# COMPOSITIONS AND METHODS FOR TREATING AND REPAIRING TENDONS

## CROSS-REFERENCE TO RELATED APPLICATION

**[0001]** This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 61/763,908 filed February 12, 2013, which application is incorporated herein by reference in its entirety.

## FIELD OF THE INVENTION

**[0002]** The present invention relates to compositions and methods for repairing tendons, and more specifically, to compositions comprising non-bulbar dermal sheath cells for use in the treatment and repair of tendons, and for the prevention of tendon injuries.

## BACKGROUND

Description of the Related Art

**[0003]** Tendons are tough bands of fibrous connective tissue that usually connect muscle to bone. Examples of common tendons include the Achilles tendon, which connects the calf muscle to the heel bone, and the Patellar tendon, which connects the patella to the tibia.

**[0004]** Tendons can be injured in a number of ways, including for example, through overuse, strain, disease and general aging. The term "tendinopathy" can be used to refer to a number of injuries, including both those caused by inflammation and micro-tears. Tendons can also be ruptured or torn, typically necessitating surgical intervention.

**[0005]** While there are a number of surgical methods that can be used to treat tendon injuries, even with such methods healing of the tendon can take several years, if they heal at all. The present invention discloses novel compositions and methods for treating tendon injuries, and further provides other related advantages.

## SUMMARY

**[0006]** Briefly stated, the present disclosure provides compositions and methods for treating or preventing tendon injuries utilizing hair follicle derived Non-Bulbar

Dermal Sheath ("NDBS") cells. In one aspect, the present disclosure provides use of a composition comprising isolated Non-Bulbar Dermal Sheath cells in the manufacture of a medicament for treating tendon injury. Within one example of the present disclosure 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 examples the NBDS cells are isolated.

**[0007]** Within one example of the disclosure methods are provided 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 example of the disclosure the vital hair is obtained by biopsy from the occipital scalp of a subject. Within another example the hair is cleaved utilizing a micromanipulator and scalpel. Within yet other examples, 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, the cells are passaged over multiple passages.

**[0008]** Within other examples of the disclosure, isolated NBDS cells are provided, 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 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.

**[0009]** Within preferred examples of the disclosure, 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, 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%, 0.1% melanocytes. Within further examples of the disclosure 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.

**[0010]** These NBDS cells (or isolated NBDS cells) may be contained within compositions with other ingredients, such as, for example, serum plasma, fibrin, and /or hyaluronic. Within other examples the NBDS cells (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 disclosure 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 however NBDS cells (and isolated NBDS cells) are provided in compositions that do not have any of the aforementioned ingredients (including for example, serum or plasma).

**[0011]** Within yet other examples of the disclosure methods are provided for treating or preventing tendon injuries, comprising the step of administering to a subject a composition comprising NBDS cells are described above (and in preferred examples isolated NBDS cells). Within one example, the subject is a mammal selected from the group consisting of humans, horses, pigs, dogs, cats, guinea pigs, rabbits, rats and mice.

**[0012]** Within various examples of the disclosure, the tendon injury is a tendon rupture or tear, or other injury selected from the group consisting of tendinosis, tenosynovitis, and avulsion. Within yet another example, the tendon is an Achilles tendon or the Patellar tendon. Within other examples, the tendon is a flexor tendon, or an extensor tendon. Within yet other examples tendon injuries should be understood to include a wide variety of tendon associated diseases (including tendinopathies,

tendinoses, tendinitis, tenosynovitis, partenonitis, paratenonitis with teninosis, and microtear*s* of a tendon) may also be treated utilizing the compositions provided herein. Representative tendons that may be treated include for example a) the Achilles tendon (e.g., M*i*d-portion Achilles tendinopathy; Achilles paratendinopathy; Insertional Achilles tendinopathy; Retrocalcaneal bursitis; Superficial calcaneal bursitis: b) Shoulder tendons *(e.g.,* Bicipital tendinopathy; Rotator cuff tendinopathy: c) Elbow tendons (e.g., Medial or Lateral epicondylitis or tennis elbow) d) Hand and Wrist: (e.g., flexor / extensor tendinopathy; flexor / Extensor tenosynovitis; De Quervain's disease; and Dupuytren's contracture; e) hamstring and patellar tendopathies with or without microtears; and f) plantar fasciitis with or without microtears.

**[0013]** The details of one or more examples 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

**[0014]** 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), 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).

**[0015]** Figure 2 is an illustration of a hair follicle depicting the origin for dermal papillae ("DP") cells, dermal sheath cup ("DSC") cells, and Non-Bulbar Dermal Sheath ("NBDS") cells.

**[0016]** Figure 3 is a photomicrograph of NBDS cells in culture.

**[0017]** Figure 4 illustrates NBDS cells which have been stained for collagen production. More specifically, a NBDS / plasma gel mixture was subjected to a mild

# CLAIMS

What is claimed is:

1. Use of a composition comprising isolated Non-Bulbar Dermal Sheath cells in the manufacture of a medicament for treating tendon injury.

2. The use according to claim 1 wherein the composition further comprises serum and/or plasma.

3. The use according to claim 1 wherein the composition does not contain serum or plasma.

4. The use according to any one of claims 1 to 4 wherein the composition further comprises fibrin, hyaluronic acid, components of the extracellular matrix, cytokines, chemokines, analgesic agents, anti-inflammatory agents or immunomodulatory agents.

5. The use according to claim 4 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, fibronectins and laminins.

6. The use according to claim 4 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).

7. The use according to any one of the preceding claims wherein said subject is a mammal selected from the group consisting of humans, horses, dogs and cats.

8. The use according to any one of claims 1 to 6 wherein said tendon injury is a tendon rupture, complete, partial or micro-tear.

9. The use according to any one of claims 1 to 6 wherein said tendon injury is selected from the group consisting of tendinosis, tenosynovitis, and avulsion.

10. The use according to any one of claims 1 to 6 wherein said tendon is the Achilles tendon, the Patellar tendon, a flexor tendon, or an extensor tendon.

11. The use of any one of the preceding claims wherein the composition is prepared according to a preparation method comprising the steps of:

a. preparing vital hair;
b. cleaving the hair prepared in step (a) to remove the hair follicle bulb;
c. isolating Non-Bulbar Dermal Sheath tissue; and
d. cultivating the isolated Non-Bulbar Dermal sheath tissue to produce NBDS cells.

12. The use of claim 11, wherein the preparation method further comprises the step of conducting enzymatic digestion of said isolated Non-Bulbar Dermal Sheath tissue.

13. The use of claim 12, wherein the enzymatic digestion is conducted with collagenase.

14. The use according to claim 11 wherein said vital hair has been obtained by biopsy from the occipital scalp of a subject.

15. The use according to claim 11 wherein said hair has been cleaved utilizing a micromanipulator and scalpel.

16. The use according to claim 11 wherein said NBDS cells have been cultivated over multiple passages in either serum containing or serum-free media.