

be engineered to control cell differentiation in the absence of growth factor supplementation of culture media.

[0035] The population of stem cells may be sourced from multiple locations in the human body including, but not limited to, fat, bone marrow, blood and muscle. Mesenchymal stem cells derived from adipose tissues (ASC) differentiate into multiple phenotypes including adipose, muscle, bone, neuronal, endothelial, hepatocyte and epithelial-like cells. Adipose tissue is an attractive *in vivo* cellular source of autologous stem cells for regenerative therapies for several reasons. Adipose-derived stem cells may be easily isolated from the stromal vasculature of subcutaneous adipose tissue by liposuction with a minimally invasive procedure and the excised adipose contains 100 to 1000 times more pluripotent cells per cubic centimeter than bone marrow. In certain embodiments, the population of stem cells may be sourced from the patient or a donor. The stem cells may be harvested by any method or technique known in the art.

[0036] The laminar construct also comprises a hydrogel matrix. In some embodiments, the hydrogel matrix comprises two or more hydrogel layers. One possible hydrogel layer may comprise a material that encourages stem cell differentiation towards blood vessel cells. Examples of such hydrogel layers include, but are not limited to, hydrogels comprising fibrin, PEGylated fibrin, hyaluronic acid and soluble degradation products thereof. Hydrogels suitable for use in the present disclosure may also comprise matrix molecules along with soluble signals known to encourage blood vessel in-growth during inflammation and wound healing. PEGylated fibrin exhibits several unique features of both synthetic hydrogels and natural materials. First, the presence of PEG provides a highly hydrated (>90% water) moist environment for managing exudate. The presence of fibrin confers biodegradability to the material; however, PEGylated fibrin is potentially more stable *in vitro* than fibrin alone. Finally, the inherent biologic activity of fibrin stimulates tissue and blood vessel in-growth.

[0037] Additionally, a layer of the hydrogel matrix may comprise a material that encourages stem cell differentiation towards dermal fibroblasts. This type of material includes, but is not limited to, types I, II, III, IV and V collagen; adhesion proteins such as fibronectin, tenascin and vitronectin; glycosaminoglycans such as chondroitin sulfate, heparan sulfate and hyaluronic acid as well as provisional matrix proteins including fibrin-based materials or combinations thereof.

[0038] The hydrogel matrix may be created by any method or technique known in the art. For example, PEGylated fibrin may be created by modifying fibrinogen (Fgn) with the benzotriazole carbonate derivative of polyethylene glycol to create secondary crosslinking. Specifically, a derivatized polyethylene glycol (PEG) has end groups that may react with peptide side chains such as amine, hydroxyl, carboxyl or thiol functionalities. The derivatized PEG is typically a carbonate, N-hydroxysuccinimide ester, epoxide, or tresylate group. The reaction of derivatized PEG with proteins may be performed under relatively mild conditions at room or body temperature in aqueous solution at pH values typically between 6.0 and 8.0. Reaction times are dependent on the reactivity of the PEG end groups and may proceed for between 5 minutes to several hours. Potential amine-reactive PEG derivatives include, but are not limited to, benzotriazole carbonate PEG, succinimidyl methylbutanoate PEG, succinimidyl propionate PEG, nitrophenyl carbonate PEG, succinimidyl carbonate PEG, succinimidyl succinate PEG, succinimidyl glutarate PEG, and suc-

cinimidyl valerate PEG. In a preferred embodiment, an end-group hydrolysis rate of about 20 minutes is used.

[0039] Once sourced, the stem cells of the present disclosure may be combined with the hydrogel matrix in a variety of ways. In one embodiment, these stem cells may be sandwiched between the two or more hydrogel layers. In another embodiment, the stem cells may be seeded uniformly within the hydrogel layers prior to gelation of the matrix. The stem cells may be seeded from suspension or following culture on microcarrier beads, such as chitosan microspheres. In a preferred embodiment, the cells are seeded on microcarriers and "sandwiched" between two hydrogel layers as shown in FIGS. 1 and 2.

[0040] The present disclosure presents a number of advantages over existing techniques. For example, if the stem cells are sourced from an individual patient, the process results in an autograft with fewer immune complications. Simpler production results from inducing the cells to differentiate towards different cell types, including but not limited to vascular and dermal cells, via properties of the hydrogel matrix rather than the culture conditions. Additionally, the present disclosure provides for the formation of blood vessels within the structure which may increase the viability of the construct. One limitation, the lack of epidermal (keratinocyte) cell population, may be overcome through in-growth or via split-thickness grafts.

[0041] To facilitate a better understanding of the present invention, the following examples of certain aspects of some embodiments are given. In no way should the following examples be read to limit, or define, the entire scope of the invention.

EXAMPLE 1

[0042] From previous work, it has been demonstrated that MSCs by themselves cannot differentiate into cells expressing EC phenotype without induction by the appropriate cytokines. Endothelial cell tubes in Matrigel™ typically disappear within a few hours. However, in PEGylated fibrin gel, MSCs maintained vascular tube-like networks until the gel degraded, approximately 7-10 days later. Murine, porcine and rat MSCs may also be capable of forming tubes within the PEGylated fibrin gels. Controls of unreactive PEG mixed with fibrinogen demonstrate CD31 and vWF negative phenotype. In addition to the effects that can be realized from embedded MSCs in PEGylated fibrin gels, the gels alone are able to support capillary ingrowth in a subcutaneous implantation in Lewis rats. FIGS. 4A-4D illustrate the results of embedding human MSCs in PEGylated fibrin. FIG. 4D compares fibrin gels alone after a 7 day gel plug implantation relative to the PEGylated fibrin gels. The PEGylated fibrin clearly supports extensive capillary in-growth.

EXAMPLE 2

[0043] Having seen that PEGylated fibrin gel can induce robust tube-like differentiation of mesenchymal stem cells, it was further investigated whether adipose derived stem cells (ASC) could differentiate into a typical vascular network. Advantages of using ASC are that they can be easily isolated from the stromal vasculature of subcutaneous adipose tissue by liposuction with minimally invasive procedures and the excised adipose is enriched in progenitor cells relative to bone marrow. Recent studies provide evidence that infused ASC *in vivo* are nonimmunogenic even when used in immunocom-