cells. Many immunosuppressants are also cytotoxic and include antimetabolites (azathioprine), alkylating agents (cyclophosphamide), and folic-acid antagonists (methotrexate or 6-MP). Other immunosuppressants include mycophenolate mofetil (CellCept® from Hoffmann-La Roche, Inc.) and cyclosporin. These drugs may cause numerous side effects including lethal infections.

[0006] Transplantation of ESC-derived cells requires the use of viable live cells that have a very limited shelf life. Maintaining cell viability requires local on site production for immediate infusion and or cryopreservation (freezing) in agents such as DMSO before overnight shipping on dry ice to prevent thawing. The frozen cells must then be thawed, washed to remove cytotoxic cryo-preservatives like DMSO, sterility rechecked, and viability reconfirmed before infusion, all of which increases expense and limits availability and practical application.

SUMMARY

[0007] Methods and compositions are provided herein for regenerating and/or repairing aged, degenerating, injured, diseased, or otherwise damaged organs and tissues in a subject using nonviable pluripotent stem cells that have been either lethally irradiated or lyophilized. In this latter aspect, pluripotent stem cells (e.g., embryonic stem cells or "ESC") are frozen and then vacuum dried to a powder that has an indefinite shelf life at room temperature and may be applied locally or dissolved into a solution before injection in an amount effective to provide a desired therapeutic effect. Lyophilized ESC may be manufactured at one site, do not require cryopreservation, have an indefinite shelf life, do not require immune suppression, and may be distributed globally to hospitals, pharmacies, or other locations to be reconstituted and injected on location similar to lyophilized proteins, antibodies, and drugs.

[0007A] In one aspect, the present invention refers to a wound healing composition comprising lyophilized pluripotent stem cells dispersed in a pharmaceutically

acceptable carrier and the lyophilized pluripotent stem cells included in a therapeutically effective amount.

[0007B] In another aspect, the present invention refers to use of a wound healing composition comprising lyophilized pluripotent stem cells in the manufacture of a medicament for promoting wound healing in a subject.

[0007C] In yet another aspect, the present invention refers to a topical anti-aging composition for the skin comprising lyophilized pluripotent stem cells dispersed in a cosmetically acceptable carrier.

[0007D] In a further aspect, the present invention refers to a method for providing an anti-aging effect to the skin of a subject, the method comprising administering an anti-aging composition comprising lyophilized pluripotent stem cells in a pharmaceutically acceptable carrier to the skin of the subject.

[0007E] In yet another aspect, the present invention refers to use of an effective amount of lyophilized human pluripotent stem cells or lethally irradiated human pluripotent stem cells in the manufacture of a medicament for regenerating cardiac muscle in a primate subject comprising administration of an effective amount of lyophilized human pluripotent stem cells or lethally irradiated human pluripotent stem cells to a damaged or aged area of the primate subject's heart.

[0007F] In a further aspect, the present invention refers to a method for promoting the *ex vivo* growth and expansion of human cell lines, the method comprising applying nonviable lethally irradiated or lyophilized pluripotent stem cells to human cells grown in culture

[0008] As described herein, in one ~~aspect~~example, an anti-aging benefit is provided to the skin by topical application of lyophilized human ESC to increase the hydration of human skin and reduce fine lines, wrinkles, pores, and skin roughness. In another

~~aspect~~example, a method is provided for promoting wound healing using either lethally irradiated ESC or lyophilized ESC powder. In yet another ~~aspect~~example, a method is provided for intracardiac, intracoronary, and/or intravenous injection into the heart to induce cardiac muscle regeneration. Nonviable lethally irradiated ESC and lyophilized ESC, vesicles, or fragments thereof are immune independent, do not require tissue typing, and may be utilized to repair any tissue or organ.

[0009] Neither the lethally irradiated ESC nor lyophilized pluripotent stem cell powder described in the compositions and methods herein are able to grow or divide *in vivo* or *in vitro*. The lyophilized stem cells are in the form of a powder of nonviable cells and or cellular contents that cannot be reconstituted into viable cells. The powdered form does not include living cells following the lyophilization or freeze vacuum desiccation process. Advantageously, the pluripotent stem cells killed with either lethal doses of irradiation or converted into a powder by lyophilization retain capability to repair damaged or degenerating organs or tissues in a subject.

[00010] A composition and method is provided for an anti-aging benefit to human skin. By one approach, the composition is a topical formulation comprising lyophilized pluripotent stem cell powder in an amount effective to provide a desired anti-aging cosmetic benefit to the skin. The method for providing an anti-aging benefit comprises administering an anti-aging or cosmetic composition comprising lyophilized pluripotent stem cells in a cosmetically acceptable carrier to the skin of the subject. For example, treatment with the lyophilized pluripotent stem cell powder is effective to provide the skin with a more youthful appearance due to increased hydration of the skin and a reduction in pores, skin roughness, and fine lines and/or wrinkles.

[00011] In yet another ~~aspect~~example, a composition is provided comprising lyophilized pluripotent stem cell powder which can be applied to a wound to promote wound healing. A method is also provided for promoting wound healing comprising administering a therapeutically effective amount of lyophilized pluripotent stem cell

powder to a wound of a subject. Promoting wound healing comprises, for example, accelerating wound closure.

[00012] In another ~~aspect~~example, pluripotent stem cell powder can be used to regenerate or repair cardiac muscle that has been damaged through, for example, age, disease, ischemia, or degeneration. In this respect, a method is provided for regenerating cardiac muscle comprising applying lyophilized pluripotent stem cell powder reconstituted in saline and injected into a damaged or aged area or vessel of the heart.

BRIEF DESCRIPTION OF THE DRAWINGS

[00013] FIG. 1 is a graph demonstrating that pluripotent (embryonic) stem cell powder as well as lethally irradiated embryonic stem cells increase the rate at which murine wounds close compared to fibroblast and media only controls.

[00014] FIG. 2 is a bar graph showing that ESC powder and lethally irradiated embryonic stem cells increase the rate at which wounds close compared to fibroblast and media only controls.

[00015] FIG. 3 is a bar graph demonstrating the reduction in skin roughness after treatment of humans with cream containing human lyophilized ESC powder.

[00016] FIG. 4 is a bar graph showing that treatment with cream containing human lyophilized ESC powder increases skin moisturization in humans.

[00017] FIG. 5 is a bar graph showing that treatment with cream containing human lyophilized ESC powder decreases skin wrinkles and fine lines in humans.

[00018] FIG. 6 is a bar graph showing that treatment with cream containing human lyophilized ESC powder decreases pore size in humans.

[00019] FIG. 7 is a bar graph demonstrating that treatment with lyophilized ESC powder as well as lethally irradiated ESC improves mouse cardiac contractility and

CLAIMS

What is claimed is:

1. A wound healing composition comprising lyophilized pluripotent stem cells dispersed in a pharmaceutically acceptable carrier and the lyophilized pluripotent stem cells included in a therapeutically effective amount.

2. The wound healing composition according to claim 1, wherein the pluripotent stem cells are embryonic stem cells.

3. The wound healing composition according to claim 1 or 2, wherein the lyophilized pluripotent stem cells do not include conditioned media.

4. The wound healing composition according to any one of claims 1 to 3, wherein the therapeutically effective amount comprises about 5×10^4 to about 1×10^8 lyophilized nonviable pluripotent stem cells or cell contents thereof.

5. Use of a wound healing composition comprising lyophilized pluripotent stem cells in the manufacture of a medicament~~A method~~ for promoting wound healing in a subject~~, the method comprising administering a wound healing composition comprising lyophilized pluripotent stem cells to a wound of a subject in need of wound healing~~.

6. The ~~method~~ use according to claim 5, further comprising preparing the wound healing composition by mixing lyophilized pluripotent stem cells with liquid media.

7. The use ~~method~~ according to claim 5 or 6, wherein the lyophilized pluripotent stem cells do not include conditioned media.

8. The use ~~method~~ according to any one of claims 5 to 7, wherein the wound healing composition is to be administered to the wound in an amount effective to accelerate wound closure as compared to a wound not treated with the wound healing composition.

9. The use ~~method~~ according to any one of claims 5 to 8, wherein the wound is an abrasion, cut, puncture, incision, laceration, ulcer, burn, or surgical or trauma wound.

10. A topical anti-aging composition for the skin comprising lyophilized pluripotent stem cells dispersed in a cosmetically acceptable carrier.

11. The topical anti-aging composition according to claim 10, wherein the lyophilized pluripotent stem cells do not include conditioned media.

12. The topical anti-aging composition of claim 10 or 11, wherein the composition is in the form of a cream, lotion, gel, salve, ointment, serum, or cosmetic.

13. The topical anti-aging composition of any one of claims 10 to 12, wherein the composition further comprises at least one active ingredient selected from the group consisting of skin penetrating agents, antioxidants, vitamins, provitamins, sunscreen, and combinations thereof.

14. The topical anti-aging composition according to any one of claims 10 to 13, wherein the anti-aging composition comprises 5×10^4 to about 1×10^8 lyophilized pluripotent stem cells per ounce of anti-aging composition.

15. A method for providing an anti-aging effect to the skin of a subject, the method comprising administering an anti-aging composition comprising lyophilized pluripotent stem cells in a pharmaceutically acceptable carrier to the skin of the subject.

16. The method according to claim 15, wherein the pluripotent stem cells are embryonic stem cells.

17. The method according to claim 15 or 16, wherein the subject is a human.

18. The method according to any one of claims 15 to 17, wherein the anti-aging composition is in a form selected from the group consisting of cream, lotion, gel, salve, ointment, serum, and cosmetic.

19. The method according to any one of claims 15 to 18, wherein the lyophilized pluripotent stem cells do not include conditioned media.

20. The method according to any one of claims 15 to 19, wherein the anti-aging composition comprises about 5×10^4 to about 1×10^8 lyophilized pluripotent stem cells per ounce of anti-aging composition.

21. Use of an effective amount of lyophilized human pluripotent stem cells or lethally irradiated human pluripotent stem cells in the manufacture of a medicament ~~A method~~ for regenerating cardiac muscle in a primate subject comprising ~~delivering~~ administration of an effective amount of lyophilized human pluripotent stem cells or lethally irradiated human pluripotent stem cells to a damaged or aged area of the primate subject's heart.

22. The ~~method~~ use according to claim 21, wherein the lyophilized pluripotent stem cells are human embryonic stem cells.

23. The ~~method~~ use according to claim 21 or 22, wherein the lyophilized pluripotent stem cells are to be administered ~~delivered~~ by injection to the damaged or aged area of the heart.

24. The ~~method~~ use according to any one of claims 21 to 23, wherein the pluripotent stem cells are to be administered ~~delivered~~ in a composition comprising a physiologically acceptable carrier or excipient.

25. The ~~method~~ use according to any one of claims 21 to 24, wherein the lyophilized pluripotent stem cells do not include conditioned media.

26. A method for promoting the *ex vivo* growth and expansion of human cell lines, the method comprising applying nonviable lethally irradiated or lyophilized pluripotent stem cells to human cells grown in culture.

27. The method according to claim 26, wherein the nonviable lethally irradiated or lyophilized pluripotent stem cells are applied in media or on a matrix or adherence scaffolding.

28. The method according to claim 26 or 27, wherein the human cells are selected from the group consisting of endothelial cells, epithelial cells, fibroblasts, smooth muscle cells, myocytes, keratinocytes, melanocytes, and combinations thereof.