METHOD OF CONNECTIVE TISSUE RESTORATION

FIELD OF THE INVENTION

[0001] The present application provides methods of restoring connective tissue in a patient, utilizing a combination of imaging techniques and minimally invasive tissue remodeling and regeneration, in combination with regenerative proteins. The methods provide a method for treating pain, mobility constraints, and stiffness issues across the interstitial layer (from head to toe), and in various joints and other areas where connective tissues are present. Also provided are kits for carrying out the various methods described herein.

BACKGROUND OF THE INVENTION

[0002] In 2018, a network of fluid-filled spaces surrounded by connective tissue throughout the human body, labeled the interstitium, was discovered. (*See, e.g.*, Benias *et al.*, "Structure and Distribution of an Unrecognized Interstitium in Human Tissues," *Scientific Reports* 8:4947 (2018)) Previously thought to be a dense wall of collagen, the interstitium is a fluid-filled highway responsible for ion diffusion and cellular protein transport and the primary source of lymph. It has also been determined to be a potential source for areas of treatment for pain, mobility constraints and stiffness issues. What is needed is a mechanism for resetting and remodeling the intersititum, including connective tissue, to treat and manage a patient's pain and mobility limitations.

~~BRIEF~~ SUMMARY ~~OF THE INVENTION~~

[0003] The present ~~invention~~ disclosure fulfills these needs, providing methods for treating connective tissues, including scar tissue internally, using a natural and non-surgical treatment to free nerves from entrapment, eliminate stubborn scar tissue and restore the anatomy to its pre-injury state.

[0003a] In one aspect, the present disclosure refers to use of a liquid composition in the manufacture of a medicament for treating a subepidermal tissue in a patient, wherein:

a. the tissue beneath a skin surface of the patient is to be visualized;

b. the tissue beneath the skin surface is to be vibrated;

c. one or more entrapped nerves is to be located within the subepidermal tissue;

d. a probe is to be introduced into the subepidermal tissue at the site of the entrapped nerve;

e. the subepidermal tissue and/or the entrapped nerve is to be manipulated with the probe; and

f. the liquid composition is to be injected into the subepidermal tissue via the probe at the site of the entrapped nerve.

[0003b] In one aspect, the present disclosure refers to use of a liquid composition comprising one or more regenerative proteins and a buffer in the manufacture of a medicament for treating a mesoderm-derived tissue in a patient, wherein:

a. the mesoderm-derived tissue beneath a skin surface of the patient is to be visualized via dynamic ultrasound imaging and real-time image analysis;

b. the connective tissue beneath the skin surface is to be vibrated;

c. one or more entrapped nerves is to be located within the connective tissue;

d. a needle probe is to be introduced into the connective tissue at the site of the entrapped nerve;

e. the tissue and/or the entrapped nerve is to be manipulated with the needle probe; and

f. the liquid composition is to be injected into the connective tissue via the needle probe.

[0004] In ~~embodiment~~examples, provided herein is a method for treating a subepidermal tissue in a patient, comprising: visualizing the tissue beneath a skin surface of the patient; vibrating the tissue beneath the skin surface; locating one or more entrapped nerves within the subepidermal tissue; introducing a probe into the subepidermal tissue at the site of the entrapped nerve;

manipulating the subepidermal tissue and/or the entrapped nerve with the probe; and injecting a liquid composition into the subepidermal tissue via the probe at the site of the entrapped nerve.

[0005] In further ~~embodiment~~examples, provided herein is a method for treating a mesoderm-derived tissue in a patient, comprising: visualizing the mesoderm-derived tissue beneath a skin surface of the patient via dynamic ultrasound imaging and real-time image analysis; vibrating the connective tissue beneath the skin surface; locating one or more entrapped nerves within the connective tissue; introducing a needle probe into the connective tissue at the site of the entrapped nerve; manipulating the tissue and/or the entrapped nerve with the needle probe; and injecting a liquid composition into the connective tissue via the needle probe, the liquid composition comprising one or more regenerative proteins and a buffer.

[0006] In still further ~~embodiment~~examples, provided herein is a kit for carrying out treatment of connective tissue of a patient, comprising: a liquid composition comprising dextrose, plasma-lyte and one or more placental proteins selected from the group consisting of: Basic fibroblast growth factor (bFGF); Epidermal growth factor (EGF); Granulocyte colony-stimulating factor (GCSF); Platelet-derived growth factor (PDGF-AA); Platelet-derived growth factor (PDGF-BB); Placental growth factor (PLGF); Transforming growth factor alpha (TGF – alpha); Transforming growth factor beta 1 (TGF-β1); interleukin 4 (IL-4); interleukin 6 (IL-6); interleukin 8 (IL-8); interleukin 10 (IL-10); Tissue inhibitor of metalloproteinase (TIMP-1); Tissue inhibitor of metalloproteinase (TIMP-2); Tissue inhibitor of metalloproteinase (TIMP-4); Growth Differentiation Factor (GDF-15); Granulocyte macrophage colony-stimulating factor (GM-CSF); Interferon gamma (IFN-γ); Interleukin 1 alpha (IL1-alpha); Interleukin 1 Beta (IL1-β); Interleukin 1 receptor antagonist (IL-1ra); Interleukin 5 (IL-5); Interleukin 7 (IL-7); Interleukin 12 p40 (IL-12p40); Interleukin 12 p70 (IL-12p70); Interleukin 15 (IL-15); Interleukin 17 (IL-17); Interleukin 16 (IL-16); Macrophage colony- stimulating factor (MCSF); Osteoprotegerin (OPG); B lymphocyte chemoattractant (CXCL13) (BLC); Chemokine ligand 1 (CCL1) (I-309); Eotaxin-2; Monocyte chemotactic protein 1 (CCL2) (MCP-1); Monokine induced by gamma interferon (CXCL9) (MIG); Macrophage inflammatory protein 1 alpha (CCL3) (MIP-1α); Macrophage inflammatory protein 1 beta (CCL4) (MIP-1β); Macrophage inflammatory protein 1D (MIP-5, CCL15) (MIP-1d); Regulated on activation, normal T cell expressed and secreted (CCL5) (RANTES); Brain-derived neurotrophic factor (BDNF); Bone morphogenetic protein 5

CLAIMS

What is claimed is:

1. Use of a liquid composition in the manufacture of a medicament ~~A method~~ for treating a subepidermal tissue in a patient, wherein ~~comprising~~:
    a. ~~visualizing~~ the tissue beneath a skin surface of the patient is to be visualized;
    b. ~~vibrating~~ the tissue beneath the skin surface is to be vibrated;
    c. ~~locating~~ one or more entrapped nerves is to be located within the subepidermal tissue;
    d. ~~introducing~~ a probe is to be introduced into the subepidermal tissue at the site of the entrapped nerve;
    e. ~~manipulating~~ the subepidermal tissue and/or the entrapped nerve is to be manipulated with the probe; and
    f. ~~injecting a~~the liquid composition is to be injected into the subepidermal tissue via the probe at the site of the entrapped nerve.
2. The use~~method~~ of claim 1, wherein the subepidermal tissue is a mesoderm-derived tissue.
3. The use~~method~~ of claim 2, wherein the mesoderm-derived tissue is a connective tissue.
4. The use~~method~~ of claim 3, wherein the connective tissue is located in the patient's neck, back, knees, hips, shoulders, elbows, feet, ankles, toes, hands, wrists, and/or fingers.
5. The use~~method~~ of claim 1, wherein the liquid composition comprises one or more regenerative proteins or cells, and a buffer.
6. The use~~method~~ of claim 1, wherein the visualization~~ing~~ comprises ultrasound imaging.
7. The use~~method~~ of claim 6, wherein the ultrasound imaging operates at a frequency of about 4-100 MHz.

8. The use~~method~~ of claim 1, wherein the subepidermal tissue is located within about 10 cm from the skin surface.
9. The use~~method~~ of claim 1, wherein the vibration~~g~~ is an acoustic radiation force impulse or a palpitation.
10. The use~~method~~ of claim 9, wherein the acoustic radiation force impulse is a shear wave.
11. The use~~method~~ of claim 1, wherein the probe is a needle probe.
12. The use~~method~~ of claim 11, wherein the needle probe includes one or more fenestrated holes to allow for delivery of the liquid composition.
13. The use~~method~~ of claim 1, wherein the manipulation~~g~~ of the entrapped nerve includes vibration and/or separation of the tissue via the needle.
14. The use~~method~~ of claim 1, wherein the visualization~~ing~~, vibration~~g~~ and/or location~~g~~ coincide with movement by the patient causing movement of the subepidermal tissue.
15. The use~~method~~ of claim 1, wherein the visualization~~ing~~ includes real-time image analysis to locate the entrapped nerves and abnormal tissue.
16. The use~~method~~ of claim 1, ~~further comprising dissipating~~ the liquid composition is further to be dissipated within the tissue via one or more of manual manipulation, percussion massaging, application of a sound wave, and application of a pulsed electromagnetic field.
17. Use of a liquid composition comprising one or more regenerative proteins and a buffer in the manufacture of a medicament ~~A method~~ for treating a mesoderm-derived tissue in a patient, ~~comprising~~wherein:
    a. ~~visualizing~~ the mesoderm-derived tissue beneath a skin surface of the patient is to be visualized via dynamic ultrasound imaging and real-time image analysis;

b. ~~vibrating~~ the connective tissue beneath the skin surface is to be vibrated;

c. ~~locating~~ one or more entrapped nerves is to be located within the connective tissue;

d. ~~introducing~~ a needle probe is to be introduced into the connective tissue at the site of the entrapped nerve;

e. ~~manipulating~~ the tissue and/or the entrapped nerve is to be manipulated with the needle probe; and

f. ~~injecting a~~the liquid composition is to be injected into the connective tissue via the needle probe~~, the liquid composition comprising one or more regenerative proteins and a buffer~~.

18. The use~~method~~ of claim 17, wherein the mesoderm-derived tissue is a connective tissue.

19. The use~~method~~ of claim 18, wherein the connective tissue is located in the patient's neck, back, knees, hips, shoulders, elbows, feet, ankles, toes, hands, wrists, and/or fingers.

20. The use~~method~~ of claim 17, wherein the liquid composition comprises dextrose, plasma-lyte and one or more placental proteins selected from the group consisting of:

Basic fibroblast growth factor (bFGF);

Epidermal growth factor (EGF);

Granulocyte colony-stimulating factor (GCSF);

Platelet-derived growth factor (PDGF-AA);

Platelet-derived growth factor (PDGF-BB);

Placental growth factor (PLGF);

Transforming growth factor alpha (TGF – alpha);

Transforming growth factor beta 1 (TGF-B1);

interleukin 4 (IL-4);

interleukin 6 (IL-6);

interleukin 8 (IL-8);

interleukin 10 (IL-10);

Tissue inhibitor of metalloproteinase (TIMP-1);

Tissue inhibitor of metalloproteinase (TIMP-2);

Tissue inhibitor of metalloproteinase (TIMP-4);

Growth Differentiation Factor (GDF-15);

Granulocyte macrophage colony-stimulating factor (GM-CSF);

Interferon gamma (IFN-γ);

Interleukin 1 alpha (IL1-alpha);

Interleukin 1 Beta (IL1-β);

Interleukin 1 receptor antagonist (IL-1ra);

Interleukin 5 (IL-5);

Interleukin 7 (IL-7);

Interleukin 12 p40 (IL-12p40);

Interleukin 12 p70 (IL-12p70);

Interleukin 15 (IL-15);

Interleukin 17 (IL-17);

Interleukin 16 (IL-16);

Macrophage colony- stimulating factor (MCSF);

Osteoprotegerin (OPG);

B lymphocyte chemoattractant (CXCL13) (BLC);

Chemokine ligand 1 (CCL1) (I-309);

Eotaxin-2;

Monocyte chemotactic protein 1 (CCL2) (MCP-1);

Monokine induced by gamma interferon (CXCL9) (MIG);

Macrophage inflammatory protein 1 alpha (CCL3) (MIP-1α);

Macrophage inflammatory protein 1 beta (CCL4) (MIP-1β);

Macrophage inflammatory protein 1D (MIP-5, CCL15) (MIP-1d);

Regulated on activation, normal T cell expressed and secreted (CCL5) (RANTES);

Brain-derived neurotrophic factor (BDNF);

Bone morphogenetic protein 5 (BMP-5);

Endocrine gland- derived vascular endothelial growth factor (EG-VEGF);

Fibroblast growth factor 4 (FGF-4);

Keratinocyte growth factor (FGF-7);

Growth hormone (GH);

Insulin-like growth factor (IGF-I);

Insulin-like growth factor binding protein – 1 (IGFBP-1);

Insulin-like growth factor binding protein – 2 (IGFBP-2);

Insulin-like growth factor binding protein – 3 (IGFBP-3);

Insulin-like growth factor binding protein – 4 (IGFBP-4); and

Insulin-like growth factor binding protein – 6 (IGFBP-6).

21. The use~~method~~ of claim 17, wherein the ultrasound imaging operates at a frequency of about 4-100 MHz.

22. The use~~method~~ of claim 17, wherein the connective tissue is located within about 10 cm from the skin surface.

23. The use~~method~~ of claim 17, wherein the vibrati on~~g~~ is an acoustic radiation force impulse or a palpitation.

24. The use~~method~~ of claim 23, wherein the acoustic radiation force impulse is a shear wave.

25. The use~~method~~ of claim 17, wherein the needle probe includes one or more fenestrated holes to allow for delivery of the liquid composition.

26. The use~~method~~ of claim 17, wherein the manipulati on~~g~~ of the entrapped nerve includes vibration and/or separation of the tissue via the needle.

27. The use~~method~~ of claim 17, ~~further comprising dissipating~~wherein the liquid composition is further to be dissipated within the connective tissue via one or more of manual manipulation, percussion massaging, application of a sound wave, and application of a pulsed electromagnetic field.

28. The use~~method~~ of claim 17, further comprising movement of the connective tissue by the patient to help aid in re-structuring the connective tissue.

29. The use~~method~~ of claim 17, ~~further comprising re-administering~~wherein the composition comprising regenerative proteins is further to be re-administered to the connective tissue at about 14-90 days after the initial ~~administering~~administration.

30. The use~~method~~ of claim 29, wherein the re-administration~~ering~~ occurs about every 14-90 days for a period of 1-12 months.

~~31. A kit for carrying out treatment of connective tissue of a patient, comprising:~~

~~a. a liquid composition comprising dextrose, plasma-lyte and one or more placental proteins selected from the group consisting of:~~

~~Basic fibroblast growth factor (bFGF);~~

~~Epidermal growth factor (EGF);~~

~~Granulocyte colony-stimulating factor (GCSF);~~

~~Platelet-derived growth factor (PDGF-AA);~~

~~Platelet-derived growth factor (PDGF-BB);~~

~~Placental growth factor (PLGF);~~

~~Transforming growth factor alpha (TGF—alpha);~~

~~Transforming growth factor beta-1 (TGF-B1);~~

~~interleukin-4 (IL-4);~~

~~interleukin-6 (IL-6);~~

~~interleukin-8 (IL-8);~~

~~interleukin-10 (IL-10);~~

~~Tissue inhibitor of metalloproteinase (TIMP-1);~~

~~Tissue inhibitor of metalloproteinase (TIMP-2);~~

~~Tissue inhibitor of metalloproteinase (TIMP-4);~~

~~Growth Differentiation Factor (GDF-15);~~

~~Granulocyte macrophage colony-stimulating factor (GM-CSF);~~

~~Interferon gamma (IFN-γ);~~

~~Interleukin-1 alpha (IL1-alpha);~~

~~Interleukin-1 Beta (IL1-β);~~

~~Interleukin-1 receptor antagonist (IL-1ra);~~

~~Interleukin-5 (IL-5);~~

~~Interleukin-7 (IL-7);~~

~~Interleukin-12 p40 (IL-12p40);~~

~~Interleukin-12 p70 (IL-12p70);~~

~~Interleukin-15 (IL-15);~~

~~Interleukin-17 (IL-17);~~

~~Interleukin-16 (IL-16);~~

~~Macrophage colony- stimulating factor (MCSF);~~

~~Osteoprotegerin (OPG);~~

~~B-lymphocyte chemoattractant (CXCL13) (BLC);~~

~~Chemokine ligand 1 (CCL1) (I-309);~~

~~Eotaxin-2;~~

~~Monocyte chemotactic protein-1 (CCL2) (MCP-1);~~

~~Monokine induced by gamma interferon (CXCL9) (MIG);~~

~~Macrophage inflammatory protein-1 alpha (CCL3) (MIP-1α);~~

~~Macrophage inflammatory protein-1 beta (CCL4) (MIP-1β);~~

~~Macrophage inflammatory protein-1D (MIP-5, CCL15) (MIP-1d);~~

~~Regulated on activation, normal T-cell expressed and secreted (CCL5) (RANTES);~~

~~Brain-derived neurotrophic factor (BDNF);~~

~~Bone morphogenetic protein-5 (BMP-5);~~

~~Endocrine gland- derived vascular endothelial growth factor (EG-VEGF);~~

~~Fibroblast growth factor-4 (FGF-4);~~

~~Keratinocyte growth factor (FGF-7);~~

~~Growth hormone (GH);~~

Insulin-like growth factor (IGF-I);

Insulin-like growth factor binding protein – 1 (IGFBP-1);

Insulin-like growth factor binding protein – 2 (IGFBP-2);

Insulin-like growth factor binding protein – 3 (IGFBP-3);

Insulin-like growth factor binding protein – 4 (IGFBP-4); and

Insulin-like growth factor binding protein – 6 (IGFBP-6); and

b. a needle probe for injecting the liquid composition into the connective tissue and for manipulating an entrapped nerve; and

c. instructions for carrying out the method of treatment of the connective tissue.

32. The kit of claim 31, wherein the needle probe includes one or more fenestrated holes to allow for delivery of the liquid composition.

33. The kit of claim 31, further comprising one or more instruments for manual manipulation, percussion massaging, application of a sound wave, or application of a pulsed electromagnetic field, to the connective tissue.