

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 DermaMatrix 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 embodiment 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.

In one aspect, there is provided a minimally polarized micro-aggregate multi-cellular composition, characterized by: a multi-dimensional support selected from the group consisting of scaffolding, collagen, matrix, particle, and fiber, and isolated living LGR expressing cells.

The disclosure provides in a further embodiment to the previous embodiment 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.

The disclosure provides in a further embodiment to any of the previous embodiments 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.

The disclosure provides in a further embodiment to any of the previous embodiments 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.

The disclosure provides in a further embodiment to any of the previous embodiments 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 either surrounding adjacent tissues.

The disclosure provides in a further embodiment to any of the previous embodiments 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 embodiment to any of the previous embodiments 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 In another aspect, there is provided a tissue graft comprising a composition described herein for application to ectodermal, mesodermal or endodermal-derived tissues systems throughout a mammalian body.

15 In another aspect, there is provided 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.

20 The disclosure provides in a further embodiment 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.

25 The disclosure is characterized in still another embodiment 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.

30 The disclosure provides in a further embodiment to any of the previous method embodiments 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

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.

5 The disclosure provides in a further embodiment to any of the previous method embodiments 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
10 restoration.

The disclosure provides in a further embodiment to any of the previous method embodiments 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
15 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.

In another aspect, there is provided a method for producing a minimally polarized micro-aggregate multi-cellular composition characterized by the steps
20 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
25 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;
- 30 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 embodiment to any of the previous

embodiments 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 embodiment to the previous embodiment 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. In one aspect, there is provided a 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.

In the context of a first embodiment 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.

A second embodiment 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 embodiment 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 embodiment 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.

A fifth embodiment 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-derived tissues systems throughout the body.

Still another embodiment 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 systems throughout the body through local or distant migration throughout the body.

A further embodiment 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 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 embodiment of the disclosure is to generate LGR expressing stem cells as micro-aggregate multi-cellular functional units exhibiting minimal 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 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

constructs using a delivery vehicle in the form of scaffolding, matrix or fiber with or without the supplementation of growth factors migratory/recruiting analytes or LGR specific binding elements such as but not limited to ligand families: R-spondin, EDGF, PDGF, Wnt, VEGF, antimicrobial peptides.

5 Use of the LGR epithelial stem cells, particularly in conjunction with a formed scaffolding substrate, provides full thickness wounds and or voids in epithelial systems with a stem cell enriched tissue substitute. Moreover, the addition of this minimally polarized functional cell unit (MPFU) to an epithelial system is enhances/improves the status that epithelium which includes the
10 growth, generation or regeneration of hair, glands, secreted anti-microbial peptides, growth factors and analytes generally required to maintain and promote the health and viability of the epithelium and local surrounding tissues elements.

 Recognizing that LGR4+, LGR5+ and LGR6+ stem cell and progenitor
15 cell proliferation kinetics remain high, especially when in contact with substrate scaffolding, complete epithelial turnover rates are typically less than 12 days (1cm inter-population distance spacing). This capacity to regenerate a sufficient tissue bi-layer and subsequent barrier function suggests a role for these cells as a type of evolving biologic dressing for complex full thickness
20 and multi- tissue wounds.

 Beyond a capacity to regenerate skin, muscle and bone quickly, the progenitors of the LGR4, LGR5 and LGR6 stem cells also have the ability to generate native anti-microbial peptides that not only reduce the basal level of microorganisms within the wound bed but also augment progenitor cells
25 amplification and differentiation, leading to a reduction in wound and peri-wound infections, faster wound closure, and hair follicle development.

 The disclosure herein describes the translational applicability of an LGR expressing epithelial stem cell -seeded scaffold in providing an immediate, deliverable and viable tissue barrier that is capable of maintaining a stem cell
30 colony focus with concomitant competent progeny. From these stem cell foci, progeny can undergo migratory proliferative-differentiation in order to stimulate epithelial tissue elements, healing and graft integration. It has been found that the LGR epithelial stem cells can be applied alone, with

scaffolding, soluble growth factors and/or additional cell lineage which promote the polarization of the scaffold bound populations as well as intrinsic tissue architecture required in epithelial healing and cellular regenerative efforts.

- 5 Broadly defined, the protocol of the disclosure involves: a) harvesting living human/mammalian tissue; b) processing the tissue element to generate a micro-aggregate multi-cellular functional units which contain LGR expressing cells; c) applying the LGR expressing cell micro-aggregate multi-cellular functional units to a delivery vehicle substrate selected from the group
- 10 consisting of scaffolding, matrix, particle, cell(s) and fiber to create a construct; d) optionally including selected additional enhancing factors; and e) applying the construct to tissues for generating, regenerating, enhancing and/or healing tissue systems including those related to ectodermal, mesodermal and/or endodermal origin tissues including but not limited to
- 15 skin, glands, hair, nerves, bone, muscle, fat, tendons, blood vessels, fascia, ocular tissues, bone marrow, lung, heart, nails, gastrointestinal tissues, oral tissues, teeth, taste buds, urogenital tissues, renal tissues, reproductive tissues, lymphatic tissues, immune system tissues/elements and such related appendages and protein cellular elements.
- 20 This disclosure contemplates the direct delivery of supported LGR expressing epithelial stem cells by application, transplantation, implantation, directed seeding, directed migration, directed tracking, in setting, laminating and/or injection of the cellular element to alter mammalian tissue(s) in therapeutics, devices, biologics, drugs and bio-engineering.

25 **Definitions**

- In this detailed description, references to "one embodiment", "an embodiment", or "in embodiments" mean that the feature being referred to is included in at least one embodiment of the invention. Moreover, separate references to "one embodiment", "an embodiment", or "embodiments" do not
- 30 necessarily refer to the same embodiment; however, neither are such embodiments mutually exclusive, unless so stated, and except as will be readily apparent to those skilled in the art. Thus, the invention can include any variety of combinations and/or integrations of the embodiments