl-8)alkyl, ~C(0)~ NH₂, -C(0)-NH-(Cl-8)alkyl, -(Cl-8)alkyl-C(0)-NH- (wherein amino is limited to phenyl unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, unsubstituted Cl-7 alkyl, hydroxy, unsubstituted Cl-7 alkoxy, (halo) 1-3 (Cl-7)alkyl, nitro, unsubstituted amino and cyano), -(Cl-7)alkyl-aryl (wherein ary1 is limited to phenyl unsubstituted or substituted with one or more substituents independently selected from the group consisting of halo, unsubstituted Cl-7 alkyl, hydroxy, Cl-7 alkoxy, (halo) 1-3 (Cl-7)alkyl, nitro, unsubstituted amino and cyano), -(Cl-7)alkyl-hydroxy, -(Cl-7)alkyl-(halo)-3, -(Cl-7)alkyl-amino (wherein amino is substituted with two substituents independently selected from the group consisting of hydrogen and --Cl-7 alkyl), -(Cl-7)alkyl-amino(Cl-7)alkylamino, -(Cl-7)alkyl-NH--C(0)-(Cl-7)alkyl, -Cl-7 alkyl-NH-S₀₂-(Cl-7)alkyl, -(Cl-7)alkyl-SH, -(Cl-7)alkyl-S-(Cl-7)alkyl, -(Cl-7)alkyl- S₀₂-(Cl-7)alkyl, -(Cl-7)alkyl-0-C(0)-(Cl-7)alkyl, -(Cl-7)alkyl-C(N), -(Cl-7)alkyl-C(NH)-NH₂, -(Cl-7)alkyl-C(0)-NH₂, -(Cl-7)alkyl-C(0)-NH₂, -(CH₂)₂2-6 -heterocyclaryl, -(CH₂)₂2-6 -T-C(V)-Z (wherein T is NH, V is O and Z is amino (wherein amino is substituted with two substituents independently selected from the group consisting of hydrogen and Cl-7 alkyl));

X is selected from the group consisting of N and CR₅;

R₃ and R₄ are independently selected from the group consisting of hydrogen, Cl-8 alkyl, C-2-8 alkenyl, C-2-8 alkynyl, CI-8 alkoxy, -C(0)H, -C(0)-(Cl-8)alkyl, -C₀₂H₃, ~C(0)-0-(Cl-8)alkyl, ~C(0)- NH₂, ~C(NH)~ NH₂, -C(0)-NH-(Cl-8)alkyl, -C(0)-N[(Cl-8)alkyl]₂, -SH, -S-(Cl-8)alkyl, -S₀₂-(Cl-8)alkyl, -S₀₂- NH₂, - S₀₂-NH-(Cl-8)alkyl, -S₀₂-N[(Cl-8)alkyl]₂, amino (substituted with two substituents independently selected from the group consisting of hydrogen, CI-8 alkyl, C-2-8 alkenyl, C-2-8 alkynyl, -(Cl-8)alkyl-NH₂, -C(0)-(Cl-8)alkyl, -C(0)-0-(Cl-8)alkyl, ~C(0)- NH₂, -C(0)-NH-
(Cl-8)alkyl, --C(0)-N[(Cl-8)alkyl]$_2$, --S02-(Cl-8)alkyl, -- C(0)$_2$-NH$_2$, -- S0$_2$-NH-(Cl-8)alkyl, -S02 --N[(Cl-8)alkyl]$_2$ and -C(NH)--NH$_2$, amino-(Cl-8)alkyl- (wherein amino is substituted with two substituents independently selected from the group consisting of hydrogen, C1-8 alkyl, C2-8 alkenyl, C2-8 alkynyl, -(Cl-8)alkyl-NH$_2$, -C(0)--(Cl-8)alkyl, -C(0)-0-(01-8)alkyl, --C(0)-NH$_2$, --C(0)-NH--(Cl-8)alkyl, -- C(0)--N[(Cl-8)alkyl]$_2$, -- S0$_2$-(Cl-8)alkyl, -- S0$_2$-NH$_2$, -- S0$_2$--NH--(Cl-8)alkyl, -- S0$_2$--N[(Cl-8)alkyl]$_2$ and - C(NH)--NH$_2$), cyano, halo, (halo)1-3-(Cl-8)alkyl-, (halo)1-3-(Cl-8)alkoxy-, hydroxy, hydroxy(C1-8)alkyl-, nitro, aryl, ~(Cl-8)alkyl-aryl, heteroaryl and ~(Cl-8)alkyl-heteroaryl;

Y and Z are independently selected from the group consisting of O, S, (H,OH) and (H,H); with the proviso that one of Y and Z is O and the other is selected from the group consisting of O, S, (H,OH) and (H,H); and

$R_5$ is selected from the group consisting of hydrogen, halogen, C1-8 alkyl, C2-8 alkenyl, C2-8 alkynyl {wherein alkyl, alkenyl and alkynyl are optionally substituted with one to two substituents independently selected from the group consisting of amino (substituted with two substituents selected from the group consisting of hydrogen and C1-8 alkyl), cyano, halo, hydroxy, nitro, oxo, aryl and heteroaryl}, aryl and heteroaryl {wherein aryl and heteroaryl are optionally substituted with one to two substituents independently selected from the group consisting of C1-8 alkyl, C1-8 alkoxy, amino (substituted with two substituents selected from the group consisting of hydrogen and C1-8 alkyl), cyano, halo, hydroxy and nitro};

or a pharmaceutically acceptable salt thereof; and

(x) Formula X:

![Formula X](image)

or a pharmaceutically acceptable salt, solvate, or prodrug thereof, wherein,

X is $\begin{array}{c} \text{N} \\ \text{N} \end{array}$ or $\begin{array}{c} \text{N} \\ \text{N} \end{array}$, wherein A is O or S;

Y is S, SO, or S0$_2$;

n is 1 or 2; and

Z is hydrogen, halo, COOH, COOR, CH$_2$COOH, or CH$_2$COOR, wherein R is alkyl.
2. The method of claim 1, wherein the formula of the Compound is:
<p>| | |</p>
<table>
<thead>
<tr>
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<tr>
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<td><img src="image2" alt="Chemical Structure 22" /></td>
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or a pharmaceutically acceptable salt, solvate, or prodrug thereof.

3. The method of claim 1, wherein the Compound is Formula IV:

4. The method of claim 3, wherein the pharmaceutical composition comprises 0.00001% to 0.0001% of the Compound of Formula IV.

5. The method of any one of claims 1 to 4, wherein the pharmaceutical composition is administered by topical administration.

6. The method of any one of claims 1 to 5, wherein the subject is also administered minoxidil or finasteride.

7. The method of any one of claims 1 to 6, wherein the integumental perturbation is by laser.

8. The method of any one of claims 1 to 6, wherein the integumental perturbation is by dermabrasion.

9. The method of claim 8, wherein the dermabrasion is to a depth of 30 µm to 200 µm.
10. A method for inducing hair growth on the scalp of a human subject with androgenetic alopecia, wherein the method comprises (a) performing dermabrasion with a depth of 30 µm to 200 µm at Day 0; (b) commencing at Day 0, topical administration of a GSK-3 inhibitor twice daily for about 7 days followed by a period without topical treatment of about 21 days; and (c) administering topically 5% minoxidil foam for at least 5 months.

11. The method of claim 9 or 10, wherein the dermabrasion is to a depth of 100 µm.

12. The method of any one of claims 1 to 11, wherein hair growth is measured as an increase in terminal hair.
Architecture of the skin

FIG. 2
Histology of human hair follicles
Cellular structure of the human hair bulb

FIG. 4
Dose Administration Schedule DA

Groups 1-5

Sampling for blood and skin

Dose administration was day 0 and day 2.

---

**FIG. 9**
**Fig. 10**

PK Exp #1

- **Groups 1-5**

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<tr>
<th>Species</th>
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<th>Dose (ug)</th>
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</tr>
<tr>
<td>5</td>
<td>16</td>
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Group 4 (100 ug)

Scab detachment
Sampling for blood and skin

T=0
T=48 h (2 days), T=96 h (5 days), T=168 h (7 days), T=240h (10 days), T=336 h (14 days)

PK Exp #2

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<tr>
<th>Groups</th>
<th># Animals / Grp</th>
<th>Dose (ug)</th>
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</tr>
<tr>
<td>2</td>
<td>16</td>
<td>0.1</td>
</tr>
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</table>

Sampling for blood and skin
FIG. 12A

FIG. 12B
FIG. 13A – 13B

SUBSTITUTE SHEET (RULE 26)
DA: Pharmacokinetic Profile CHIR 99021 in Skin

FIG. 14A

DA: Pharmacokinetic Profile of CHIR 99021 in Blood

FIG. 14B
Dose Response in Skin, Blood, 96 H
DA Model

FIG. 14C

Dose Response in Skin, Blood, 96 H
FTE Model

FIG. 14D
FIG. 15A

FIG. 15B
FIG. 15C
CHIR 99021

Total Germs Per Mouse

Treatment Groups (ug)

FIG. 16
CHIR 99021

Area of Germ Forming Region

FIG. 17
CHIR 99021

Germ Density in Germ Forming Region

FIG. 18
CHIR 99021

Total Wound Area

Treatment Groups (ug)

FIG. 19
CHIR 99021

Germ Density in Total Wound Area

Parametric 1-way ANOVA ($P < 0.05$):
- 10 vs. 0.01
- 10 vs. 0.1

FIG. 20
CHIR 99021

% Germ Forming Region in total wound area

Treatment Groups (ug)
Parametric 1-way ANOVA (P<0.05): 10 vs. Placebo 10 vs. 0.01 10 vs. 0.1
Non-Parametric 1-way ANOVA (P<0.05): 10 vs. 0.01 10 vs. 0.1

FIG. 21
Coverage for the three highest responding mice from 10 ug CHIR 99021 group.

FIG. 23
**Active Treatment**

- GSK3 Inhibitor
- Topical Application
- Day 0
- Day 6

- ROGAINE FOAM (minoxidil 5%)
- Topical Application
- Day 28
- Day 84
- Day 168
- Day 182

- Dermabrasion
- Photography

**Control Treatment**

- Placebo Gel
- Topical Application
- Day 0
- Day 6

- ROGAINE FOAM (minoxidil 5%)
- Topical Application
- Day 28
- Day 84
- Day 168
- Day 182

- Dermabrasion
- Photography

Fig. 28
FIG. 31

FIG. 32
FIG. 43

A

PLA/PLG: 0/100
(Prepared by cotton candy machine)

B

Percent IL+ Cumulative Release

120 100 80 60 40 20 0

Time in Days

25 20 15 10 5
Polyethylene oxide-succinimidyl glutarate

\[ \text{Polyethylene oxide-amine} \quad \text{pH 7.4-8} \]

Hydrolytically Labile Ester Linkages

CROSSLINKED NETWORK

Crosslink
FIG. 45

Graph showing the relationship between pH and gel time (in minutes).
y = 1.8715x^2 - 21.876x + 68.444

FIG. 47
FIG. 49
Viscosity of PVA Solutions as a Function of Temperature

Shear Viscosity (cP)

Concentration of PVA (% wt/V)

- VISCOSITY AT 25 DEG C
- VISCOSITY AT 30 DEG C

FIG. 50
FIG. 55A

Proportion of stageable follicles ≥ Stage 5

Proportion of follicles at Stage 4 and below

FIG. 55B
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 31/505 (2012.01)
USPC - 514/275

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 31/505 (2012.01)
USPC: 514/275

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/352, 397 (text search) Find search terms below

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
Electronic Database searched: PUBLWEST (PGP,B,USPT,USOC,EPAB,JPAB), Google. Search Terms Used: hair growth, dermabrasion, minoxidil, carbonitrile, imidazoles, pyrimidinS, pharacemS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Category</th>
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<td>US 2009/0259418 A1 (ALBECK et al.) 29 October 2009 (29.10.2009) entire document especially para [0015]-[0016]; [0025]; [0027]; [0084]; [0085]; [0130]; [0238]</td>
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<td>Y</td>
<td>US 2005/0222220 A1 (PADILLA et al.) 06 October 2005 (06.10.2005) especially para [0061], [0072]-[0073]</td>
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<td>Y</td>
<td>US 2009/0074886 A1 (BENNETT et al.) 19 March 2009 (19.03.2009) especially para [0003]; [004]; [0150]; [0153]</td>
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<td>US 2005/0037038 A1 (GUPTA) 17 February 2005 (17.02.2005) especially para [0001]; [0016]; [0017]</td>
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Relevant to claim No. 1-5 and 10

Date of the actual completion of the international search 10 March 2012 (10.03.2012)

Date of mailing of the international search report 2 3 MAR 2012

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer: Lee W. Young
PCT Helpdesk: 571-272-4300
PCT O/S: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)
**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.: 6-9, 11-12  
   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/2 10 (continuation of first sheet (2)) (July 2009)