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- (71) **Applicant:** PARCELL LABORATORIES, LLC
[US/US]; 21 Strathmore Road, Natick, MA 01760 (US).
- (72) **Inventors:** CRAWFORD, Keith D.; 5 Downey Terrace,
Westwood, Massachusetts 02090 (US). GARVEY, John;
5 Angelo Lane, Londonderry, New Hampshire 03053 (US).
WHITE, Colin; 74 OakRidge Terrace, Lynnfield, Mas-
sachusetts 01940 (US). LAYTON, Pamela; 26 Longmead-
ow Dr, Westwood, Massachusetts 02090 (US).
- (74) **Agents:** GUTERMAN, Sonia, K. et al.; Lawson &
Weitzen, LLP, 88 Black Falcon Avenue, Suite 345, Bo-
ston, Massachusetts 02210 (US).

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(54) **Title:** COMPOSITIONS HAVING EARLY LINEAGE ADULT STEM CELLS AND EXTRACTS THEREOF

(57) **Abstract:** The present invention provides various dermatological and cosmetic compositions for topical application to a subject that include early lineage adult stem cells (ELA cells) and extracts or formulations thereof. Such compositions can effectively improve the health or appearance of a subject's skin.



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Compositions having early lineage adult stem cells and extracts thereof

Related application

5 This application claims the benefit of U.S. provisional application serial number 61/861,303 filed August 1, 2013 entitled, "Compositions having early lineage adult stem cells and extracts thereof", which is incorporated by reference herein in its entirety.

Technical field

10 The present invention relates to formulations and methods for dermal and connective tissue rejuvenation.

Background

 Skin is subject to a number of conditions that can lead to loss of elasticity, wrinkling and age spots, and a resultant desire for cosmetic enhancement. Such conditions include
15 aging due to chronological aging or photoaging from exposure to the sun, or both. Skin aging results in wrinkling, the appearance of pigmented areas ("age spots"), thinning of the skin, loss of elasticity, and other undesirable characteristics. Other skin conditions, which can be improved by cosmetic approaches, include reduction in visible scarring, for example, from acne or other causes, uneven pigmentation, and the like.

20 A variety of procedures have been developed for improving skin appearance. Examples of such procedures include treatment with Botulinum Toxin Type A ("botox"), retinoids and derivatives thereof and especially retinoic acid (all-trans and 13-cis) and retinol, and the use of hydroxy acids. Although progress has been made in the use of cosmetic compositions for the treatment of skin, response to treatment is variable and often a condition
25 is only marginally to moderately responsive to treatment. Unfortunately, once applied to the skin, some of these agents can cause itching, stinging and tightness, which may lead to considerable discomfort, and a progressive immune reaction to the product. For many subjects, sensitivity to sun is enhanced. The use of these products by consumers with sensitive skin is often prevented. Many of the same considerations apply to other methods for
30 cosmetic treatment of skin, such as laser resurfacing or dermabrasion. Thus, there remains a need for cosmetic and/or dermatological compositions and methods for the treatment of skin that does not cause immune reaction to product in subjects and that can be used by subjects with sensitive skin.

Summary

An embodiment of the invention provides a formulation including, a cosmetic base having from about 0.01% to 10% w/w of an ELA cell preparation. In some embodiments the ELA cell preparation is devitalized, extracted and or fractionated. In other embodiments the ELA cell preparation is lyophilized. Some embodiments further include a tissue growth factor. Other embodiments further include a tissue growth factor and cell membrane fraction(s).

In some embodiments of the invention the ELA cell preparation includes expanded ELA cells. Some embodiments of the invention further includes from about 0.01% to 10% w/w of cell-free medium conditioned by growth of ELA cells and/or ELA-lineage cells. In some embodiments the media is lyophilized. In some embodiments the invention is substantially free from non-human animal products.

Another embodiment of the invention provides use of ELA cells (ECCS), lysates (ECL) and/or ELA cell products (ELP) in the manufacture of a medicament. In an embodiment of the invention the use thereof results in improvement in the appearance or health of the skin of a subject.

Another embodiment of the invention provides a method of treating a skin condition including: wrinkling, folds, sagging, age spots, uneven pigmentation, thinning, elasticity, scarring, surface roughness, surface roughness, surface vessels, redness, and pore size, including administering to a subject having one or more of the aforesaid conditions, a dose of a formulation including, a cosmetic base having from about 0.01% to 10% w/w of an ELA cell preparation.

Another embodiment of the invention provides a method of tissue augmentation, including inducing tissue growth and recovery from standard cosmetic procedures by administering to a subject subdermally, a preparation of 10^2 to 10^6 live ELA cells.

Another embodiment of the invention provides a method of tissue augmentation, including inducing tissue growth and recovery from standard cosmetic procedures by administering to a subject externally, the formulation including, a cosmetic base having from about 0.01% to 10% w/w of an ELA cell, cell extract, or cell-free media preparation.

Another embodiment of the invention provides a method of tissue augmentation, including inducing tissue growth and recovery from standard cosmetic procedures by administering to a subject subdermally, a preparation of 10^2 to 10^6 live ELA cells and subsequently administering externally one or more doses of the formulation including, a

cosmetic base having from about 0.01% to 10% w/w of an ELA cell, cell extract, or cell-free media preparation.

In some embodiments the mode of administration is topical, and optionally, other cosmetics or devices, may be in contact on the surface or below the dermal surface at the same time. In some embodiments the cosmetic base is a lotion. In other embodiments the cosmetic base is a cream. In some embodiments the cosmetic base includes a pigment. In another embodiment the cosmetic base includes a sunscreen. In some embodiments the cosmetic base includes at least one anti-acne agent. In other embodiments the cosmetic base includes liposomes. In some embodiments the cosmetic base includes at least one antioxidant. In other embodiments the cosmetic base includes a platelet-rich fibrin matrix. An embodiment of the invention provides a composition including, a cosmetic base having from about 0.01% to 10% w/w of one or more of: an ELA cell preparation, ELA cell extract, and ELA cell-conditioned medium; the ELA cell-conditioned medium generated in an environmentally-closed cell culture system. In an embodiment of the invention the medium is filter-sterilized. In an embodiment of the invention the medium is concentrated.

Brief description of drawings

Fig. 1 is a bar graph showing increased cytokine release from cultured ELA cells, into the conditioned media. ELA cells were cultured and the conditioned medium was collected after 72 hours. The cells were harvested and counted and the supernatants were centrifuged at 14,000× g for 5 min to remove cellular debris. Immunoreactive VEGF, IL-6 and IL-8 protein levels were measured in the conditioned media using commercial ELISA kits in accordance with the manufacturer's instructions. Pre-conditioned medium was used as control and cytokine levels in the control were analyzed by ELISA. The data collected from unconditioned media and conditioned media was analyzed. The cytokine levels in ELA cell conditioned medium were observed to be significantly higher by at least about two-fold than cytokine levels in the pre-conditioned medium.

Detailed description

The present invention includes skin rejuvenating compositions that result in improvements to skin health and/or appearance, including but not limited to improvements with respect to wrinkling, folds, sagging, age spots, uneven pigmentation, thinning, elasticity, scarring, surface roughness, surface roughness, surface vessels, redness, and pore size.

According to one embodiment, such a composition is achieved by incorporating a population of fresh or expanded ELA cells, or extracts or derivatives thereof.

Early lineage adult stem cells (ELA) are adult progenitor cells that have extravasational and tissue regenerative properties. ELA stem cells are capable of differentiating into tissues of endodermal, mesodermal and ectodermal origin. The source, isolation, characterization and certain uses and formulations of ELA cells are described in patent applications: U.S. provisional application serial numbers: 60/927,596, 61/247,236, 61/247,242, 61/249,172, and 61/501,846, as well as U.S. patent application serial number 12/598,047 and PCT applications serial numbers PCT/US2008/005742 and PCT/US2010/050288. These are each incorporated herein by reference in their entirety. The ELA cells are described by their expression of certain stem cell specific pluripotency genes (e.g., Oct-4, KFL-4, Nanog, Sox-2, Rex-1, GDF-3 and Stella). ELA cells are distinct from embryonic stem cells (ESC) and other types of early lineage adult cells such as MSC, VSEL, MAPC and cord blood derived progenitor cells because the ELA stem cells do not appear to detectably express the various stem cell markers CD34, CD44, CD45, CD49a, CD66A, CD73, CD90, CD105, CSCR4, SSEA, or MHC class I or MHC class II structures, found on other cell types.

For the purposes of this disclosure, ELA cells are may be derived from multiple sources including, bone marrow stroma, blood, dermis, periosteum and tissues, but a useful exemplary source is synovial fluid (SF). The above patent applications detail the manner in which ELA cells can be obtained and propagated. In various embodiments, ELA cells are extracted from patient intended to receive the cellular therapy (i.e., an autologous transplant). ELA cells can be culture expanded prior to their introduction into the patient. Growth of ELA cells in vitro can be used, for example, to increase the number of ELA cells available for implantation or injection. In non-limiting examples, ELA cell numbers are increased about two fold or greater, about ten fold or greater, or about twenty fold or greater or more, depending on the desired number of cells. Patient-specific master cell banks, for example can include ten or more passages of expanded autologous cells, which increases ELA cell counts by many orders of magnitude in comparison to primary isolates. In various embodiments, growing ELA cells in vitro can include expansion in a cell culture medium, which includes nutrients, buffers, salts, proteins, vitamins and/or growth factors, which promote ELA cell growth. A useful cell culture medium is RPMI 1680 supplemented with 10% serum, and appropriate antibiotics such as penicillin/streptomycin and G418. Human serum is preferred for expanding the ELA cells for human transplant preparations, but fetal bovine serum has

produced acceptable yields of the ELA cells in culture. A currently preferred culture system is an environmentally controlled, closed-culture system, commonly known as a bioreactor, which is particularly useful for collection of ELA cell-conditioned media. Other systems are suitable for culture of ELA cells, the expansion of donor cells, and the creation of master
5 ELA cell banks. These should comply with Good Tissue Practices and ideally should be cGMP. The expanded cells are commonly but not necessarily frozen prior to implantation, and a suitable cryogenic medium that is acceptable to the FDA for such purposes is CryoStorCS10, available from BioLife Solutions, Bothell, WA.

In one embodiment, of the cosmetic composition having ELA cells is substantially
10 free from non-human animal products. Another embodiment of the invention provides for the use of a medium conditioned (ELA cellular cytokine suspension or ECCS) by growth of ELA cells to prepare a medicament or cosmetic for administration to skin of a subject as a cellular rejuvenation compound. Another embodiment of the invention provides for the use of lysates from devitalized ELA cells to prepare a medicament for a cosmetic or for the
15 administration to a subject as a cellular rejuvenation compound. Another embodiment of the invention provides for the use of ELA cell derived products from primary, expanded or transgene expressing ELA cells to prepare a medicament for the administration to a subject as a cellular rejuvenation compound. Methods of expressing transgene products from transfected ELA cells are described in PCT application serial number PCT/US2008/005742.

20 The present invention provides various dermatological and cosmetic compositions for topical application to an individual that includes ELA cell, ECCS, ELA cell lysates derived (ECL), and ELA cell products (ECP), each alone or in combination.

According to one embodiment of the invention, the composition is formulated for application to the skin of an individual. The amount of ELA cells, ECCS, ECL, and ECP in
25 this dermatological or cosmetic composition is an amount capable of providing a detectable improvement in the health or appearance of an individual's skin compared to untreated skin of the individual. Generally, a 0.01% to 10% w/w of cell extract, cell conditioned media or lyophilized ELA cells, applied over a 30 day period, results in detectable improvement. In certain embodiments, the compositions of the invention include lotions, creams, oils, gels,
30 including hydrogels, powders, serums, salves, foundations, facial masks, lip care products, sunscreens, hair care products, such as shampoos, conditioners, including deep conditioners, hair care treatments, hot oil treatments, skin cleansers, exfoliants, ointments, compact formulations, or the like.

The term cosmeceutical refers to a formulation or composition including at least one biologically active ingredient that has an effect on the user of the product and at least one cosmeceutically-acceptable carrier. Cosmeceuticals may be viewed as cosmetics that, in certain applications and under appropriate conditions, provide medicinal or drug-like benefits. In certain applications, for example, cosmeceuticals affect the underlying structure of the skin, decrease wrinkle depth, or reverse or ameliorate the effect of photooxidation or aging on the skin. Cosmeceuticals are particularly useful as skin care products, hair care products, and sun care products. In certain embodiments, cosmeceutical compositions include delivery systems including at least one of liposomes, cyclodextrins, polymer systems, or hyaluronic acid or related compounds. Cosmeceutical compositions include cosmeceutically-acceptable carriers.

A topical cosmetic or cosmeceutical ointment, lotion, or gel composition typically contains an effective amount of ELA cells, ECCS, ECL cells, and cell products derived therefrom. The cosmeceutical ointment may also include other active and inert ingredients in a cosmetically- or a cosmeceutically-acceptable carrier, such as a pharmaceutical cream base, an oil-in-water emulsion, a water-in-oil emulsion, or a gel. Various cosmetic and cosmeceutical compositions for topical use include drops, tinctures, lotions, creams, salves, serums, solutions, and ointments containing conditioned media or extracts, and an appropriate carrier. The optimal percentage of the conditioned media or extract in each composition varies according to the composition's formulation and the therapeutic effect desired. The skilled artisan in the formulation arts will understand that the inventive compositions may include any of a number of cosmetically-, cosmeceutically-, or pharmaceutically-acceptable formulations, depending on the type of product, the nature of the composition, the location of composition's use, the desired effect, and the like. While proprietary formulations are common in the formulation arts, formulators of ordinary skill will be able to determine or readily select appropriate formulations for specific applications without undue experimentation.

Discussions of cosmetic- and cosmeceutically-acceptable ingredients and formulations are found in the FDA Cosmetics Handbook, U.S. Food and Drug Administration; Handbook of Cosmetic and Personal Care Additives, Ash and Ash, compilers, 1994, Chemical Publishing, New York, N. Y.; Bennett's Cosmetic Formulary, 1993, Chemical Publishing Co.; Harry's Cosmeticology, 7^{sup}.th ed.5 Wilkinson & Moore, 1982 and 8^{sup}.th ed., Rieger, 2000, Chemical Publishing; Cosmetic Bench Reference-2001, Allerud Publishing Corp.; CTFA Compendium of Cosmetic Ingredient Composition,

Nikitakis and McEwen, eds., 1990, Cosmetic, Toiletry, and Fragrance Association, Washington, D.C., Surfactant Encyclopedia, 2nd revised edition, Rieger, 1996, Allured Publishing; The Chemistry and Manufacture of Cosmetics, 2nd ed., De Navarre, Van Nostrand, Princeton, N.J.; Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics, Leung, 1996, John Wiley; A Consumer's Dictionary of Cosmetic Ingredients, 5th ed., Winter, 1999, Three Rivers Press, New York, N. Y.; Cosmeceuticals: Active Skin Treatment, 1998, Allured Publishing; Handbook of Cosmetic Science and Technology, Knowlton and Pearce, 1993, Elsevier Advanced Technology, Oxford, UK; Personal-Care Formulas, 1997, Allured Publishing; Beginning Cosmetic Chemistry, Scheuller and Romanowski, 1999, Allured Publishing; and Skin Permeation: Fundamentals and Application, Zatz, 1993, Allured Publishing. Discussions of pharmaceutically-acceptable ingredients and formulations may be found in, among other places, Remington's Pharmaceutical Sciences, 18th ed., Gennaro, ed., 1990, Mack Publishing.

The methods of the invention have wide applicability to cosmetic conditions. The mode of administration for cosmetic applications is typically topical, but administration and dosage regimens vary depending on the cosmetic condition for which modulation is sought.

The present invention provides methods, compositions, and kits for cosmetic use with individuals. The term "individual" as used herein includes humans as well as other mammals.

In some embodiments, the compositions, methods, and/or kits are used to provide a cosmetic treatment to an individual desiring and/or in need of cosmetic treatment (e.g., young children subject to burn or other scarring may not desire treatment but may nonetheless be in need of treatment). The term "treating" or "treatment" as used herein includes achieving a cosmetic benefit. By cosmetic benefit is meant any desired modulation of the cosmetic condition being treated. For example, in an individual with wrinkling, cosmetic benefit includes eradication or lessening of the appearance of wrinkling. Also, a cosmetic benefit is achieved with the eradication or amelioration of one or more of the psychological symptoms associated with the underlying condition such that an improvement is observed in the patient, notwithstanding the fact that the patient may still be affected by the cosmetic condition. For example, ELA cells, ECCS, ECL, ECP or combination thereof provides cosmetic benefit not only when a cosmetic defect is eradicated, but also when an improvement is observed in the individual with respect to the cosmetic defect and its attendant consequences, such as psychological consequences. In some cases, methods and compositions of the invention may be directed at achieving a prophylactic benefit. A "prophylactic," or "preventive" effect includes prevention

of a condition, retarding the progress of a condition (e.g., skin aging), or decreasing the likelihood of occurrence of a condition. As used herein, "treating" or "treatment" includes prophylaxis.

As used herein, the term "effective amount" encompasses an amount sufficient to
5 effect beneficial or desired cosmetic results. An effective amount is administered in one or more administrations. In terms of cosmetic treatment, an "effective amount" of ELA cells, ECCS, ECL, ECP or a combination thereof of the invention is an amount that is sufficient to palliate, ameliorate, stabilize, reverse or slow the progression of a cosmetic condition, or to provide a desired effect such as cosmetic augmentation of a soft tissue. An "effective
10 amount" may be of a ELA cells, ECCS, ECL, and ECP or a combination thereof used alone or in conjunction with one or more agents used to modulate a cosmetic condition.

The skin is subject to a number of cosmetic conditions that result in alterations of function and/or appearance that are considered undesirable, and the manifestation of these conditions can lead to psychological discomfort or harm. In some cases, no defect may be
15 present however, it may be desirable to the subject to augment or alter the skin in such a way as to produce a cosmetically pleasing effect. Examples of cosmetic conditions that may be modulated by the present invention include, but are not limited to, skin aging, cosmetic defect, undesired pigmentation, and post-cosmetic procedure damage.

Skin aging includes chronological aging as well as photoaging, and may appear as
20 wrinkling, lack of elasticity (for example sagging), uneven pigmentation, thinning of the skin and/or collagen so that veins and other underlying structures become more prominent, and the like. Skin aging is a major example of a skin condition that involves a decrease in cell proliferation and in cell function. As used herein, "skin aging" refers to alterations in the appearance and function of skin that occur with aging, such as wrinkling, loss of elasticity,
25 sagging, uneven pigmentation (for example, "age spots" or "liver spots"), and loss of underlying tissue mass. Such conditions may be accelerated and/or exacerbated by exposure to ultraviolet radiation ("photoaging") and other environmental conditions.

With age and/or exposure to UV radiation, the epidermis thins and the skin appendages atrophy. Hair becomes sparse and sebaceous secretions decrease, with
30 consequent susceptibility to dryness, chapping, and fissuring. The dermis diminishes with loss of elastic and collagen fibers. Moreover, keratinocyte proliferation (which correlates with skin thickness and skin proliferative capacity) decreases with age.

An increase in keratinocyte proliferation and collagen production is believed to counteract skin aging, i.e., wrinkles, thickness, elasticity and repair. According to the present

invention, ELA cells, ECCS, ECL, and ECP therefrom can be used cosmetically to counteract, at least for a time, the effects of aging on skin. A formulation containing ELA cells, ECCS, ECL, and ECP or a combination thereof may be applied topically in areas where it is desired to counteract skin aging.

5 Also included in the skin conditions that may be treated by the methods of the invention are cosmetic defects that, while not pathological or physiologically harmful, may nonetheless cause psychological distress, in some cases to the extreme. In these cases it is desirable to correct a particular feature or features causing distress or, alternatively, enhance a feature considered desirable. In addition to skin aging, such conditions include, e.g., striae
10 gravidorum and striae distensiae ("stretch marks), atrophic scarring (e.g., acne scarring), wound (e.g., traumatic wounds, chronic wounds, or burn wounds) or surgical scarring, thickened and cracked skin (especially on the feet), and hair loss. In the latter embodiments, the invention relates to the use of preparations that comprise ELA cells, ECCS, ECL, and ECP or combination thereof to enhance hair growth. Cells from which the hair is produced
15 grow in the bulb of the follicle. They are extruded in the form of fibers as the cells proliferate in the follicle. Hair "growth" refers to the formation and elongation of the hair fiber by the dividing cells. In some embodiments, the methods of the invention provide a means for altering the dynamics of the hair growth cycle to induce proliferation of hair follicle cells, particularly stem cells of the hair follicle. The subject compositions and method can be used
20 to increase hair follicle size and the rate of hair growth in individuals, such as humans, e.g., by promoting proliferation of hair follicle stem cells. In one embodiment, the method comprises administering to the skin in the area in which hair growth is desired an amount of ELA cells, ECCS, ECL, and ECP or combination thereof sufficient to increase hair follicle size and/or the rate of hair growth in the animal, e.g., human. Typically, the composition is
25 administered topically as a cream or lotion, and is applied on a daily basis until hair growth is observed and for a time thereafter sufficient to maintain the desired amount of hair growth.

Undesired pigmentation includes pigmentation over an area of the body that is different than the pigmentation desired by the individual. Undesired pigmentation can be the result of, e.g., photoaging, reaction to inflammation, or reaction to trauma such as surgical or
30 accidental skin breakage, and the like. Undesired pigmentation includes altered or undesired pigmentation over small areas such as freckles, as well as altered or undesired pigmentation over larger areas, such as, for example, uneven pigmentation or larger areas of undesired pigmentation.

Further cosmetic uses of the methods of the invention include tissue augmentation through, generally, topical application, such as for lip enhancement. By "augment" is meant to include giving the appearance of greater fullness, generally through an increase in the tissue of the skin or underlying tissue. Any suitable skin area may be selected for
5 augmentation by the methods of the invention.

In addition, the methods of the invention may be employed to enhance and/or accelerate recovery from standard cosmetic procedures, which are damaging to skin and/or underlying tissues. In addition, these procedures may be associated with lengthy recovery time and suboptimal results. Such procedures include chemical peel, dermabrasion, laser
10 resurfacing, ablative resurfacing, nonablative resurfacing, photodynamic therapy, noncoherent light phototherapy, breast lift, face lift, eyelid lift, forehead lift, neck lift, thigh lift, buttock lift, tummy tuck, and scar revision. As will be apparent to those of skill in the art, some of these procedures can require further skin firming (e.g., "lifting" procedures) while others are more extensively damaging to the surface of the skin and require assistance for
15 healing in a timely and optimal fashion (e.g., chemical peel, dermabrasion, ablative and non-ablative skin resurfacing). In some embodiments, the methods of the invention provide a method for achieving firming and lifting of the eyelids; this may be done either in place of or in conjunction with a conventional eyelid lift procedure. The methods of the invention may be used in conjunction with both types of procedures to enhance and/or accelerate healing
20 and recovery. ELA cells, ECCS, ECL, and ECP or combination thereof may be administered in any cosmetically acceptable carrier, as described in more detail below. In embodiments of methods of the invention, the concentration of ELA cells, ECCS, cell lysates derived therefrom, and cell products derived therefrom or a combination thereof used may be more than about 0.00001, 0.00005, 0.0001, 0.001, 0.01, 0.1, 1, 5, 10 or 20% on a w/w basis. In
25 some embodiments, the concentration of ELA cells, ECCS, ECL, and ECP or combination thereof is more than about 0.00005%. The concentration of ELA cells, ECCS, ECL, and ECP or combination thereof may be less than about 0.0001, 0.001, 0.001, 0.01, 0.1, 1, 5, 10, or 20% (all concentration percentages given herein are w/w unless otherwise indicated). In some
30 embodiments, the ELA cells, ECCS, ECL, and ECP or a combination thereof are used at a concentration of about 0.00001% to about 1%; or about 0.00001% to about 0.1%; or about 0.0001% to about 0.01%; or about 0.0005% to about 0.005%; or about 0.0005% to about 0.002%; or about 0.001%. In some embodiments, lower and higher concentrations are contemplated. Skin coverage may also be described in terms of total μL of ELA cells, ECCS, ECL, and ECP or combination thereof/ cm^2 of skin; in these terms, a typical coverage per

administration would be more than about 5, 10, 100, 1000, 10,000, 50,000, or 500,000 μL / cm^2 of skin; less than about 1,000,000, 500,000, 50,000, 5000, 500, 50, or 5 μL / cm^2 of skin; or about 5 μL / cm^2 to about 500 μL / cm^2 of skin; or about 50 μL / cm^2 of skin.

The methods of the invention typically utilize topical administration, which may be
5 by any suitable means that brings the ELA cells, ECCS, ECL, and ECP or combination thereof and, optionally, other cosmetic or dermatological agents, in contact with the surface of the skin. This would include application as a gel, lotion, cream, or liposomal preparation with or without occlusion, or application as a plaster, patch, mask, glove, or similar device for extended contact with an affected area of skin. For modulation of cosmetic conditions or to
10 produce a desired cosmetic effect, the frequency and duration of administration of a formulation comprising a ELA cells, ECCS, ECL, and ECP or combination thereof is dependent on factors including the nature of the formulation (e.g., concentration, presence or lack of other cosmetic or dermatological agents, vehicle type), the severity and extent of the condition, and in some cases the judgment of a skin care professional, e.g., a health care
15 professional such as a dermatologist, or a cosmetologist.

Topical application may be applied more than once, twice, three times, four times, five times, or six times per week, or more than once, twice, three times, four times, five times, or six times per day. Frequency of application may be less than about twice, three times, four times, five times, or six times per week, or less than about once, twice, three
20 times, four times, five times, or six times per day. Some embodiments of the invention provide a method for cosmetic treatment of the skin of an individual by topical administration of an effective amount of a ELA cells, ECCS, ECL, and ECP therefrom or a combination thereof. In some embodiments, the formulation is administered an average of about once per day; in some embodiments, the formulation is administered an average of about once or twice
25 per day; in some embodiments, the formulation is administered an average of about once to three times per day; in some embodiments, the formulation is administered an average of more than about three times per day. In one embodiment, the formulation is administered an average of about twice per day, typically in the morning upon rising and in the evening before retiring. Topical administration may be without a covering. Alternatively, topical
30 administration may include the use of a covering over the formulation, which may be occlusive or non-occlusive. For example, administration in the evening before retiring may include covering the administered area with an occlusive or non-occlusive covering, which may remain in place during sleep.

The duration of treatment generally will depend on the response of the skin to cosmetic treatment. Treatment may continue at the discretion of the individual being treated. In some cases, administration or application of a formulation containing ELA cells, ECCS, ECL, and ECP or combination thereof may be more frequent at the beginning of treatment and less frequent as treatment continues and the condition is ameliorated or the desired effect is achieved. In some cases, treatment may continue indefinitely in order to maintain a condition in abeyance or in an improved state, to delay onset of a cosmetic condition (e.g., skin aging), or to slow the progression of a cosmetic condition. These modifications of frequency and duration are easily accomplished by the individual being treated.

Some embodiments of cosmetic treatment of skin employ topical administration of a lotion, in some embodiments a mixture of emulsifying lanolin alcohols, waxes, and oils (e.g., EUCERIN™) or a mixture of petrolatum or mineral oil, a quaternary ammonium compound, a fatty alcohol, and a fatty ester emollient (e.g., CUREL, as described below, comprising ELA cells, ECCS, ECL, and ECP or combination thereof. The lotion containing the ELA cells, ECCS, ECL, and ECP or combination thereof is applied at a frequency of once to three times per day, in some embodiments once per day, until the desired result, e.g., reduction or elimination of wrinkling, sagging, and the like, is observed, followed by topical application once to three times per week, in some embodiments once per week, thereafter.

Some embodiments of cosmetic treatment of skin employ topical administration of a cream, in some embodiments a mixture of emulsifying lanolin alcohols, Water/Aqua/Eau, Petrolatum, Glycerin, Isostearyl Palmitate, Butylene Glycol, or Glyceryl Stearate (e.g., Creme LuXury™) or a mixture of, comprising of ELA cells, ECCS, ECL, and ECP or combination thereof. This composition is applied at a frequency of once to three times per day, in some embodiments once per day, until the desired result, e.g., reduction or elimination of wrinkling, sagging, and the like, is observed, followed by topical application once to three times per week, in some embodiments once per week, thereafter.

Some embodiments involves the cosmetic treatment of the eyelashes by employing a mascara in some embodiments a mixture of mineral oils, linseed oil, castor, eucalyptus, lanolin, or beeswax (e.g. COVERGIRL® NatureLuxe Mascara) or a mixture of, comprising of ELA cells, ECCS, ECL, and ECP or combination thereof. This composition is applied at a frequency of once to three-times per day, in some embodiments once per day, until the desired result, e.g. lengthening, thicken and/or curling of the eyelashes. In some instances,

mascara may contain nanofibers, nanotubals, ELA cells, ECCS, ECL, and ECP or combination thereof to further lengthen, thicken, and/or curl the eyelashes.

The skilled artisan will understand that the appropriate carriers of the inventive compositions typically may other ingredients typically found in the cosmetic and
5 cosmeceutical fields: oils, waxes or other standard fatty substances, or conventional gelling agents and/or thickeners; emulsifiers; moisturizing agents; emollients; sunscreens; hydrophilic or lipophilic active agents, such as ceramides; agents for combating free radicals; bactericides; sequestering agents; preservatives; basifying or acidifying agents; fragrances; surfactants; fillers; natural products or extracts of natural product, such as aloe or green tea
10 extract; vitamins; or coloring materials. The amounts of these various ingredients will vary depending on the use of the composition and the cosmetic or cosmeceutical effect desired.

If the ELA cells, ECCS, ECL, and ECP or combination thereof is used in combination with another skin care method or composition, any suitable combination of the ELA cells, ECCS, ECL, and ECP or combination thereof and the additional method or composition may
15 be used. Thus, for example, if use of a ELA cells, ECCS, ECL, and ECP or combination thereof is in combination with another cosmetic or dermatological agent, the two may be administered simultaneously, consecutively, in overlapping durations, in similar, the same, or different frequencies, etc. In some cases a composition will be used that contains a ELA cells, ECCS, ECL, and ECP or combination thereof in combination with one or more other
20 cosmetic or dermatological agents.

Other dermatological or cosmetic agents that may be used in methods of the invention are described in more detail below. Dosages, routes of administration, administration regimes, and the like for these agents are well-known in the art.

As the cosmetic compositions are for topical use, they need not be sterile; however, if
25 sterility is desired it is readily accomplished by filtration through sterile filtration (0.22 micron) membranes, or by other art-accepted means.

If desired, further cosmetic or dermatological ingredients may be incorporated in the formulations. The nature of the other ingredient(s) will depend on the cosmetic condition to be modulated and/or cosmetic result desired. These are described more fully below.

30 The ELA cells, ECCS, ECL, and ECP or combination thereof may be used neat (e.g., with an occlusive dressing so that the ELA cells, ELA cells, ECCS, ECL, and ECP or combination thereof is dissolved or dispersed in perspiration at the site), but generally is prepared in a vehicle suitable for topical administration. Compositions of the invention

include ELA cells, ECCS, ECL, and ECP or combination thereof in a vehicle suitable for topical administration.

Numerous vehicles for topical application of cosmetic compositions are known in the art. See, e.g., Remington's Pharmaceutical Sciences, Gennaro, A R5 ed., 20^{sup}.th edition, 2000: Williams and Wilkins PA, USA. All compositions usually employed for topically administering cosmetic compositions may be used, e.g., creams, lotions, gels, dressings, shampoos, tinctures, pastes, ointments, salves, powders, liquid or semi-liquid formulation, patches, liposomal preparations, and the like. Application of said compositions may, if appropriate, be by aerosol e.g. with a propellant such as nitrogen carbon dioxide, a freon, or without a propellant such as a pump spray, drops, lotions, or a semisolid such as a thickened composition which can be applied by a swab. In particular compositions, semisolid compositions such as salves, creams, lotions, pastes, gels, ointments and the like will conveniently be used. The ELA cells, ECCS, ECL, and ECP or combination thereof may optionally be dissolved or diluted in a small amount of an appropriate solvent, such as ethanol or DMSO, before dispersion in the vehicle; however, this is not required. Compositions known in the art, preferably hypoallergenic and pH-controlled are especially preferred for topical administration, and include toilet waters, packs, lotions, skin milks or milky lotions. The preparations contain, besides the ELA cells, ECCS, ECL, and ECP or combination thereof and, optionally, other active ingredients, components usually employed in such preparations. Examples of such components are oils, fats, waxes, surfactants, humectants, thickening agents, antioxidants, viscosity stabilizers, chelating agents, buffers, preservatives, perfumes, dyestuffs, lower alkanols, and the like.

Examples of oils include fats and oils such as olive oil and hydrogenated oils; waxes such as beeswax and lanolin; hydrocarbons such as liquid paraffin, ceresin, and squalene; fatty acids such as stearic acid and oleic acid; alcohols such as cetyl alcohol, stearyl alcohol, lanolin alcohol, and hexadecanol; and esters such as isopropyl myristate, isopropyl palmitate and butyl stearate. As examples of surfactants there may be cited anionic surfactants such as sodium stearite, sodium cetylsulfate, polyoxyethylene laurylether phosphate, sodium N-acyl glutamate; cationic surfactants such as stearyldimethylbenzylammonium chloride and stearyltrimethylammonium chloride; ampholytic surfactants such as alkylaminoethylglycine hydrochloride solutions and lecithin; and nonionic surfactants such as glycerin monostearate, sorbitan monostearate, sucrose fatty acid esters, propylene glycol monostearate, polyoxyethylene oleylether, polyethylene glycol monostearate, polyoxyethylene sorbitan monopalmitate, polyoxyethylene coconut fatty acid monoethanolamide, polyoxypropylene

glycol (e.g. the materials sold under the trademark "Pluronic"), polyoxyethylene castor oil, and polyoxyethylene lanolin. Examples of humectants include glycerin, 1,3-butylene glycol, and propylene glycol; examples of lower alcohols include ethanol and isopropanol; examples of thickening agents include xanthan gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, polyethylene glycol and sodium carboxymethyl cellulose; examples of antioxidants include butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, citric acid and ethoxyquin; examples of chelating agents include disodium edetate and ethanehydroxy diphosphate; examples of buffers include citric acid, sodium citrate, boric acid, borax, and disodium hydrogen phosphate; and examples of preservatives are methyl

5 parahydroxybenzoate, ethyl parahydroxybenzoate, dehydroacetic acid, salicylic acid and benzoic acid. These substances are merely exemplary, and those of skill in the art will recognize that other substances may be substituted with no loss of functionality.

For preparing compositions for topical administration, the concentration of ELA cells, ECCS, ECL, and ECP or combination thereof may be more than about 0.00001, 0.00005,

15 0.0001, 0.001, 0.01, 0.1, 1, 5 or 10% by weight. In some embodiments, the concentration of the ELA cells, ECCS, ECL, and ECP or combination thereof is more than about 0.00005%. The concentration of ELA cells, ECCS, ECL, and ECP or combination thereof may be less than about 0.0001, 0.001, 0.001, 0.01, 0.1, 1, 5, 10 or 20% (all concentration percentages given herein are w/w unless otherwise indicated). In some embodiments, lower and higher

20 concentrations are contemplated. In some embodiments employing ointments, lotions, or creams, the pharmaceutically acceptable "base" is a carrier for example, that can contain 1 to 20%, in particular 5 to 15% of a humectant, 0.1 to 10% in particular from 0.5 to 5% of a thickener and water; or said carrier may consist of 70 to 99%, in particular 20 to 95% of a surfactant, and 0 to 20%, in particular 2.5 to 15% of a fat; or 80 to 99.9% in particular 90 to

25 99% of a thickener; or 5 to 15% of a surfactant, 2- 15% of a humectant, 0 to 80% of an oil, very small (<2%) amounts of preservative, coloring agent and/or perfume, and water. In water, the carrier for example consists of 2 to 10% of a lower alcohol, 0.1 to 10% or in particular 0.5 to 1% of a surfactant, 1 to 20%, in particular 3 to 7% of a humectant, 0 to 5% of a buffer, water and small amounts (<2%) of preservative, dyestuff and/or perfume. In a

30 skin milk, the carrier typically consists of 10-50% of oil, 1 to 10% of surfactant, 50-80% of water and 0 to 3% of preservative and/or perfume.

In some embodiments, ELA cells, ECCS, ECL, and ECP or combinations thereof, optionally with other active ingredients, are dissolved, mixed, or suspended in a mixture of emulsifying lanolin alcohols, waxes, and oils (e.g., EUCERIN™ Lotion) or a mixture of

petrolatum or mineral oil, a quaternary ammonium compound, a fatty alcohol, and a fatty ester emollient (e.g., CUREL™ Lotion), or lotions that are substantially similar in composition. EUCERIN™ Dry Skin Therapy Original Moisturizing Lotion comprises water, mineral oil, isopropyl myristate, PEG-40 sorbitan peroleate, glyceryl lanolate, sorbitol, propylene glycol, cetyl palmitate, magnesium sulfate, aluminum stearate, lanolin alcohol, BHT, methylchloroisothiazolinone, and methylisothiazolinone. CUREL™ Fragrance Free Daily Moisturizing Lotion comprises water, glycerin, distearyldimonium chloride, petrolatum, isopropyl palmitate, cetyl alcohol, dimethicone, sodium chloride, methylparaben, and propylparaben.

Some embodiments of compositions of the invention comprise ELA cells, ECCS, ECL, and ECP or combination thereof in a lotion comprising a mixture of emulsifying lanolin alcohols, waxes, and oils (e.g., EUCERIN™ Dry Skin Therapy Original Moisturizing Lotion) at a concentration greater than about 0.00005%. Some embodiments of compositions of the invention comprise ELA cells, ECCS, ECL, and ECP or combinations thereof in a lotion comprising a mixture of emulsifying lanolin alcohols, waxes, and oils (e.g., EUCERIN™ Dry Skin Therapy Original Moisturizing Lotion) at a concentration of about 0.0001% to about 0.01%.

Some embodiments of compositions of the invention comprise ELA cells, ECCS, ECL, and ECP or combinations thereof in a lotion comprising a mixture of petrolatum or mineral oil, a quaternary ammonium compound, a fatty alcohol, and a fatty ester emollient (e.g., CUREL™ Fragrance Free Daily Moisturizing Lotion) at a concentration greater than about 0.00005%. Some embodiments of compositions of the invention comprise a ELA cells, ECCS, ECL, and ECP or combination thereof in a lotion comprising a mixture of petrolatum or mineral oil, a quaternary ammonium compound, a fatty alcohol, and a fatty ester emollient (e.g., CUREL™ Fragrance Free Daily Moisturizing Lotion) at a concentration of about 0.0001% to about 0.01%.

In addition, ELA cells, ECCS, ECL, and ECP or combinations thereof and, optionally, other active ingredients, may be formulated in liposome-containing compositions. Liposomes are artificial vesicles formed by amphipathic molecules such as polar lipids, for example, phosphatidyl cholines, ethanolamines and serines, sphingomyelins, cardiolipins, plasmalogens, phosphatidic acids and cerebroside. Liposomes are formed when suitable amphipathic molecules are allowed to swell in water or aqueous solutions to form liquid crystals usually of multilayer structure comprised of many bilayers separated from each other by aqueous material (also referred to as coarse liposomes). Another type of liposome known

to be consisting of a single bilayer encapsulating aqueous material is referred to as a unilamellar vesicle. If water-soluble materials are included in the aqueous phase during the swelling of the lipids they become entrapped in the aqueous layer between the lipid bilayers. The incorporation of ELA cells, ECCS, ECL, and ECP or combination thereof into liposomal preparations suitable for topical application can be achieved by a number of methods. With respect to liposomal preparations, any known methods for preparing liposomes for treatment of a condition may be used. See, for example, Bangham et al., J. Mol Biol, 23: 238-252 (1965) and Szoka et al., Proc. Natl Acad. Sci. 75: 4194-4198 (1978). Ligands may also be attached to the liposomes to direct these compositions to particular sites of action.

Liposomes containing ELA cells, ECCS, ECL, and ECP or combination thereof and, optionally, other ingredients can be employed directly or they can be employed in a suitable pharmaceutically acceptable carrier for topical administration. The viscosity of the liposomes can be increased by the addition of one or more suitable thickening agents such as, for example xanthan gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose and mixtures thereof. The aqueous component may consist of water alone or it may contain electrolytes, buffered systems and other ingredients, such as, for example, preservatives. Suitable electrolytes which can be employed include metal salts such as alkali metal and alkaline earth metal salts. Exemplary metal salts are calcium chloride, sodium chloride and potassium chloride. The concentration of the electrolyte may vary from zero to 260 mM, preferably from 5 mM to 160 mM. The aqueous component is placed in a suitable vessel which can be adapted to effect homogenization by effecting great turbulence during the injection of the organic component. Homogenization of the two components can be accomplished within the vessel, or, alternatively, the aqueous and organic components may be injected separately into a mixing means which is located outside the vessel. In the latter case, the liposomes are formed in the mixing means and then transferred to another vessel for collection purpose. The organic component consists of a suitable non-toxic, cosmetically acceptable solvent such as, for example ethanol, glycerol, propylene glycol and polyethylene glycol, and a suitable phospholipid which is soluble in the solvent. Suitable phospholipids which can be employed include lecithin, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, lysophosphatidylcholine and phosphatidyl glycerol, for example. Other lipophilic additives may be employed in order to selectively modify the characteristics of the liposomes. Examples of such other additives include stearylamine, phosphatidic acid, tocopherol, cholesterol and lanolin extracts. In addition, other ingredients which can prevent oxidation of the phospholipids may be added to the

organic component. Examples of such other ingredients include tocopherol, butylated hydroxyanisole, butylated hydroxytoluene, ascorbyl palmitate and ascorbyl oleate. Preservatives such as benzoic acid, methyl paraben and propyl paraben may also be added.

Although ELA cells, ECCS, ECL, and ECP or combinations thereof are generally capable of penetrating cell membranes and reaching the deep layers of skin, it may be useful in some embodiments to also include a penetration enhancer in the formulations of the invention. A penetration enhancer is a substance that improves cutaneous penetration of a bioactive substance. Suitable penetration enhancers include, for example, dimethyl sulfoxide (DMSO), DMSO-like compounds, ethanolic compounds, pyroglutamic acid esters and other solvents or compounds known to those skilled in the pharmaceutical art which facilitate dermal penetration of the drugs or chemicals chosen for the pharmaceutical composition. Other penetration enhancers include amphiphiles such as L-amino acids, anionic surfactants, cationic surfactants, amphoteric surfactants, nonionic surfactants, fatty acids and alcohols. Additional penetration enhancers are disclosed in Remington: The Science and Practice of Pharmacy, 19th Edition (1995) on page 1583. The penetration enhancer chosen and the relative proportion of the penetration enhancer with respect to the active drugs or chemicals depends on the desired rate of delivery of the drugs or chemicals into the skin, which in turn depends on the condition being treated and the outcome sought. More specifically, the type and amount of enhancer is chosen so that a sufficiently high concentration of active drugs or chemicals is attained in the skin to treat the condition within the time period considered desirable. Apart from the above-described compositions, use may be made of covers, e.g. plasters, bandages, dressings, gauze pads, patches and the like, containing an appropriate amount of ELA cells, ECCS, ECL, and ECP or a combination thereof and, optionally, other ingredients. In some cases use may be made of plasters, bandages, dressings, gauze pads, patches and the like which have been impregnated with a topical formulation containing the therapeutic formulation. Additional cosmetic and dermatological agents. Other cosmetic or dermatological agents may be included in methods and formulations of the invention. A "cosmetic or dermatological agent" as used herein, includes any substance whose administration for treatment of a cosmetic condition or to achieve a desired cosmetic effect, results in an status of the condition that is better than the status that would exist without the use of the cosmetic or dermatological agent.

Anti-wrinkling agents are one form of cosmetic or dermatological agents. Anti-skin wrinkling agents include a variety of agents, often in combination, that prevent or treat wrinkling through a variety of actions. Many approaches are taken to reduce the appearance

of facial and other wrinkles based on the understanding of the molecular basis of wrinkle formation. Such treatments include cosmetic products, drug therapy and surgical procedures. For example, many cosmetic products contain hydroxy acids, which may stimulate collagen synthesis. Another common treatment utilizes retinol, retinoic acid, retinol palmitate, a
5 derivative of vitamin A, (or prescribed versions, Retin-A and Renova) which may directly or indirectly stimulate collagen production or retard collagen degradation. Bicyclic aromatic compounds with retinoid-type activity, which are useful in particular in preventing or treating various keratinization disorders, are described in EP 679 630. These compounds are particularly active for repairing or combating chronological or actinic ageing of the skin, for
10 example such as in anti- wrinkle products. Antioxidants such as vitamin C and E and coenzyme Q-10 are believed to counteract free radicals, which damage cells and cause aging and have been used in treatments of wrinkles. Recently, the FDA approved cosmetic use of Botox (an extremely purified form of botulinum toxin) to treat glabella frown lines.

Thus cosmetic or dermatological agents that complement cosmetic treatment of skin
15 with the methods or compositions of the invention include, alone or in combination, the bicyclic aromatic compounds defined above, other compounds which have retinoid- type activity, free-radical scavengers, hydroxy or keto acids or derivatives thereof.

The term "free-radical scavenger" refers to, for example, alpha-tocopherol, superoxide dismutase, ubiquinol (e.g., coenzyme Q10) or certain metal-chelating agents. Hydroxy acids
20 include, e.g., alpha-hydroxy acids such as lactic acid and glycolic acid or beta-hydroxy acids such as salicylic acid and salicylic acid derivatives such as the octanoyl derivative; other hydroxy acids and keto acids include malic, citric, mandelic, tartaric or glyceric acids or the salts, amides or esters thereof. Other anti-wrinkling agents and anti-skin aging agents useful in the invention include sulfur-containing D and L amino acids and their derivatives and salts,
25 particularly the N-acetyl derivatives, a preferred example of which is N-acetyl-L- cysteine; thiols, e.g. ethane thiol; fat-soluble vitamins, ascorbyl palmitate, ceramides, pseudoceramides (e.g., pseudoceramides described in U.S. Pat. Nos. 5,198,210; 4,778,823; 4,985,547; 5,175,321, all of which are incorporated by reference herein), phospholipids (e.g., distearoyl lecithin phospholipid), fatty acids, fatty alcohols, cholesterol, plant sterols, phytic acid, lipoic
30 acid; lysophosphatidic acid, and skin peel agents (e.g., phenol and the like), and mixtures thereof. Preferred fatty acids or alcohols are those that have straight or branched alkyl chains containing 12-20 carbon atoms. A particularly preferred fatty acid is linoleic acid since linoleic acid assists in the absorption of ultraviolet light and furthermore is a vital component of the natural skin lipids. Other non-limiting examples of suitable anti-wrinkle actives for use

herein are described in U.S. Pat. No. 6,217,888, which description is incorporated herein by reference.

Compositions for cosmetic treatment of skin may further include sunscreens to lower skin's exposure to harmful UV rays. Sunscreens include those materials commonly employed to absorb or block ultraviolet light. Illustrative compounds are the derivatives of PABA, cinnamate and derivatives of salicylate (other than ferulyl salicylate). For example, octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone (also known as oxybenzone) can be used. Octyl methoxycinnamate and 2-hydroxy-4-methoxy benzophenone are commercially available under the trademarks, PARSOL MCX and BENZOPHENONE-3, respectively. Dermascreen may also be used.

Many other sunscreens are known to those of skill in the art. In some embodiments, sunscreens are FDA-approved or approved for use in the European Union. For example, FDA-approved sunscreens may be used, singly or, preferably, in combination. See, e.g., U.S. Pat. Nos. 5,169,624; 5,543,136; 5,849,273; 5,904,917; 6,224,852; 6,217,852; and Segarin et al., chapter VII, pages 189 of *Cosmetics Science and Technology*, and Final Over-the-Counter Drug Products Monograph on Sunscreens (Federal Register, 1999:64:27666-27963), all of which are incorporated herein by reference. The exact amount of sunscreen employed in-the compositions can vary depending upon the degree of protection desired from the sun's UV radiation.

Cosmetic compositions of the invention may further include anti-acne agents. Anti-acne agents include benzoyl peroxide, antibiotics, e.g., erythromycin, clindamycin phosphate, 5,7-dichloro-8-hydroxyquinoline, resorcinol, resorcinol acetate, salicylic acid, azaleic acid, long chain dicarboxylic acids, sulfur, zinc, retinoids, antiandrogens, and various natural agents such as those derived from green tea, tea tree oil, and mixtures thereof. Other non-limiting examples of suitable anti-acne agents for use herein are described in U.S. Pat. No. 5,607,980, which description is incorporated herein by reference.

Other cosmetic and dermatological agents include anticellulite agents. Anticellulite agents include isobutylmethylxanthine, caffeine, theophylline, theobromine, aminophylline, yohimbine, and mixtures thereof. Yet other cosmetic or dermatological agents that complement cosmetic treatment of skin include .alpha. -interferon, estradiol; progesterone; pregnanalone; methylsolanomethane (MSM); copper peptide (copper extract); plankton extract (phytosome); broparoestrol; estrone; adrostenedione; androstanediols; etc.

The compositions of the present invention may contain a wide range of additional components. The CTFA Cosmetic Ingredient Handbook, Seventh Edition, 1997 and the

Eighth Edition, 2000, which are incorporated by reference herein in their entirety, describes a wide variety of ingredients commonly used in skin care compositions, which are suitable for use in the compositions of the present invention. Other topically-applied compounds are listed in Remington's Pharmaceutical Sciences, 20th Ed., Lippincott Williams & Wilkins, Baltimore, Md. (2000) (hereinafter Remington's), U.S.

Pharmacopeia and National Formulary, The United States Pharmacopeial Convention, Inc., Rockville, Md. and Physician's Desk Reference, Medical Economics Co., Inc., Oradell, N.J. incorporated herein by reference. The concentration of the other active ingredient in formulations provided by the invention is that which provides an effective amount of the other active ingredient; these concentrations are well-known in the art.

Examples

Example 1: Materials and Methods

ELA[®] cells are early lineage adult stem cells that are isolated in an undifferentiated and highly plastic state. ELA[®] cells were cultured in TheraPEAK[™] MSCGM-CD[™] Mesenchymal Stem Cell Medium, Chemically Defined (Lonza Ltd.) with or without 1% Fetal Bovine Serum (FBS) or in DMEM or in an appropriate medium. ELISA kits for detecting levels of cytokine were obtained from Qiagen.

Example 2: Purification of ELA stem cells

ELA stem cells are obtained from blood of mammalian subjects, for example human individual which is harvested, diluted in serum-free medium (GIBCO[™] AIM V Medium liquid; Invitrogen, Carlsbad, CA), and centrifuged three times at 200 g for ten minutes at room temperature. The cells are then re-suspended in serum-free medium, and are layered over a combined gradient of Ficoll-Paque and Stem Cells Technologies Granulocyte gradient commercially available from ROSETTE SEP DM-M; Stem Cell Technologies, Vancouver, British Columbia. Granulocytes are prevented from entering the gradient and red blood cells and stem cells are obtained in to pellet. The sample is centrifuged at 500 g for 30 minutes at room temperature. The Ficoll-Stem Cell Technology gradients separate the cells into a buffy layer, an intermediate layer, and pelleted layer.

The desired ELA stem cell population is isolated from the pelleted layer, washed in phosphate-buffered saline, and resuspended in culture medium. See U.S. utility application serial number 12/598,047 filed October 29, 2009, publication US 2010/0291042 published November 18, 2010.

Example 3: ELA cells stimulate cytokine production

ELA cells were cultured and cell-free conditioned media were collected after an period of cell growth sufficient to permit expression and production of secreted cell factors (e.g., 24-72 hours). Cells were harvested by centrifugation at 72 hours, counted and supernatants were centrifuged at 14,000× g for 5 min to remove cellular debris. Concentrations of each of Vascular Endothelial Derived Growth Factor (VEGF), Interleukin-6 (IL-6) and Interleukin-8 (IL-8) protein levels were measured in the conditioned medium using commercial Enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions. Pre-conditioned control medium cytokine levels were analyzed by ELISA. The data collected from control pre-conditioned medium and conditioned medium was analyzed as shown in Fig.1.

The cytokine levels in ELA cell conditioned medium were observed to be consistently and significantly higher than cytokine levels in unconditioned media. The cytokines VEGF, IL-6 and IL-8 play a role in anti-aging. VEGF stimulates angiogenesis and IL-6 and IL-8 reduce inflammation. It is here envisioned that ELA cells combat symptoms of aging by stimulating increase in production of cytokines and that ELA cell conditioned media might be used either as a source for production or directly following sterilization by filtration in a cosmetic preparation.

Example 4: Assay of additional growth factors including keratinocyte growth factor, dermal fibroblasts growth factor, and endothelial cells growth factor

ELA cells are cultured and the cell-free ELA-conditioned media is collected after an period of cell growth sufficient to permit expression and production of secreted cell factors (e.g., 24-72 hours). Cells are harvested by centrifugation at 72 hours, counted and supernatants are centrifuged at 14,000× g for 5 min to remove cellular debris. Each of Vascular Endothelial Derived Growth Factor (VEGF), Keratinocyte growth factor and dermal fibroblasts growth factor protein levels are measured in the conditioned media using commercial Enzyme-linked immunosorbent assay (ELISA) kits in accordance with the manufacturer's instructions. Pre-conditioned control medium growth factor levels are analyzed by ELISA. The data collected from pre-conditioned media and ELA-conditioned media is analyzed.

The protein levels of VEGF, Keratinocyte growth factor and dermal fibroblasts growth factor etc. is greater than control unconditioned medium. It is here envisioned that

increased production of e.g., Keratinocyte growth factor and dermal fibroblast and other growth factors produced therein stimulates increase in tissue production of endogenous cells, collagen and elastin. It is also envisioned that increased production of VEGF stimulates angiogenesis. Other cytokines have immune-mediating effects.

5 Therefore, it is here proposed that ELA cells combat aging symptoms by stimulating increase in production of e.g., VEGF, Keratinocyte growth factor and dermal fibroblasts growth factor and other secreted soluble growth factors and cytokines, and that ELA cell-conditioned media is useful as a source of these factors, or directly in a cosmetic preparation. Addition of ELA cell extracts and fractions are useful as such membrane fractions add fatty
10 acids and membrane receptor proteins, and microsomal fractions contain bioactive polypeptides and microRNAs, which have further tissue regenerative effects on target tissues. Such ELA cell extracts and/or ELA cell-conditioned media are effective at ranges of 0.01% to 10% w/w. Specific concentrations will depend on the fraction, e.g., growth factors are effective in lower concentrations than fatty acids, as will be apparent to those of skill in the
15 art.

What is claimed is:

1. A formulation comprising, a cosmetic base having from about 0.01% to 10% w/w of an ELA cell preparation.
2. The formulation of claim 1, wherein the ELA cell preparation includes devitalized
5 ELA cells.
3. The formulation of claim 2, wherein the ELA cells are lyophilized.
4. The formulation of claim 2, further comprising an ELA cell membrane fraction.
5. The formulation of claim 1, further comprising a tissue growth factor.
6. The formulation of claim 1, wherein the ELA cell preparation includes expanded ELA
10 cells.
7. The formulation of claim 1, further comprising from about 0.01% to 10% w/w of medium conditioned by growth of ELA cells.
8. The formulation of claim 7, wherein the media is lyophilized.
9. The formulation of claim 1, substantially free from non-human animal products.
- 15 10. Use of ELA cells (ECCS), lysates (ECL) and/or ELA cell products (ELP) in the manufacture of a medicament.
11. A formulation comprising, a cosmetic base having from about 0.01% to 10% w/w of an ELA cell extract and/or ELA cell-conditioned media.
12. A method of treating a skin condition including: wrinkling, folds, sagging, age spots,
20 uneven pigmentation, thinning, elasticity, scarring, surface roughness, surface roughness, surface vessels, redness, and pore size, comprising administering to a subject having one or more of the aforesaid conditions, a dose of a formulation of claim 1.
13. A method of tissue augmentation, comprising inducing tissue growth and recovery from standard cosmetic procedures by administering to a subject subdermally, a preparation
25 of 10^4 to 10^7 live ELA cells.
14. A method of tissue augmentation, comprising inducing tissue growth and recovery from standard cosmetic procedures by administering to a subject externally, the formulation of claim 1.

15. A method of tissue augmentation, comprising inducing tissue growth and recovery from standard cosmetic procedures by administering to a subject subdermally, a preparation of 10^4 to 10^7 live ELA cells and subsequently administering externally one or more doses of the formulation of claim 1.
- 5 16. The method of claim 14, wherein the mode of administration is topical, and optionally, other cosmetics or devices, may be in contact on the surface or below the dermal surface at the same time.
17. The formulation of claim 1, wherein the cosmetic base is a lotion.
18. The formulation of claim 1, wherein the cosmetic base is a cream.
- 10 19. The formulation of claim 1, wherein the cosmetic base includes a pigment.
20. The formulation of claim 1, wherein the cosmetic base includes a sunscreen.
21. The formulation of claim 1, wherein the cosmetic base includes at least one anti-acne agent.
22. The formulation of claim 1, wherein the cosmetic base includes at least one
15 antioxidant.
23. The formulation of claim 1, wherein the cosmetic base includes a platelet-rich fibrin matrix.
24. A composition comprising, a cosmetic base having from about 0.01% to 10% w/w of one or more of: an ELA cell preparation, an ELA cell extract, and an ELA cell-conditioned
20 medium; the ELA cell-conditioned medium is generated in an environmentally-closed cell culture system.
25. The composition according to claim 24 wherein the medium is filter-sterilized.
26. The composition according to claim 24 wherein the medium is concentrated.