

glands and hair -- which truly defines skin. This failure, in turn, leads to monolayer fragility, epithelial instability, barrier breakdown, and scar.

Alternatively, the more robust acellular matrices such as ALLODERM® from LifeCell Corporation, INTEGRA® from Integra LifeSciences Corporation and DERMATRIX® a product from Musculoskeletal Transplant Foundation, although excellent reconstructive options, lack those properly placed lineage specific stem cell populations which are necessary to develop functional native tissues.

The inventor herein has already written about the relatively recent recognition of LGR5 and LGR6 as markers of both intestinal and epidermal stem cells in mammals. In *Stimulation of the Follicular Bulge LGR5+ and LGR6+ Stem Cells with the Gut-Derived Human Alpha Defensin 5 Results in Decreased Bacterial Presence, Enhanced Wound Healing, and Hair Growth from Tissues Devoid of Adnexal Structures*, *Plast. Reconstr. Surg.* 132: 1159, 2013, Leucine-rich repeat-containing G-protein—coupled receptor (LGR) is a seven-pass transmembrane protein receptor with significant sequence and structural homology to the follicle-stimulating hormone, thyroid-stimulating hormone, and luteinizing hormone receptor families.

In that study, it was recognized that human alpha defensin 5 peptide significantly enhanced wound healing and reduced basal bacterial load compared with human beta defensin 1 and sulfadiazine. Human alpha defensin 5 was the only therapy to induce LGR stem cell migration into the wound bed. In addition, gene heat mapping showed significant mRNA up-regulation of key wound healing and Wnt pathway transcripts such as Wnt1 and Wisp1. So it was concluded that human alpha defensin 5 could be used for enhanced wound healing due to the observed increase of LGR stem cell migration into wound beds and associated bacterial reduction and hair production through the augmentation of key Wnt and wound healing transcripts. In short, this and other work led to the recognition of the potential for using LGR4+, LGR5+ and LGR6+ expressing epithelial stem cells in direct biomedical engineering soft tissue constructs.

Summary

The disclosure provides in a first example a minimally polarized micro-

aggregate multi-cellular composition including isolated living LGR expressing cells and a multi-dimensional support selected from the group consisting of scaffolding, collagen, matrix, particle, and fiber.

5 The disclosure provides a composition, comprising: (i) a multi-dimensional support selected from the group consisting of scaffolding, collagen, matrix, particle, and fiber; and (ii) living LGR-expressing epithelial stem cells; wherein said composition is a minimally polarized, micro-aggregate, multi-cellular composition.

10 In one aspect, the disclosure provides a composition, comprising: a minimally polarized functional unit comprising a dermal section, an epidermal section, and a follicular section, from a mammalian cutaneous tissue specimen, wherein the follicular section comprises living LGR-expressing stem cells and wherein a stem cell niche is maintained;

15 wherein said composition is capable of assembling functional polarized cutaneous tissue.

The disclosure provides in a further example to the previous example a minimally polarized micro-aggregate multi-cellular composition including isolated living LGR expressing cells and a multi-dimensional support selected from the group consisting of scaffolding, collagen, matrix, particle, and fiber where the LGR expressing cells are supplemented with growth factors and where the LGR expressing cells are selected from the group consisting of LGR4, LGR5 and LGR6.

20 The disclosure provides in a further example to any of the previous examples a minimally polarized micro-aggregate multi-cellular composition including isolated living LGR expressing cells and a multi-dimensional support selected from the group consisting of scaffolding, collagen, matrix, particle, and fiber where the LGR expressing cells are supplemented with migratory/recruiting analytes and the LGR expressing cells being selected from the group consisting of LGR4, LGR5 and LGR6.

30 The disclosure provides in a further example to any of the previous examples a minimally polarized micro-aggregate multi-cellular composition including isolated living LGR expressing cells and a multi-dimensional support selected from the group consisting of scaffolding, collagen, matrix, particle, and fiber where the LGR expressing cells are supplemented with LGR specific binding elements selected from the group consisting of ligand families, R-spondin, EDGF, PDGF, Wnt, VEGF, and antimicrobial peptides and where the LGR expressing cells are selected from the group consisting of LGR4, LGR5 and LGR6.

35 The disclosure provides in a further example to any of the previous examples a minimally polarized micro-aggregate multi-cellular composition including isolated living LGR expressing cells and a multi-dimensional support selected from the group consisting of scaffolding, collagen, matrix, particle, and fiber where the composition is used as a therapeutic construct for a select target consisting of a tissue region, wound, void, defect tissue, or blood for alteration of
45 either surrounding adjacent tissues.

The disclosure provides in a further example to any of the previous examples a minimally polarized micro-aggregate multi-cellular composition characterized by a isolated living LGR expressing cells transplanted to damaged tissue to accelerate healing thereof.

5 The disclosure provides in a further example to any of the previous examples a minimally polarized micro-aggregate multi-cellular composition for tissue system repair or restoration throughout the body comprising a support scaffolding with isolated LGR containing cells secured thereto.

10 The disclosure provides a tissue graft comprising a composition described herein for application to ectodermal, mesodermal or endodermal-derived tissues systems throughout a mammalian body.

15 The disclosure provides a method for obtaining a minimally polarized micro-aggregate multi-cellular composition characterized by the steps of growing and isolating living LGR expressing cells for transplantation to a select mammalian target tissue.

The disclosure provides a method of producing a minimally polarized micro-aggregate multi-cellular composition, the method comprising:

- 20 a) extracting at least one minimally polarized functional unit containing LGR-expressing cells from a mammalian tissue specimen;
- b) processing of hypodermis and subdermal fat cellular components of the mammalian tissue specimen to provide a mesenchymal-derived cellular population;
- 25 c) adding at least a portion of the mesenchymal-derived cellular population to the at least one minimally polarized functional unit to create at least one epithelial stem cell functional singularity unit;
- d) enriching the at least one epithelial stem cell functional singularity unit; and
- e) adding the at least one enriched epithelial stem cell functional singularity unit to a delivery substrate.

30 The disclosure provides in a further example to the foregoing method a method for obtaining a minimally polarized micro-aggregate multi-cellular composition characterized by the steps of growing and isolating living LGR expressing cells for transplantation to a select mammalian target tissue further characterized by the step of affixing the isolated living LGR expressing cells to a multi-dimensional support selected from the group consisting of scaffolding, collagen, matrix, particle, and fiber.

35 The disclosure is characterized in still another example by a method for obtaining a minimally polarized micro-aggregate multi-cellular composition characterized by the steps of growing and isolating living LGR expressing cells for transplantation to a select mammalian target tissue further characterized by the step of selecting the LGR expressing cells from the group consisting of LGR4, LGR5 and LGR6.

40 The disclosure provides in a further example to any of the previous method examples the step of applying the minimally polarized micro-aggregate multi-cellular to one of the group consisting of epithelial systems, glands, hair, nerves, bone, muscle, fat, tendons, blood vessels, fascia, ocular tissues and peptide secreting cellular elements employing delivery by a technique selected from the

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group consisting of application, transplantation, implantation, directed seeding, directed migration, directed tracking, in setting, laminating and/or injection of the cellular element generating, regenerating, enhancing and healing.

The disclosure provides in a further example to any of the previous method examples a method for obtaining a minimally polarized micro-aggregate multi-cellular composition characterized by the steps of growing and isolating living LGR expressing cells for transplantation to a select mammalian target tissue further characterized by the step of applying the minimally polarized micro-aggregate multi-cellular composition directly to a tissue *in vivo* for tissue restoration.

The disclosure provides in a further example to any of the previous method examples a method for obtaining a minimally polarized micro-aggregate multi-cellular composition characterized by the steps of growing and isolating living LGR expressing cells for transplantation to a select mammalian target tissue further characterized by the step indirectly applying the minimally polarized micro-aggregate multi-cellular composition via the blood stream for tissue restoration in a body.

The disclosure provides a method for producing a minimally polarized micro-aggregate multi-cellular composition characterized by the steps of:

- a) obtaining a tissue specimen;
- b) extracting minimally polarized functional units containing LGR expressing cells from the specimen;
- c) processing of hypodermis and subdermal fat cellular components from an appropriate source;
- d) adding the processed hypodermis and subdermal fat components to the extracted minimally polarized functional units to create epithelial stem cell singularity units;
- e) enriching the epithelial stem cell singularity units;
- f) adding the epithelial stem cell singularity units to a construct scaffold; and
- g) verifying the maintenance of minimum polarization of the obtained composition.

The disclosure provides in a further example to any of the previous

examples a media formulation used in obtaining minimally polarized micro-aggregate multi-cellular compositions using cell sustaining media composition for reducing the viability of micro-organisms during transport and processing of tissues, characterized by: a) a mixture of epithelial cells and keratinocytes; b) at least one agent selected from the group consisting of penicillin, streptomycin, and amphotericin B; and c) fibrinogen.

The disclosure provides in a further example to the previous example of a cell sustaining media composition for reducing the viability of microorganisms during transport and processing of tissues, characterized by: a) a mixture of epithelial cells and keratinocytes; b) at least one agent selected from the group consisting of penicillin, streptomycin, and amphotericin B; and c) fibrinogen, where the fibrinogen is human and where the agent includes both an antibiotic and an antimycotic for stabilizing human tissues. The disclosure provides a cell sustaining media composition for reducing the viability of microorganisms during transport and processing of tissues, characterized by a) a mixture of epithelial cells and keratinocytes, b) at least one agent selected from the group consisting of penicillin, streptomycin, and amphotericin B, and c) fibrinogen.

In the context of a first example of the disclosure, it is characterized by LGR expressing cells being applied to scaffolding matrix, and/or fiber to thereby establish micro-aggregate multi-cellular grafts for tissue engineering applications, cell therapy applications, regenerative medicine applications, medical/therapeutic applications the grafts being directly applied to tissue or blood for improvement and or alteration of epithelial systems throughout the body.

In one example, there is provided a composition produced by the disclosed methods.

In one aspect, there is provided a composition of the disclosure for use in therapy.

In another aspect, the disclosure provides use of a composition of the disclosure in the manufacture of a medicament for:

- i) the treatment of wounds, wherein the composition accelerates healing of the wounds; and/or
- ii) the treatment of tissue, wherein the composition repairs or restores tissue; and/or
- iii) treating any one of a tissue region, wound, void, defect tissue, or blood, wherein the composition is to be used as a therapeutic construct; and/or
- iv) a treatment to alter surrounding adjacent tissues, wherein the composition is to be used as a therapeutic construct.

In one example, there is provided a composition of the disclosure for use:

- i) in the treatment of wounds, wherein the composition accelerates healing of the wounds; and/or
- ii) in the treatment of tissue, wherein the composition repairs or restores tissue; and/or
- iii) as a therapeutic construct for treating any one of a tissue region, wound, void, defect tissue, or blood; and/or
- iv) as a therapeutic construct in a treatment to alter surrounding adjacent tissues.

A second example of the disclosure is characterized by LGR expressing cells being applied to scaffolding, matrix, and/or fiber with/or without additional enhancing factors or analytes before or after either being applied to tissue or blood for improvement and or alteration of *in vivo* epithelial systems.

A further example of the disclosure is characterized by LGR expressing cells altered by enhancing factors or analytes, being applied as targets within the body, tissue or blood for improvement and/or alteration of epithelial systems through local or distant migration throughout the body and/or to restore gland and hair growth.

A fourth example of the disclosure is characterized by transplanting LGR expressing cells from tissue, blood or culture for alteration of surrounding adjacent tissues or distant tissues such as but not inclusive of LGR expressing cells applied to scaffolding, matrix, and fiber before or after either

being applied to tissue or blood for improvement and or alteration of ectodermal, mesodermal or endodermal- derived tissues systems throughout the body.

5 A fifth example of the disclosure is characterized by directly applying LGR expressing cells to a delivery substrate vehicle selected from a group consisting of scaffolding, matrix, and fiber with/or without additional enhancing factors or analytes before or after either being applied to tissue or blood for improvement and or alteration of ectodermal, mesodermal or endodermal-
10 derived tissues systems throughout the body.

 Still another example of the disclosure is characterized by combining LGR expressing cells altered by enhancing factors or analytes, with a delivery support substrate as targets within the body, tissue or blood for improvement and or alteration of ectodermal, mesodermal or endodermal-derived tissues
15 systems throughout the body through local or distant migration throughout the body.

 A further example of the disclosure is characterized by adhering LGR expressing cells to a support substrate for the delivery, application, transplantation, implantation, directed seeding, directed migration, directed
20 tracking, in setting, laminating and/or injection of the cellular element generating, regenerating, enhancing and/or healing epithelial systems, glands, hair, nerves, bone, muscle, fat, tendons, blood vessels, fascia, ocular tissues and peptide secreting cellular elements.

 A final stated example of the disclosure is to generate LGR expressing stem cells as micro-aggregate multi-cellular functional units exhibiting minimal
25 polarization for transplantation and direct application to a target within a mammalian body, tissue or blood to enhance and accelerate tissue generating, regenerating, enhancing and/or healing.

 In most general terms, the disclosure herein contemplates the transplanting and/or delivery of isolated LGR expressing cells (Leucine-rich
30 repeat-containing G-protein coupled receptor) for the generation, regeneration, recruitment or enhancement of an epithelial system, hair, gland bone. The disclosure also contemplates application to both local/proximate and distant/remote tissue in clinical medicine, bioengineering and/or research
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I claim:

1. A composition, comprising:

a minimally polarized functional unit comprising a dermal section, an epidermal section, and a follicular section, from a mammalian cutaneous tissue specimen, wherein the follicular section comprises living LGR-expressing stem cells and wherein a stem cell niche is maintained;

wherein said composition is capable of assembling functional polarized cutaneous tissue.

2. The composition of claim 1, further comprising soluble growth factors.

3. The composition according to any one of claims 1 to 2, further comprising LGR-specific binding elements selected from R-spondin, an epidermal-derived growth factor (EDGF), a platelet-derived growth factor (PDGF), a Wnt protein, a vascular endothelial growth factor (VEGF), and an antimicrobial peptide.

4. The composition according to any one of claims 1 to 3, wherein the living LGR-expressing stem cells are selected from LGR4-expressing cells, LGR5-expressing cells and LGR6-expressing cells.

5. The composition according to any one of claims 1 to 4, further comprising a delivery substrate.

6. The composition according to claim 5, wherein the delivery substrate is selected from the group consisting of scaffolding, collagen, matrix, particle, and fiber.

7. A composition as defined in any one of claims 1 to 6 for use in therapy.

8. Use of a composition as defined in any one of claims 1 to 6 in the manufacture of a medicament for:

i) the treatment of wounds, wherein the composition accelerates healing of the wounds; and/or

- ii) the treatment of tissue, wherein the composition repairs or restores tissue;
and/or
- iii) treating any one of a tissue region, wound, void, defect tissue, or blood,
wherein the composition is to be used as a therapeutic construct; and/or
- 5 iv) a treatment to alter surrounding adjacent tissues, wherein the composition is
to be used as a therapeutic construct.