

LAMINAR CONSTRUCT FOR TISSUE-ENGINEERED DERMAL EQUIVALENT

STATEMENT OF GOVERNMENT INTEREST

[0001] This invention was made with support under Grant Number W81XWH-08-C-0062 awarded by the US Army Medical Research and Materiel Command. The U.S. government has certain rights in the invention.

BACKGROUND

[0002] Skin grafts are often necessary for the treatment of severe, full-thickness burns, non-healing skin ulcers and other surgical operations where there is loss of skin or a need for skin coverage of soft tissue. The graft procedure involves placing a layer of healthy skin on the wound site. The graft serves to close the wound, protecting the underlying tissue to facilitate healing. Two main classifications of skin graft surgery, autograft and allograft, depend on the source of the donor tissue. In an autograft operation, the skin graft is harvested from a different location on the patient. In an allograft operation, the graft is harvested from an external source such as another donor (e.g., cadaver) or is prepared artificially (e.g., dermal equivalent).

[0003] Previous work done in the field of tissue engineering has produced skin grafts that may be used in surgery. Generally, the tissue graft may be produced by seeding a collagen matrix or other biocompatible material with the appropriate cells to create the desired culture. As the cells proliferate, the matrix degrades and is eventually replaced by a layer of healthy tissue. This layer of tissue or the matrix seeded with cells may be used in skin graft surgery.

[0004] Recent advances in tissue engineering based wound dressings have resulted in the emergence of a range of dermal, epidermal and even complete skin equivalents. Advances in cellular biology and knowledge in wound healing and growth factors have provided a wide variety of choices to attack the problem of the complex wound. Continued research and new developments have improved the level of care in the field of complex burn wound care and has resulted in the availability of epidermal, dermal and total skin substitutes.

[0005] For example, Epicel, a cultured epidermal autograft (CEA), is one of the early tissue engineered products. It is an epidermis cultured in vitro and not a true skin equivalent which may limit its range of potential uses. CEA requires several weeks to produce, has a low rate of graft take, is very fragile, is susceptible to infection, and is not suitable for use without a dermal layer. To address the above problems, composites consisting of dermal equivalents in combination with epidermal components were developed. A typical engineered skin substitute (ESS) is composed of an epidermal substitute of autologous keratinocytes, attached to a dermal analogue of collagen or collagen-glycosaminoglycan combination populated with autologous fibroblasts. Following in vitro culture prior to grafting, ESS demonstrates morphogenesis similar to native human skin.

[0006] However, current efforts to produce suitable dermal equivalents are complicated by the multiple layers and corresponding functions that must be present. For example, the external layer must be capable of closing the wound and providing protection to the underlying tissue. Internal layers must be conducive to the formation of blood vessels and circulation. The state-of-the-art techniques in tissue engi-

neered skin rely on the sequential culture of dermal fibroblasts and keratinocytes on a collagen matrix. The culture process relies on a relatively long culture time to increase cell numbers within the construct before a suitable dermal equivalent has been produced.

SUMMARY

[0007] The present disclosure generally relates to laminar constructs and more specifically, to laminar constructs for tissue-engineered dermal equivalents.

[0008] In one embodiment, the present disclosure provides a laminar construct comprising a hydrogel matrix comprising at least a first hydrogel layer and a second hydrogel layer, and a plurality of mesenchymal stem cells.

[0009] In another embodiment, the present disclosure provides a method of creating a laminar construct comprising: providing a hydrogel matrix comprising at least a first hydrogel layer and a second hydrogel layer, and introducing a plurality of mesenchymal stem cells to the hydrogel matrix.

[0010] In yet another embodiment, the present disclosure provides a method comprising preparing a dermal equivalent for an allograft operation for a patient, wherein the dermal equivalent comprises a hydrogel matrix comprising at least a first hydrogel layer and a second hydrogel layer, and a plurality of mesenchymal stem cells.

[0011] The features and advantages of the present invention will be readily apparent to those skilled in the art. While numerous changes may be made by those skilled in the art, such changes are within the spirit of the invention.

DRAWINGS

[0012] A more complete understanding of this disclosure may be acquired by referring to the following description taken in combination with the accompanying figures in which:

[0013] FIG. 1 illustrates the creation of a bilayer dermal construct, according to one embodiment.

[0014] FIG. 2 is a schematic of a layered construct according to one embodiment which could provide both the vascular as well as dermal fibroblast component for treatment of wounds.

[0015] FIG. 3 illustrates the creation of a laminar skin equivalent, according to one embodiment.

[0016] FIGS. 4A and 4B depict immunohistochemical staining against CD31 and vWF, respectively, of human MSCs embedded in PEGylated fibrin after 7 days. The images use nuclear counterstain with DAPI (20x). FIG. 4C depicts PCR showing that entrapped MSCs highly expressed VEGF and vWF (lane 2 and 5), expressed CD31 (lane 6), but did not express CD 34 (lane 4). FIG. 4D depicts a gel plug assay using 1 ml preformed gel plugs after a 7 day subcutaneous implant. The left image is fibrin gel only while the right is PEGylated fibrin. The arrow denotes blood vessel in the gel interior.

[0017] FIG. 5 depicts the differentiation of ASC into vascular like structures in PEGylated fibrin.

[0018] FIG. 6 depicts the proliferation timecourse of ASC in PEGylated fibrin.

[0019] FIGS. 7A-7L depict Confocal Z-stacked images of tube-like structures formed by ASC in PEGylated fibrin gel. ASC when seeded in PEGylated fibrin exhibit an endothelial phenotype expressing both von Willebrand factor (B) and CD31 (C). FIG. 7D shows the merged image of 7B and 7C