Over time this leads to skin laxity clinically visible as fine or sometimes more pronounce wrinkles on the face, the hands, the lower arms, the décolleté and elsewhere.

[0006] While there are a number of surgical and non-surgical methods that can be used to treat skin, but none of those techniques addresses the issue of a cellular deficit of functional fibroblasts in the aging skin.

[0007] The present invention discloses novel compositions and methods for treating skin aging and injuries, and further provides other related advantages.

SUMMARY

[0008] Briefly stated, the present invention provides compositions and methods for treating or preventing skin aging or skin injuries utilizing hair follicle derived Non-Bulbar Dermal Sheath ("NDBS") cells. Within one aspect of the present invention methods are provided comprising the steps of (a) preparing vital (i.e., 'living') hair; and (b) culturing the vital hair such that a population of NBDS cells can be obtained. Within preferred embodiments the NBDS cells are isolated. In one aspect, the present disclosure refers to use of a composition comprising isolated Non-Bulbar Dermal Sheath (NBDS) cells in the manufacture of a medicament for treating or preventing skin injury, wherein the NBDS cells are prepared by a method comprising: (a) cleaving vital hair above the bulbar portion of the hair root (i.e., above the dermal papillae and dermal sheath cup cells, i.e., above the end bulbs), but below the base of the sebaceous gland canal, to remove the hair follicle bulb; (b) isolating NBDS tissue; (c) cultivating the isolated NBDS tissue to produce the NBDS cells; (d) passaging the NBDS cells over at least four passages; wherein the isolated NBDS cells are at least 95% pure, and have at least one contaminating cell type within the cell population; and wherein the NBDS cells are isolated from ultraviolet na'ive tissue. In another aspect, the present disclosure refers to a cosmetic method of preventing or treating skin aging comprising administration of a composition comprising isolated Non-Bulbar Dermal Sheath (NBDS) cells, wherein the NBDS cells are prepared by a method comprising (a) cleaving vital hair above the bulbar portion of the hair root (i.e., above the dermal papillae and dermal sheath cup cells, i.e., above the end bulbs), but below the base of the sebaceous gland canal, to remove the hair follicle bulb; (b) isolating NBDS tissue; (c) cultivating the isolated NBDS tissue to produce the NBDS cells; (d) passaging the

NBDS cells over at least four passages; wherein the isolated NBDS cells are at least 95% pure, and have at least one contaminating cell type within the cell population; and wherein the NBDS cells are isolated from ultraviolet na'ive tissue.

[0009] Within one ~~aspect of the invention~~example methods are ~~provided~~ described for isolating NBDS cells, comprising the steps of: (a) preparing vital hair; (b) cleaving the hair prepared in step (a) to remove the hair follicle bulb (which contains the dermal sheath cup and dermal papilla); (c) isolating Non-Bulbar Dermal Sheath tissue; and (d) cultivating the isolated Non-Bulbar Dermal sheath tissue to produce NBDS cells. Within one ~~embodiment of the invention~~example the vital hair is obtained by biopsy from the occipital scalp of a subject. Within another example ~~embodiment~~ the hair is cleaved utilizing a micromanipulator and scalpel. Within yet other examples~~embodiments~~, the methods provided herein further comprise the step of conducting enzymatic digestion of the isolated Non-Bulbar Dermal Sheath tissue, optionally, with, for example collagen digesting enzymes such as collagenase, dispase, and leupeptin. Within further examples~~embodiments~~, the cells are passaged over multiple passages.

[0010] Within other examples~~aspects of the invention~~, isolated NBDS cells are ~~provided~~described, optionally prepared according to the methods described above, wherein the cells are isolated in order to provide a population which are primarily positive for one or more of: CD 90, CD73 and CD49b, and / or primarily negative for one or more of CD34, CD45 and KRT14 (optionally before or after culturing). Within preferred examples ~~embodiments~~ the isolated NBDS cells are at least 70%, 80%, 90%, 95%, 98% or 100% positive for

one or more of the positive markers described above, and/or at least 80%, 90%, 95%, or 98% negative for one of the negative markers described above.

[0011] Within preferred examples~~embodiments of the invention~~, isolated NBDS cells have less than 15%, 10%, 5%, or 1% keratinocytes within the cell population and/or less than 15%, 10%, 5%, or 1% melanocytes within the cell population. However, within further examples~~embodiments~~, the isolated NBDS cell population is derived from a population of dermal cells (preferably from a hair follicle) that have some contaminating cell types, including for example, at least 1, 5, 10, .0.01%, 0.1%, or 1% keratinocytes in the cell population, and /or at least 5, 10, 0.1%, 0.01% melanocytes. Within further ~~embodiments~~ examples~~of the invention~~ the isolated NBDS cells are at least 95% pure, and have at least one contaminating cell type (e.g., at least one keratinocyte) within the cell population.

[0012] These NBDS cells (or expanded or isolated NBDS cells) may be contained within compositions with other ingredients, such as, for example, serum plasma, fibrin, and /or hyaluronic acid. Within other examples ~~embodiments~~ the NBDS cells (or expanded or isolated NBDS cells) may be constituted in a composition suitable for injection, e.g., Lactated Ringer's or a buffered saline solution. Other ingredients which may be utilized to form the compositions of the present invention include, for example, components of the extracellular matrix (e.g., glycosaminoglycans (GAGs), heparin sulfate, chondroitin sulfate, keratin sulfate, hyaluronic acid, albumin (e.g., human albumin), elastin, fibronectins and laminins), cytokines and chemokines (e.g., transforming growth factor beta (TGF-beta) and its isoforms, insulin-like growth factor (IGF) and its isoforms, granulocyte-macrophage colony-stimulating factor (GM-CSF), parathyroid-hormone-related protein, hepatocyte growth factor/scatter factor (HGF/SF), macrophage stimulating protein (MSP), epidermal growth factor (EGF), interleukin 6 (IL-6), stromal cell-derived factor 1 (SDF-1), platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) and /or various therapeutic agents (e.g., analgesic agents, anti-inflammatory agents and immunomodulatory agents). Within other examples ~~embodiments~~ however NBDS cells (and isolated NBDS cells) are ~~provided~~ described in compositions that do not have any of the aforementioned ingredients (including for example, serum or plasma).

[0013] Within yet other examples~~aspects of the invention~~ methods are ~~provided~~ described for treating the skin of a subject, comprising the step of administering to the skin of a

subject a composition comprising NBDS cells as described herein. Within one embodiment, the subject is a mammal selected from the group consisting of humans, horse, dogs and cats. Within various examples ~~embodiments~~ the treatment is due to a skin injury. Within certain examples~~embodiments~~, the skin injury can result from external trauma (e.g., a surgical procedure or wound, burn, radiation, or an accident), or an acute or chronic wound or scar. Within other examples ~~embodiments~~ the skin injury is a predisposition of spontaneous or induced bruises on the extremities. Within yet other examples ~~embodiments~~ the skin injury is due to acne and/or other inflammatory reactions leading to scars, inherent disease with fragile skin such as epidermolyis bullosa dystrophica or other inherent or acquired blistering diseases with chronic wounds incurred by diabetes mellitus, arteriosclerosis or varicosis, acute or chronic viral, mycotic or bacterial infections, chronic autoimmune inflammatory disease such as Scleroderma and variants, Borelliosis infection, Lupus erythematosis and variants, Lichen planus, the negative effect of smoking and UV-light (extrinsic aging) and general aging (intrinsic aging). Within yet another example~~embodiment~~, the skin is on the entire human body, or a selected portion of the body (such as the hands, face, and neck. Within other examples~~embodiments~~, the skin can be aged/damaged by sun (UV-light) or smoking. Within related examples ~~embodiments~~ the aged skin suffers from a conditions selected from the group consisting of fine or coarse wrinkles, decreased skin thickness, decrease elasticity and firmness. Within other examples ~~embodiments~~ the skin is treated for cosmetic purposes (e.g., to reduce the appearance of cellulite). Within yet other examples~~embodiments~~, there is no current visible injury or trauma to the skin, but the skin may nevertheless be treated in order to prevent or delay the incidence of normal aging, or to improve the appearance (and texture) of normal skin.

[0014] Within another example ~~aspect of the invention~~ compositions are ~~provided~~ disclosed comprising NBDS cell culture supernatants. Within one example~~embodiment~~, the NBDS cell culture supernatant is prepared from cultured NBDS cells as described herein. Within other example ~~embodiment~~ the NBDS cell culture supernatant can be stored by freezing, lyophilisation, or other suitable methods. The NBDS cell culture supernatant can be utilized in a variety of methods, including for example, applied topically, injected (e.g., small injections to the skin or other body parts).

[0015] Within various examples~~embodiments~~, the NBDS cell culture supernatant can be prepared and utilized as is (e.g., after filtration), diluted with suitable buffers or

excipients, concentrated, or with various components separated out (which may be utilized separately (*e.g.,* stem cell factor, wnt-factors, other growth factor, cytokines or chemokines). Within one example ~~embodiment~~ the NDBS cell culture supernatant is utilized as is, diluted or concentrated, and combined or admixed with a compound or compositions suitable for administration (e.g., a gel, crème, or polymer-based composition). Representative examples include hyaluronic acid, collagen, extracellular matrix, scaffolds, polymer-based compositions (e.g., polyethylene glycols, poly-lactic acids, and poly caprolactones)~~,~~. ~~Other~~ suitable carriers for the NBDS cell culture supernatants include pastes, and other liquids utilized in the cosmetic industry (e.g., emulsions, laquers, tonics, shampoos, sprays). Within other examples~~embodiments~~, the NDBS cell culture supernatants may be placed in manufactured or harvested extracellular matrices (see e.g. US 20100047305 or US 20100124573). Within other examples~~embodiments~~, the cells may be utilized on a medical device, or placed on or within a non-biodegradable, or, biodegradable scaffold, or other structure. Particularly preferred scaffolds or structures include biodegradable scaffolds (e.g., collagen- based scaffolds, such as, for example, meshes). Representative examples of suitable scaffolds include, for example, U.S. Patent Nos., 5,736,372, 5,759,830, 8,039,258 and 8,105,380, all of which are incorporated by reference in their entirety.

[0016] The details of one or more embodiments are set forth in the description below. Other features, objects and advantages will be apparent from the description, the drawings, and the claims. In addition, the disclosures of all patents and patent applications referenced herein are incorporated by reference in their entirety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Figure 1 illustrates the dissection of a human hair follicle. Figure 1A shows an isolated human hair follicle, which can be cleaved above the bulbar portion of the hair root (i.e., above the dermal papillae and dermal sheath cup cells, i.e., above the end bulbs), but below the base of the sebaceous gland canal, in order to obtain an isolated dermal sheath (see Figure 1B). The structure depicted in Figure 1B can be separated into at least two separate components, as shown in Figures 1C and 1D. Figure 1C depicts the hair fiber and associated inner root sheath, and outer root sheath which contain predominantly keratinocytes, and Figure 1D is the dermal sheath containing NBDS cells (also occasionally referred to as the connective-tissue sheath, upper dermal

CLAIMS

What is claimed is:

1. Use of a composition comprising isolated Non-Bulbar Dermal Sheath (NBDS) cells in the manufacture of a medicament for treating or preventing skin injury, wherein the NBDS cells are prepared by a~~A~~ method ~~for isolating NBDS cells,~~ comprising:

(a) cleaving ~~preparing~~ vital hair above the bulbar portion of the hair root (i.e., above the dermal papillae and dermal sheath cup cells, i.e., above the end bulbs), but below the base of the sebaceous gland canal, to remove the hair follicle bulb;

~~(b) cleaving the hair prepared in step (a) to remove the hair follicle bulb;~~

(~~c~~b) isolating ~~Non-Bulbar Dermal Sheath~~NBDS tissue; ~~and~~

(~~d~~c) cultivating the isolated NBDS ~~Non-Bulbar Dermal sheath~~ tissue to produce the NBDS cells;

(d) passaging the NBDS cells over at least four passages;

wherein the isolated NBDS cells are at least 95% pure, and have at least one contaminating cell type within the cell population; and wherein the NBDS cells are isolated from ultraviolet na'ive tissue.

2. A cosmetic method of preventing or treating skin aging comprising administration of a composition comprising isolated Non-Bulbar Dermal Sheath (NBDS) cells, wherein the NBDS cells are prepared by a method comprising

(a) cleaving vital hair above the bulbar portion of the hair root (i.e., above the dermal papillae and dermal sheath cup cells, i.e., above the end bulbs), but below the base of the sebaceous gland canal, to remove the hair follicle bulb;

(b) isolating NBDS tissue;

(c) cultivating the isolated NBDS tissue to produce the NBDS cells;

(d) passaging the NBDS cells over at least four passages; wherein the isolated NBDS cells are at least 95% pure, and have at least one contaminating cell type within the cell population; and wherein the NBDS cells are isolated from ultraviolet na'ive tissue.

2. The method according to claim 1 wherein said vital hair is obtained by biopsy from a hair on the scalp of a subject.
3. The method according to claim 1 wherein isolated NBDS cells can either be used autologous or allogeneic.
4. The method according to claim 1 wherein said hair is cleaved utilizing a micromanipulator and scalpel, or scissors,
5. The method according to claim 1, further comprising the step of conducting enzymatic digestion of said isolated Non-Bulbar Dermal Sheath tissue.
6. The method according to claim 5 wherein said enzymatic digestion is conducted with collagenase hyaluronidase, DNAse, elastase, papain, protease type XIV, trypsin, and dispase.
7. The method according to claim 1 wherein said NBDS cells are passaged over multiple passages.
8. A composition comprising isolated Non-Bulbar Dermal Sheath cells prepared according to the method of any one of claims 1 to 7.
9. A composition comprising isolated Non-Bulbar Dermal Sheath cells.
10. A composition according to claims 8 or 9, further comprising serum plasma or platelet-rich plasma (PRP).
11. A composition according to claims 8 or 9, further comprising fibrin and/or hyaluronic acid.
12. A composition according to claims 8 or 9, further comprising components of the extracellular matrix, cytokines, chemokines and therapeutic agents.
13. The composition according to claim 12 wherein said components of the extracellular matrix are selected from the group consisting of glycosaminoglycans (GAGs), heparin sulfate, chondroitin sulfate, keratin sulfate, hyaluronic acid, elastin, collagens, fibronectins and laminins.
14. The composition according to claims 8 or 9, further comprising a scaffold.
15. The composition according to claim 14, wherein said scaffold is a biodegradable scaffold.
16. The composition according to claim 12 wherein said cytokines are selected from the group consisting of transforming growth factor beta (TGF-beta) and its isoforms, insulin-like growth factor (IGF) and its isoforms, granulocyte-macrophage colony-stimulating factor (GM-CSF), parathyroid-hormone-

related protein, hepatocyte growth factor/scatter factor (HGF/SF), macrophage stimulating protein (MSP), epidermal growth factor (EGF), interleukin 6 (IL-6), stromal cell-derived factor 1 (SDF-1), platelet derived growth factor (PDGF) and fibroblast growth factor (FGF).

17. The composition according to claim 12 wherein said therapeutic agents are selected from the group consisting of analgesic agents, anti-inflammatory agents antibiotic, antiviral, antimycotic, and immunomodulatory agents.

18. A method for treating the skin, comprising the step of administering to the skin of a subject a composition according to any one of claims 8 to 17.

19. The method according to claim 18 wherein said subject is a mammal selected from the group consisting of humans, horse, dogs and cats.

20. The method according to claim 18 wherein said treatment is due to a skin injury

21. The method according to claim 20 wherein said skin injury is an acute or chronic wound or a scar.

22.3. The method use according to claim 18 1 wherein said the skin injury is a traumatic wound incurred by surgery, a burn, radiation, or accident.

23. The method according to claim 18 wherein said skin injury is a predisposition of spontaneous or induced bruises on the extremities.

24.4. The method according to claim 18 2 wherein said the skin is aged skin.

25. The method according to claim 24 wherein said aged skin suffers from a condition selected from the group consisting of fine or coarse wrinkles, decreased skin thickness, decrease elasticity and firmness.

26. The method according to claim 18 wherein said skin is a subportion of the skin on a human subject selected from the group consisting of, the face, neck and hands.

5. The use according to claim 1 or the method of claim 2, wherein the isolated NBDS cells are autologous.

6. The use according to claim 1 or the method of claim 2, wherein the isolated NBDS cells are allogeneic.

7. The use according to claim 1 or the method of claim 2, where the composition further comprises NBDS cell supernatant.

8. The use or method according to claim 7, wherein the composition further comprises an excipient, and optionally a polymer.

9. The use or method according to claim 8, wherein the composition is formed into a paste, creme or gel.

10. The use according to claim 1 or the method of claim 2, wherein the composition further comprises serum plasma or platelet-rich plasma (PRP).

11. The use according to claim 1 or the method of claim 2, wherein the composition further comprises fibrin and/or hyaluronic acid.

12. The use according to claim 1, or the method of claim 2, wherein the composition further comprises a scaffold.

13. The use or the method of claim 12, wherein the scaffold is a biodegradable scaffold.

14. The use according to claim 1 or the method of claim 2, wherein cultivating the isolated NBDS tissue comprises passaging the NBDS cells over multiple passages in serum-free media.

15. The use according to claim 1 or the method of claim 2, wherein cultivating the isolated NBDS tissue comprises culturing the NBDS cells under conditions of low oxygen tension.

16. The use according to claim 1 or the method of claim 2, further comprising sorting the isolated NBDS cells to obtain a population of isolated NBDS cells.

17. The use or the method of claim 16, wherein the sorting is by flow cytometry.

18. The use according to claim 1 or the method of claim 2, wherein the isolated NBDS cells are in combination with keratinocytes.

~~27. A composition comprising NBDS cell supernatant.~~

~~28. A composition comprising NBDS cell supernatant made from the NBDS cells cultured according to the method of any one of claims 1 to 7.~~

~~29. The composition according to claims 27 or 28, wherein said composition is diluted or concentrated.~~

~~30. The composition according to any one of claims 27 to 29, further comprising an excipient.~~

~~31. The composition according to claim 30 wherein said excipient is a polymer.~~

~~32. The composition according to claim 30 formed into a paste, crème or gel.~~

~~33. Use of a composition according to any one of claims 27 to 32, for treatment of skin.~~

~~34. A method for treating skin, comprising administering to the skin of a subject a composition according to any one of claims 27 to 32.~~