VASCULAR TISSUE ENGINEERING

Inventors: Dan Gazit, Jerusalem (IL); Yossi Gafni, Ramat Gan (IL); Gadi Turgeman, Jerusalem (IL); Gadi Pelled, Rishon Leziyon (IL)

Correspondence Address:
Eitan, Pearl, Latzer & Cohen Zedek, LLP.
Suite 1001
10 Rockefeller Plaza
New York, NY 10020 (US)

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ABSTRACT

The invention relates to tubularized tissue, which in one embodiment is a vascular tissue made by seeding cells on the exterior surface of a scaffold and incubating the cells so as to form a tubular tissue with the scaffold inside. In another embodiment the invention relates to a hybrid tissue. In another embodiment, the invention relates to methods of using the same, for example, for enhancing blood vessel formation in a patient, for promoting angiogenesis and vasculogenesis, for replacing damaged blood vessel and for determining cellular or tissue function of an agent.
FIG. 2

FIG. 3A

FIG. 3B
VASCULAR TISSUE ENGINEERING

[0001] This application is a Continuation-in Part Application of PCT/IL02/00336, filed Apr. 30, 2002 which claims priority from provisional Application No. 60/287,003, filed Apr. 30, 2001, which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Blood vessels are the means by which oxygen and nutrients are supplied to living tissues and waste products removed from living tissue.

[0003] The need for re-vascularization is emphasized in diabetic patients. 143 million people suffer from Diabetes, worldwide, and their number is estimated to be more than doubled by the year 2025. In the U.S. alone, 200,000-400,000 diabetic patients develop foot ulcers due to poor blood flow to the extremities. These ulcers might turn into foot gangrene. From 1993 to 1995, about 67,000 amputations were performed each year among diabetic patients.

[0004] Vascular development occurs in two stages: a) an early stage of vasculogenesis, by which the primary capillary network is formed from mesoderm-derived precursors, hemangioblasts, through a process of differentiation and proliferation in situ within a previously avascular organ or tissue and b) a coalescence of these cells to form a primitive tubular network. The later stage has been termed angiogenesis, which refers to the formation of new capillary vessels from pre-existing micro-vessels by remodeling and maturation of the primary plexus. A balance between pro and anti-angiogenic molecules regulates this process. While vasculogenesis occurs primarily during early embryogenesis, angiogenesis in the adult occurs as a stage of every inflammatory process (1,2).

[0005] Angiogenesis is the process by which new blood vessels are formed (Folkman and Shing, J. Biol. Chem. 267 (16), 10931-10934 (1992). It is essential in reproduction, development and wound repair. However, inappropriate angiogenesis can have severe consequences. For example, it is only after many solid tumors are vascularized as a result of angiogenesis that the tumors begin to grow rapidly and metastasize. The angiogenesis process is believed to begin with the degradation of the basement membrane by proteases secreted from endothelial cells (EC) activated by mitogens such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). The cells migrate and proliferate, leading to the formation of solid endothelial cell sprouts into the stromal space, then, vascular loops are formed and capillary tubes develop with formation of tight junctions and deposition of new basement membrane.

[0006] There is a wide recognition that there is a great clinical need for a readily available, small or medium diameter vascular graft. These grafts are especially required in cardiology for atherosclerotic blood vessels replacement and ischemic heart treatment. Approximately 12.6 million people alive today have a history of heart attack, angina, or both. Arterial replacement is a common treatment for this disease. Autogenous vessels, particularly internal mammary arteries and saphenous veins, remain the “gold standard” for coronary grafting. However, 30% of the patients in need of arterial bypass do not have veins suitable for grafting due to diseased veins. In addition, significant morbidity, surgical costs and restenosis have been associated with the harvest of autologous vessels.

[0007] In addition, in the presence of an injury or a defect in other organs in the body, surgical approaches to correcting defects in the body, in general, involve the implantation of structures made of biocompatible, inert materials, that attempt to replace or substitute for the defective function. Non-biodegradable materials will result in permanent structures that remain in the body as a foreign object. Implants that are made of resorbable materials are suggested for use as temporary replacements where the object is to allow the healing process to replace the resorbed material. However, these approaches have met with limited success for the long-term correction of structures in the body.

[0008] Therefore, there is a need for developing tubular tissues, in order to transplant or replace narrow and/or thick vessels such as a blood vessel or tracts such as gastrointes- tinal tract and genitourinary tract, whereby the scaffold will be degraded after transplantation into the body.

SUMMARY OF THE INVENTION

[0009] The invention relates to cells which are grown on the exterior surface of a scaffold in a way permitting tubularized tissue with a degradable scaffold contained by methods of using the same for transplanting or replacing damaged tracts or vessels in the body and methods of using the same for diagnostic or screening purposes.

[0010] In one embodiment, the invention provides a tubularized tissue made according to the steps of: seeding a cell on the exterior surface of scaffold so as to obtain a scaffold encircled by cells; incubating said scaffold encircled by said cells so as to form a tubularized tissue; thereby obtaining a tubularized tissue.

[0011] In another embodiment, the invention provides a method of maturing cells into a tubularized tissue comprising the following steps: seeding a cell on the exterior surface of scaffold so as to obtain a scaffold encircled by cells; incubating said scaffold encircled by said cells so as to form a tubularized tissue; thereby obtaining a tubularized tissue.

[0012] In another embodiment, the invention provides a method of enhancing blood vessel formation in a patient in need thereof, comprising the following steps: selecting the patient in need thereof; isolating a cell from the patient; seeding a cell on the exterior surface of scaffold so as to obtain a scaffold encircled by cells; incubating said scaffold encircled by said cells so as to form a tubularized tissue; and re-administering said tubularized tissue to the patient in need thereof, thereby enhancing blood vessel formation in a patient in need thereof.

[0013] In another embodiment, the invention provides a hybrid tissue made according to the steps of: seeding a cell that will form a first tissue type on the exterior surface of a branched scaffold so as to obtain a scaffold encircled by cells with spaces in between; adding into said spaces in between, a cell that will form a second tissue type so as to form a hybrid tissue; and incubating so as to obtain a hybrid tissue.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] FIG. 1: b-End-2 Endothelial Cell Line Expressing GFP, In Vitro Characterization and In Vivo Detection. a) b-End-2 cells in 3D ECM culture, show endothelial cells (ECs) phenotype, forming tubular structures. (b) Retro-GFP transduction (MOI: 5) of b-End-2 ECs, yields 94% trans-
duction efficiency detected by FACS analysis. (c) b-End-2 ECs induce spontaneous formation of blood containing cavities (arrows) when injected s.c. in CD1 nude mouse ear, (d) Fluorescence microscopy of cultured transduced b-End-2 ECs, reveals GFP expression. (e) GFP positive cells (b-End-2) detected in vivo (s.c. transplanted, 70 days) by immunohistochemistry. (e-bottom) negative control.

[0015] FIG. 2: Filament-Like Polymeric Scaffold Product (polyester anhydrate) Based on Lactic, Glycolic and Rici-noleic Acids

[0016] FIG. 3: b-End-2 Endothelial Cells Cultured on Filament-like Polymeric Scaffold in Rotary Bioreactor (day 14). (a) Microphotograph; and Histology (b) H&E of endo-thelialized filament like polymeric scaffold indicate formation of primitive micro-capillary network structure in 14 days.

[0017] FIG. 4: b-End-2 Endothelial Cells Cultured on Filament-like Polymeric Scaffold Form a Primitive Micro-capillary Network Ex Vivo, and In Vivo (day 28) (a) Microphotograph of s.C. transplant, 2 weeks after transplantation; (b) Histology H&E, of transplanted: (c) b-End-2 ECs cultured on filament-like polymeric scaffold; (d) b-End-2 ECs cultured without polymeric scaffold; (d) naked filament-like polymeric scaffold. P: filament-like Polymeric scaffold; Arrows: b-End-2 ECs.

[0018] FIG. 5: b-End-2 ECs Cultured 7 days in Rotary Bioreactor on Filament-like Polymeric Scaffold, Transplanted S.C. into CD1 Nude Mouse for 56 days. (a) Microphotograph of s.c.transplant d.42, showing blood vessels (BV) in the implant; (b) Frozen sections GFP Fluorescent microscopy and (c) H&E Histochemistry, showing co-localized b-end-2 ECs adjacent to filament-like polymeric scaffold (arrows). (d) Histology H&E on paraffin sections, showing b-End-2 ECs (arrows) lining the filament-like polymeric scaffold (P).

[0019] FIG. 6: b-End-2 ECs Cultured 7 days in Rotary Bioreactor on Filament-like Polymeric Scaffold, Transplanted S.C. into CD1 Nude Mouse for 56 days. (a) Microphotograph of s.c.transplant d.56, showing blood vessels (BV) in the implant; (b) Histology H&E (c,d) and Reticulum Staining. Note: monolayer of b-End-2 ECs (arrows) lining the filament-like polymeric scaffold (P). Erythrocytes (asterisks) are evident in the lumen formed due to degradation of polymeric scaffold and basement membrane (BM) surrounding endothelial cells (d). (e) Reticulum staining of the host blood vessel basement membrane as a positive control, showing the appearance of basement membrane in mature blood vessel.

[0020] FIG. 7: MRI Analysis of Implant Vessel Functionality (VF):b-End-2 Endothelial Cells Cultured 60 days in Rotary Bioreactor, Transplanted S.C. into CD1 Nude Mouse for 5 days. Representative coronal gradient echo image of the implant. Increased implant vascularity is reflected by reduction of the mean signal intensity (dark area inside the implant). Note: vascularization (arrows), and the increased vessel functionality (VF) in the implant.

[0021] FIG. 8: b-End-2 endothelial cells cultured 7 days in bioreactor, transplanted S.C. into CD1 nude mouse for 28 days (H&E staining and anti CD31 [PECAM] paraffin sections).

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0022] The invention relates to cells which are grown on the exterior surface of a scaffold permitting a tubularized tissue with a scaffold contained by, methods of using the same for transplanting or replacing damaged tracts or vessels in the body and methods of using the same for diagnostic or screening purposes. In another embodiment, the invention can be used to generate a hybrid tissue grafts composed of at least two tissue types co-arranged in a functional architecture.

[0023] The invention relates to cells, which are grown so as to from a tubularized tissue analogous to tissue counterparts in vivo.

[0024] The term “tubularized tissue” refers hereinabove to a tissue which is in a form of a tube such as vessels, for example without being limited, blood vessels, or tracts such as for example genitourinary tract or gastrointestinal tract, tissues for hernia repair, tendons and ligaments.

[0025] The different biological structures described below have several features in common. They are all tubular structures primarily composed of layers of stromal tissue with an inner lining of epithelium (gastrointestinal and genitourinary) or endothelium (blood vessels). Their connective tissues also contain layers of smooth muscle with varying degrees of elastic fibers, both of which are especially prominent in arterial blood vessels. By including and sustaining these components in the tubular tissue according to the invention, the tissues they compose can attain the special structural and functional properties they require for proper physiological functioning in vivo. They can then serve as replacements for damaged or diseased tubular tissues in a living body.

[0026] Arteries—Arteries are tubes lined with a thin layer of endothelial cells and generally composed of three layers of connective tissue: the intima (which is not present in many muscular arteries, particularly smaller ones), media, and adventitia, in order from inside to outside.

[0027] The main cellular component of the inner two layers is an undifferentiated smooth muscle cell, which produces the extracellular protein elastin. The internal elastic lamina, which lies just interior to the media, is a homogenous layer of elastin. The abundance of elastin in their walls gives arteries the ability to stretch with every contraction of the heart. The intima and media also contain some fibroblasts, monocytes, and macrophages, as well as some collagen. The adventitia is composed of more ordinary connective tissue with both elastic and collagenic fibers. Collagen in this layer is important in preventing overstretching. While all the layers of the arterial wall are connective tissue, there is a compositional and functional difference between the adventitia and the inner two coats, the intima and the media. Consequently, it may be advantageous in accordance with the invention to grow these different layers in separate meshes. Whether the intima and media are grown in separate meshes, or combined in one, depends on how distinct these layers are in the particular artery into which the tubularized tissue is to be implanted.

[0028] For example, according to the invention fibroblasts can be isolated from the adventitia of a patient’s artery and used to inoculate a three-dimensional matrix, and grown to
sub-confluence. Cells can be isolated from tissue rich in elastin-producing undifferentiated smooth muscle cells, also containing some fibroblasts, from the intima and media of the same artery. Endothelial cells can be isolated from the same patient.

Veins—The layers of the connective tissue comprising the walls of veins are less well delineated than those of arteries, and contain much more collagen and less elastin. Consequently, a single three-dimensional culture can be grown, for example, from a single inoculum of cells. These cells consisting mostly of fibroblasts with some smooth muscle cells, can be isolated from the walls of a vein of the patient. When the appropriate degree of confluence is reached, endothelial cells, isolated from the same patient, for example, can be seeded on top of the stromal layer and grown to confluence.

The gastrointestinal tract comprises several different organs, but all have the same general histological scheme.

Mucous Membrane: The mucous membrane is the most interior layer of the gastrointestinal tract, and is composed of three sub-layers. The absorptive surfaces particularly are highly folded to increase the surface area. The lumen is lined with a thin layer of epithelium, which is surrounded by the lamina propria, a connective tissue which contains fibroblasts, some smooth muscle, capillaries, as well as collagenic, reticular, and some elastic fibers. Lymphocytes are also found here to protect against invasion, especially at absorptive surfaces where the epithelium is thin. The third sub-layer, the muscularis mucosa, consists of two thin layers of smooth muscle with varying amounts of elastic fibers. The smooth muscle fibers of the inner layer are arranged circularly, and the outer layer is arranged longitudinally.

Submucosa: This layer consists of loose connective tissue including elastic fibers as well as larger blood vessels and nerve fibers.

Muscularis Externa: This layer consists of two thick layers of smooth muscle, providing the motion, which advances material along through the gastrointestinal tract. The muscle fibers of the inner layer are arranged circularly, while in the outer layer they are longitudinal. An exception is the upper third of the esophagus, which contains striated muscle allowing for the voluntary contractions associated with swallowing.

Serosa (or Adventitia): This outermost layer consists of loose connective tissue, covered by squamous mesothelium where the tract is suspended freely.

Ureter—Like the gastrointestinal tract, the ureter also has a mucous membrane as its inner layer. Despite not having an absorptive surface, the interior surface of the ureter is highly folded to form a stellate conformation in cross-section. The epithelial lining, however, is four to five cells thick. The lamina propria, which lies beneath the epithelium, contains abundant collagen, some elastin, and occasional lymph nodules.

Surrounding the mucous membrane is a muscular coat, whose inner layer contains longitudinally arranged smooth muscle fibers, while those of the outer layer are circularly arranged. The outermost layer, the adventitia, consists of fibroelastic connective tissue.

Urethra—The urethra consists simply of a lamina propria, which is lined with epithelium and surrounded by two layers of smooth muscle fibers. In the inner layer, the fibers are arranged longitudinally, while in the outer layer they are circular. The connective tissue of the lamina propria is rich in elastic fibers and contains many venules. Since the urethra has only one stromal layer, a monolayer culture may suffice for its construction in vitro in accordance with the invention.

In one embodiment, the invention provides a tubularized tissue prepared according to the steps of: obtaining a cell; obtaining a scaffold; seeding said cell exterior to said scaffold so as to obtain a scaffold encircled by cells; growing said scaffold encircled by said cells so as to form a tubularized tissue with said scaffold contained by, so as to support said tubularized tissue; thereby obtaining a tubularized tissue. Upon administration of the tubularized tissue with the scaffold contained by, the scaffold is slowly degraded so as to enable the formation of the lumen inside the tubular tissue.

The cells of the present invention are supported by a scaffold, which in one embodiment is in a form of a tube.

In another embodiment, the scaffold can be in different diameters, according to the use. The following are examples to the various diameters of different elements in the vascular systems:

<table>
<thead>
<tr>
<th>Diameter</th>
<th>mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elastic artery</td>
<td>&gt;1 cm</td>
</tr>
<tr>
<td>Muscular artery (large)</td>
<td>2-10 mm</td>
</tr>
<tr>
<td>Muscular artery (small)</td>
<td>0.1-2 mm</td>
</tr>
<tr>
<td>Arteriole</td>
<td>10-100 um</td>
</tr>
<tr>
<td>Capillary</td>
<td>4-10 um</td>
</tr>
<tr>
<td>Post capillary venule</td>
<td>10-50 um</td>
</tr>
<tr>
<td>Muscular venule</td>
<td>50-100 um</td>
</tr>
<tr>
<td>Small vein</td>
<td>0.3-1 mm</td>
</tr>
<tr>
<td>Medium vein</td>
<td>1-10 mm</td>
</tr>
<tr>
<td>Large vein</td>
<td>&gt;1 cm</td>
</tr>
</tbody>
</table>

The dimensions of a red blood cell range from 6 to 10 um (diameter) x 2.6 um. The diameters can vary from 5 um to 300 um, for the formation of a narrow blood vessel or a thick blood vessel, respectively, wherein the artery diameter is 10 um ->1 cm, the diameter of a vein is 10 um ->1 cm and the capillary diameter is in the range of 4-10 um. In another embodiment the invention provides a method for the formation or repair of a lymphatic vessel. In another embodiment, the invention provides a method for the formation of small vessels without blocking the lumen.

In another embodiment, the invention provides a method for the formation of capillary bed by obtaining a cell; obtaining a scaffold; seeding said cell exterior to said scaffold so as to obtain a scaffold encircled by cells; growing said scaffold encircled by said cells so as to form a capillary bed with said scaffold contained by, so as to support said capillary bed; thereby obtaining a capillary bed. Upon administration of the capillary bed tissue with the scaffold contained by, the scaffold is slowly degraded so as to enable the formation of the lumen inside the capillary bed.

In another embodiment, the invention provides a method for the formation or repair of a bile duct and in
another embodiment the invention provides a method for the formation or repair of a pancreatic duct (main and accessory).

[0044] In another embodiment, the tubularized tissue of the invention may be the large intestine. In this embodiment, the diameter of the scaffold should be between 4-8 mm. In another embodiment, the tubularized tissue may be the small intestine. The diameter of the scaffold for this embodiment is 1.5-3.5 cm.

[0045] In one embodiment, the tubularized tissue is a monolayer tubular tissue. For the formation of a capillary for example, a monolayer tubular tissue is required, made of endothelial cells.

[0046] In another embodiment the tubularized tissue is a multi-layer tubularized tissue comprising more than one type of cells. The term “multi-layer” refers hereinabove to more than one layer and is use. For obtaining such a multi-layer tubularized tissue it is required either to seed more than one type of cells on the scaffold or in another embodiment to grow the first layer till confluence and than to add another type of cell on top of the first layer. For a capillary formation, for example, a first cell layer of endothelial cells is required, whereas the second layer comprises pericytes. In the case of a GI tract the inner layer will be a epithelial cell and the outer layer will be smooth muscle cells arranged in to layers of circular and longitudinal orientations. The same for urinary, bile and lymph vessels. In accordance with the invention, stromal cells, such as fibroblasts, are inoculated and grown on a exterior to a scaffold, wherein the scaffold is contained by. The scaffold may be configured into the shape of the connective structure desired. Stromal cells may also include other cells found in loose connective tissue such as smooth muscle cells, endothelial cells, macrophages/monocytes, adipocytes, pericytes, reticular cells found in bone marrow stroma, chondrocytes, etc.

[0047] In another embodiment, the tubularized tissue is a vascularized tissue. The invention enables the formation of blood capillary, artery or vein either as a single vessel, or several vessels or a capillary network. For the capillary network, the scaffold has to be structured with a number of branches so as to enable a network, which is then transplanted into the body. For the preparation of a capillary blood vessel, a monolayer comprises endothelial cells is required. For the preparation of a capillary, a first layer of endothelial cells is required with at least one layer of pericytes on top of the endothelial cells. In another embodiment, it is possible to transplant a monolayer tubularized tissue and the pericytes from the body will than attached to said monolayer tubularized tissue upon its introduction to the body, whereas the first layer is served as an adherent Layer.

[0048] In another embodiment, the newly formed tissue can be a hybrid tissue. The term “hybrid tissue” is referred herein under to a tissue, which is composed of cells which are differentiated to form at least two different tissues. An example for an hybrid tissue is, without being limited, vacuolated-bone, vacuolated-liver and the like. The hybrid tissue can grow in one embodiment on top of a scaffold. According to this embodiment at least one layer first cell type, for example without limitation, a cell that will be differentiated into a vascular tissue, will grow on the top of the scaffold and at least another cell type will grow on top of the first cell type. The other cell type can be without limitation a bone or a liver. In another embodiment, the scaffold is a branched scaffold. The first cell type, grown on the branched scaffold will create a network of cells, with spaces in between, wherein the other cell type will grow said spaces.

[0049] For obtaining the three dimensional tubularized tissue the cells are seeded on the exterior surface of the scaffold and are incubated in a bioreactor. Other methods for obtaining three-dimensional tissue are static tissue culture dish, tissue culture dish placed on a shaker, a tube placed on a shaker and a spinner flask.

[0050] The cells of the invention can be any cell derived from a blood vessel (artery, vein or capillary) or a tract (the genitourinary tract or the gastrointestinal tract) the body.

[0051] In another embodiment the cell used in the invention is a mesenchymal stem cell, mesodermal progenitor cell, endothelial precursor cell or neonatal dermal micro vascular endothelial cells (foreskin). In another embodiment, at least for the first layer, or for a tissue composed of a monolayer of cells, the cell is capable of differentiating into an endothelial cell.

[0052] For the GI tract the inner cells are not endothelial cells, the cells are mainly epithelial cells.

[0053] In another embodiment, the cell is isolated from peripheral blood. In one embodiment the cell is obtained from the subject in need and than is re-administered to the subject after being grown into a tubular tissue. In another embodiment, the invention provided a possibility of a universal donor, i.e. a bank of tubular tissues at various sized and diameters for a universal use. This can be done for example with cells, which are known as non-immunogenic such as bone marrow/peripheral blood MSC, and the neonatal dermal micro vascular endothelial cells (foreskin) cell.

[0054] To obtain the EC progenitors from peripheral blood about 5 ml to about 500 ml of blood is taken from the patient. In another embodiment about 50 ml to about 200 ml of blood is taken.

[0055] EC progenitors can be expanded in vivo by administration of recruitment growth factors, e.g., GM-CSF and IL-3, to the patient prior to removing the progenitor cells.

[0056] In another embodiment, the cell of the invention can be engineered in one embodiment to express a therapeutic agent and/or in another embodiment to express macromolecules necessary for cell growth, morphogenesis, differentiation, and tissue building can also be added to the biopolymer molecules or to the biopolymer fibrils to further promote cell in growth and tissue development and organization of the cells.

[0057] The term “macromolecules necessary for cell growth, morphogenesis, differentiation, and tissue building” refers to those molecules, e.g., macromolecules such as proteins, which participate in the development of tissue. Such molecules contain biological, physiological, and structural information for development or regeneration of the tissue structure and function. Examples of these macromolecules include, but are not limited to, growth factors, extracellular matrix proteins, proteoglycans, glycosaminoglycans and polysaccharides. Alternatively, the scaffold can include
extracellular matrix macromolecules in particulate form or extracellular matrix molecules deposited by cells or viable cells.

The term “growth factors” is art recognized and is intended to include, but is not limited to, one or more of platelet derived growth factors (PDGF), e.g., PDGF AA, PDGF BB; insulin-like growth factors (IGF), e.g., IGF-I, IGF-II; fibroblast growth factors (FGF), e.g., acidic FGF, basic FGF, beta-endothelial cell growth factor, FGF 4, FGF 5, FGF 6, FGF 7, FGF 8, and FGF 9; transforming growth factors (TGF), e.g., TGF-β1, TGF-β2, TGF-β3, TGF-β5; bone morphogenetic proteins (BMP), e.g., BMP 1, BMP 2, BMP 3, BMP 4; vascular endothelial growth factors (VEGF), e.g., VEGF, placenta growth factor; epidermal growth factors (EGF), e.g., EGF, amphiregulin, betacellulin, heparin binding EGF; interleukins, e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14; colony stimulating factors (CSF), e.g., CSF-G, CSF-GM, CSF-M; nerve growth factor (NGF); stem cell factor; hepatocyte growth factor, and ciliary neurotrophic factor. The term encompasses presently unknown growth factors that may be discovered in the future, since their characterization as a growth factor will be readily determinable by persons skilled in the art.

The therapeutic agent can be selected for example without being limited anti-infective, a hormone, an antiseptic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

For anti-neoplastic therapies, for example, the cells can be transfected with or coupled to cytotoxic agents, cytokines or co-stimulatory molecules to stimulate an immune reaction, other anti-tumor drugs or angiogenesis inhibitors. For treatment of regional ischemia, angiogenesis could be amplified by prior transfection of cells to achieve constitutive expression of angiogenic cytokines and/or selected matrix proteins. In addition, the cells may be labeled, e.g., radio-labeled, administered to a patient and used in the detection of ischemic tissue or vascular injury.

The cells can also be used to deliver genes to enhance the ability of the immune system to fight a particular disease or tumor. For example, the cells can be used to deliver one or more cytokines (e.g., IL-2) to boost the immune system and/or one or more antigens.

These cells may also be used to selectively administer drugs, such as an antiangiogenesis compound such as 3-O-chloroacetethyl carbamoyl fumagillin (TNP-470). Preferably the drug would be incorporated into the cell in a vehicle such as a liposome, a timed released capsule, etc. The cells would then selectively home in on a site of active angiogenesis such as a rapidly growing tumor where the compound would be released. By this method, one can reduce undesired side effects at other locations.

In another embodiment, the invention provides a method of recovering and remodeling or supporting damaged vessels. The tubularized tissue formed by the method of the invention can be used as a support for the damaged tissue, wherein the cells may comprise in one embodiment nucleic acid sequences that encodes to a protein which is active in degradation of without being limited a plaque, LDL.

The cells used for obtaining the tubularized tissue of the invention may also be modified ex vivo. For example without being limited the cells can be engineered inhibit or enhance angiogenesis. This can be accomplished, for example, by introducing DNA encoding angiogenesis inhibiting agents to the cells, using for example the gene transfer techniques mentioned herein. Angiogenesis inhibiting agents include, for example, proteins such as thrombospondin (Dameron et al., Science 265:1582-1584 (1994)), angiostatin (O’Reilly et al., Cell 79:315-328 (1994), IFN-alpha (Folkman, J. Nature Med. 1:27-31 (1995)), transforming growth factor beta, tumor necrosis factor alpha, human platelet factor 4 (PF4); substances which suppress cell migration, such as proteinase inhibitors which inhibit proteases which may be necessary for penetration of the basement membrane, in particular, tissue inhibitors of metalloproteinase TIMP-1 and TIMP-2; and other proteins such as proline which has demonstrated angioiastatic properties, decoy receptors, drugs such as analogues of the angioinhibin fumagillin, e.g., TNP-470 (Ingber et al., Nature 348:555-557, (1990), antibodies or antisense nucleic acid against angiogenic cytokines such as VEGF. Alternatively, the cells may be coupled to such angiogenesis inhibiting agent.

If the angiogenesis is associated with neoplastic growth the EC progenitor cell may also be transfected with nucleic acid encoding, or coupled to, anti-tumor agents or agents that enhance the immune system. Such agents include, for example, TNF, cytokines such as interleukin (IL) (e.g., IL-2, IL-4, IL-10, IL-12, interferons (IFN) (e.g., IFN-gamma) and co-stimulatory factor (e.g., B7). Preferably, one would use a multivalent vector to deliver, for example, both TNF and IL-2 simultaneously.

The nucleic acids are introduced into the cells by any method, which will result in the uptake and expression of the nucleic acid by the cells. These can include vectors, liposomes, naked DNA, adjuvant-assisted DNA, catheters, gene gun, etc.

Vectors include chemical conjugates such as described in WO 93/04701, which has targeting moiety (e.g. a ligand to a cellular surface receptor), and a nucleic acid binding moiety (e.g. polylysine), viral vector (e.g. a DNA or RNA viral vector), fusion proteins such as described in PCT/US 95/02140 (WO 95/22618) which is a fusion protein containing a target moiety (e.g. an antibody specific for a target cell) and a nucleic acid binding moiety (e.g. a protamine), plasmids, phage, etc. The vectors can be chromosomal, non-chromosomal or synthetic.


[0069] Pox viral vectors introduce the gene into the cells cytoplasm. Avipox vector viruses result in only a short term expression of the nucleic acid. Adenovirus vectors, adeno-associated virus vectors and herpes simplex virus (HSV) vectors are preferred for introducing the nucleic acid into neural cells. The adeno-associated virus vector results in a shorter term expression (about 2 months) than adeno-associated virus (about 4 months), which in turn is shorter than HSV vectors. The particular vector chosen will depend upon the target cell and the condition being treated. The introduction can be by standard techniques, e.g. infection, transfection, transduction or transformation. Examples of modes of gene transfer include e.g., naked DNA, CaPO4 sub precipitation, DEAE dextran, elecetrooration, proteoplast fusion, lipofection, cell microinjection, viral vectors and use of the “gene gun”.

[0070] To simplify the manipulation and handling of the nucleic acid encoding the protein, the nucleic acid is preferably inserted into a cassette where it is preferably linked to a promoter. The promoter must be capable of driving expression of the protein in cells of the desired target tissue. The selection of appropriate promoters can readily be accomplished. Preferably, one would use a high expression promoter. An example of a suitable promoter is the 763-base-pair cytoplasmagovirus (CMV) promoter. The Rous sarcoma virus (RSV) (Davis, et al., Hum Gene Ther 4:151 (1993)) and MMT promoters may also be used. Certain proteins can express using their native promoter. Other elements that can enhance expression can also be included such as an enhancer or a system that results in high levels of expression such as a tat gene and tar element. This cassette can then be inserted into a vector, e.g., a plasmid vector such as pUC118, pBR322, or other known plasmid vectors, that includes, for example, an E. coli origin of replication. See, Sambrook, et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory press, (1989). The plasmid vector may also include a selectable marker such as the beta-lactamase gene for ampicillin resistance, provided that the marker polypeptide does not adversely effect the metabolism of the organism being treated. The cassette can also be bound to a nucleic acid binding moiety in a synthetic delivery system, such as the system disclosed in WO 95/22618.

[0071] If desired, the pre selected compound, e.g. a nucleic acid such as DNA may also be used with a micro delivery vehicle such as cationic liposomes and adenoviral vectors. For a review of the procedures for liposome preparation, targeting and delivery of contents, see Mamino and Gould-Fogerite, BioTechniques, 6:682 (1988). See also, Felgner and Holm, Bethesda Res. Lab. Focus, 11(2):21 (1989) and Maurer, R. A., Bethesda Res. Lab. Focus, 11(2):25 (1989).


[0073] The effective dose of the nucleic acid will be a function of the particular expressed protein, the target tissue, the patient and his or her clinical condition. Effective amount of DNA are between about 1 and 4000 mug, more preferably about 1000 and 2000, most preferably between about 2000 and 4000.

[0074] Other delivery techniques can include 1) the electroporation technique and 2) the Adeno Associated Virus (AAV)

[0075] Alternatively, the cells for the tubularized tissue may be used to inhibit angiogenesis and/or neoplastic growth by delivering to the site of angiogenesis a cytotoxic moiety coupled to the cell. The cytotoxic moiety may be a cytotoxic drug or an enzymatically active toxin of bacterial, fungal or plant origin, or an enzymatically active polypeptide chain or fragment (“A chain”) of such a toxin. Enzymatically active toxins and fragments thereof are preferred and are exemplified by diptheria toxin A fragment, non-binding active fragments of diptheria toxin, exotoxin A (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, maclecin A chain, alphasarcin, certain Aloceries foxti proteins, certain Dianthin proteins, Phytolecom acarna proteins (PAP, PAP1 and PAP-S), Momordica charantia inhibitor, curcin, eritin, Saponaria officinalis inhibitor, gelokin, melagustin, restrictocin, phenomycin, and enomycin, Ricin A chain, Pseudomonas aeruginosa exotoxin A and PAP are preferred. Conjugates of the cells and such cytotoxic moieties may be made using a variety of coupling agents. Examples of such reagents are N-succinimidyl-3-(2-pyridyllythio) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters such as dimethyl adipimidate HCl, active esters such as disuccinimidyl subrate, aldehydes such as glutaradehyde, bis-azido compounds such as bis(p-diazoniumbenzoyl)-ethylene diamine, disocyanates such as tolylene 2,6-disocyanate, and bis-active fluorine compounds such as 1,5-difluoro-2,4-dinitrobenzene.

[0076] In another embodiment the scaffold is a filamenteous scaffold:

[0077] The generation of a filamenteous scaffold according to the present invention takes into account the following considerations: (i) Filaments must remain intact during cell adherence and growth thereupon, but at the same time must rapidly erode within 20-30 days thereafter to obtain a functional blood vessel with a continuous lumen which supports blood flow. (ii) Efficient adherence of cells to the filamenteous scaffold followed by cell proliferation around the filamentous scaffold to form a continuous and uniform cell layer. Preferably, the filamenteous scaffold of the present invention is a solid scaffold capable of supporting cell growth thereupon. Such a scaffold can mimic a blood vessel lumen and form a blood vessel having even small capillary diameter of 5-50 microns. (iii) The filamentous scaffold must be strong and flexible enough to allow formation of flexible thin filaments having a diameter ranging between 5-500 microns.

[0078] Various types of biodegradable polymers meet these criteria, including, for example, thin cellulose fibers. Cellulose fibers can be modified by oxidation with, for example, periodate in aqueous medium, rendering the fibers more susceptible to hydrolytic degradation (biodegradation). The degree of oxidation determines the strength of the fiber and its degradation profile. These oxidized fibers can be further modified by impregnation with a biodegradable
polymer such as poly(lactide-glycolide) so as to be more susceptible to biological degradation. Optionally, fiber aldehyde groups can be reacted with amino containing hydrophilic or hydrophobic safe molecules including amino acids.

Alternatively, polymers and copolymers based on hydroxy alkyl acid polyesters, polyphosphazene, poly(carbonates) and poly(phosphate esters), can also be used. Polymers based on lactide and glycolide acids are better suited for use with the filamentous scaffold of the present invention since it has been previously shown that such materials are capable of supporting cell growth and can be safely transplanted in humans (Shand and Heggie 2000).

These polymers can also be modified to meet the requirements described above. For example, block and random copolymers of lactide acid and glycolic acid having a molecular weight greater than 10,000, can be spun into thin filaments. To prevent accelerated erosion when exposed to an environment enriched with degradative enzymes, copolymers including 30 to 70% lactic acid may be used to delay degradation to a few weeks post transplantation.

Increased flexibility of the filaments can be obtained by adding plasticizing agents such as, for example, tributyl citrate, tributyl citrate acetate, phospholipids, oleate esters and the like to the polymer blend or by incorporating agents, such as, for example, ricinoleic acid into the polymer chain.

The mechanical properties of the filamentous scaffold must be maximized when supporting formation of a blood vessel such as an artery, which has to exhibit resistance to high blood pressure. This can be achieved by various cross-linking methods, interlinking the filamentous polymers, described herein above.

Preferably, cross-linking is achieved via stereo-complexation which utilizes stereoisomers, such as the stereoisomers of copolymer of lactide acid, as linker molecules for stereo-cross-linking the polymer backbone (see Example 1 of the Examples section for further detail).

The continuous scaffold is designed so as to support tissue formation in and around the filamentous scaffold. The continuous scaffold can be composed of any of the polymers described hereinabove and/or any other polymers suitable for supporting structural tissue colonization/proliferation.

For example, polysaccharide such as cross-linked dextran, arabinogalactan, chitosan, polyactide-glycolide, alginites, pullulan, hyaluronic acid, and the like, and proteins such as gelatine, collagen, fibrin, fibrinogen, albumin, and the like, can be is used to form a continuous (cross-linked) scaffold with a predetermined pore size. Alternatively, synthetic polymers such as, lactide and glycolide foams can also be used.

Preferably, the continuous scaffold component is generated under mild conditions. This enables to form the continuous scaffold component over a filamentous scaffold component, which is already seeded with cells. Compositions based on viscous hyaluronic acid solutions, alginites cross-linked by calcium salts and proteins cross-linked by denaturation or non-harmful molecules can be used to form the continuous scaffold component over an already seeded filamentous scaffold component.

Alternatively, stereo-complexed hydrophilic polymers including, natural polysaccharides, proteins, and polymers based on ethylene and propylene glycol and mixtures thereof can also be used.

In another embodiment biodegradable polymeric scaffold is non-toxic and bioabsorbable when introduced into a living organism and any degradation products of the biopolymer should also be non-toxic to the organism.

The source of molecules, which form biopolymers, include mammals such as pigs, e.g., near-term fetal pigs, sheep, fetal sheep, cows, and fetal cows. Other sources of the molecules, which can form biopolymers, include both land and marine vertebrates and invertebrates. In one embodiment, the collagen can be obtained from skins of near-term, domestic porcine fetuses, which are harvested intact, enclosed in their amniotic membranes. Collagen or combinations of collagen types can be used in the mat and mat compositions described herein. Examples of collagen or combinations of collagen types include collagen type I, collagen type II, collagen type III, collagen type IV, collagen type V, collagen type VI, collagen type VII, collagen type VIII, collagen type IX, collagen type X, collagen type XI, collagen type XII, collagen type XIII, and collagen type XIV. A preferred combination of collagen types includes collagen type I, collagen type III, and collagen type IV. Preferred mammalian tissues from which to extract the molecules, which can form biopolymer, include entire mammalian fetuses, e.g., porcine fetuses, dermis, tendon, muscle and connective tissue. As a source of collagen, fetal tissues are advantageous because the collagen in the fetal tissues is not as heavily cross-linked as in adult tissues. Thus, when the collagen is extracted using acid extraction, a greater percentage of intact collagen molecules is obtained from fetal tissues in comparison to adult tissues. Fetal tissues also include various molecular factors, which are present in normal tissue at different stages of animal development.

In another embodiment, the scaffold of the invention can support new vascular tissue or tubularized tissue formation by serving as a template to the development of a tissue in the direction outlined by the scaffold.

In another embodiment, the tissue is formed ex vivo and the transplantation into the human body is conducted the entodelial cells will sprout form new blood vessels which direction will be paved by the filament that will slowly degrade.

In another embodiment, the scaffold is a biodegradable scaffold and is degraded upon being introduced to the body, so as to permits a lumen in which nutrients, excretions and gases can pass through. The scaffold is degradable upon exposure to predetermined environmental conditions such as the conditions exist in the body, namely, hydrolytic enzymes, presence of a low pH, namely pH with is less than 5 and reducing conditions.

In another embodiment, the scaffold comprises thermo-regulated polymers that change mechanical properties as a result to changes in temperature thus allowing degradation (liquified state) to be controlled.

In another embodiment of the invention, the scaffold further comprising macromolecules necessary for cell
growth, morphogenesis, differentiation, and tissue building can also be added to the biopolymer molecules or to the biopolymer fibrils to further promote cell in growth and tissue development and organization of the cells. The term “macromolecules necessary for cell growth, morphogenesis, differentiation, and tissue building” refers to those molecules, e.g., macromolecules such as proteins, which participate in the development of tissue. Such molecules contain biological, physiological, and structural information for development or regeneration of the tissue structure and function. Examples of these macromolecules include, but are not limited to, growth factors, extracellular matrix proteins, proteoglycans, glycosaminoglycans and polysaccharides. Alternatively, the biopolymer mats, matrix composites, and matrix compositions of the invention can include extracellular matrix macromolecules in particulate form or extracellular matrix molecules deposited by cells or viable cells.

[0096] The term “growth factors” is art recognized and is intended to include, but is not limited to, one or more of fibroblast growth factors (FGF), e.g., PDGF, PDGF AA, PDGF BB; insulin-like growth factors (IGF), e.g., IGF-I, IGF-II; fibroblast growth factors (FGF), e.g., acidic FGF, basic FGF, beta-endothelial cell growth factor, FGF 4, FGF 5, FGF 6, FGF 7, FGF 8, and FGF 9; transforming growth factors (TGF), e.g., TGF-P3, TGF-betas 1, 2, TGF-beta 2, TGF-beta 3, TGF-beta 5; bone morphogenetic proteins (BMP), e.g., BMP 1, BMP 2, BMP 3, BMP 4; vascular endothelial growth factors (VEGF), e.g., VEGF, placenta growth factor; epidermal growth factors (EGF), e.g., EGF, amphiregulin, betacellulin, heparin binding EGF; interleukins, e.g., IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14; colony stimulating factors (CSF), e.g., CSF-G, CSF-GM, CSF-M; nerve growth factor (NGF); stem cell factor; hepatocyte growth factor, and ciliary neurotrophic factor. The term encompasses presently unknown growth factors that may be discovered in the future, since their characterization, as a growth factor will be readily determinable by persons skilled in the art.

[0097] In another embodiment the scaffold comprises a therapeutic agent. Those can be selected for example without being limited anti-infective, a hormone, an analgesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

[0098] In another embodiment, the invention provides a method of enhancing blood vessel formation in a patient in need thereof, comprising the following steps: selecting the patient in need thereof; isolating a cell from the patient; obtaining a scaffold; seeding said cell exterior to said scaffold so as to obtain a scaffold encircled by cells; growing the scaffold encircled by the cells so as to form a tubularized tissue with the scaffold contained by, so as to support the tubularized tissue; thereby obtaining a tubularized tissue; and re-administering the tubularized tissue to the patient in need thereof, thereby enhancing blood vessel formation in a patient in need thereof.

[0099] As was shown in the Examples section, Endothelial b-Enl-2 cells were cultured on polymeric filamentous scaffolds for 7 and 14 days in a rotary bioreactor. It was observed that endothelial monolayer had coated all the surface area of the polymeric filament after 7 days of culturing (FIG. 4g). The coated cells had the morphology of flat endothelium lining the surface of the scaffold. The vascular tissue formed ex vivo was transplanted subcutaneous into nude mice. 28, 42, 56, and 70 days after transplantation mice were sacrificed and samples were analysed microscopically. Analysis revealed the beginning of maturation of the primitive vessels allowing the flow of red blood cells inside the forming lumen of the vessels as the polymer degrades (FIG. 7h). Moreover, Reticulum stainings have shown the formation of basement membrane, which is typical for blood vessels, surrounding the engineered vessels (FIGS. 7c & 7d).

[0100] In another embodiment the cell is provided from a different i.e. universal donor, or in another embodiment, the cell is derived from a different animal and is than seeded exterior to the scaffold so as to obtain a scaffold encircled by cells; the next steps are growing the scaffold encircled by the cells so as to form a tubularized tissue with the scaffold contained by, so as to support the tubularized tissue; thereby obtaining a tubularized tissue; and administering the tubularized tissue to the patient in need thereof, thereby enhancing blood vessel formation in a patient in need thereof.

[0101] Post-natal neovascularization is believed to result exclusively from the proliferation, migration, and remodeling of fully differentiated endothelial cells (ECs) derived from pre-existing native blood vessels. This adult paradigm, referred to as angiogenesis, contrasts with vasculogenesis, the term applied to Formation of embryonic blood vessels from EC progenitors. In contrast to angiogenesis, vasculogenesis typically begins as a cluster formation, or blood island, comprised of EC progenitors (e.g. angioblasts) at the periphery and hematopoietic stem cells (HSCs) at the center (3). In addition to this intimate and predictable spatial association, such EC progenitors and HSCs share certain common antigenic determinants, including flk-1, tie-2, and CD-34. Consequently, these progenitor cells have been interpreted to derive from a common hypothetical precursor, the hemangioblast.

[0102] In accordance with the invention, the tubularized tissue can be used in a method for regulating angiogenesis, i.e., enhancing or inhibiting blood vessel formation, in a selected patient. For example, the tubularized tissue can be used to enhance angiogenesis or to deliver an angiogenesis modulator, e.g. anti- or pro-angiogenic agents, respectively to sites of pathologic or utilitarian angiogenesis.

[0103] If it is desirable to further enhance angiogenesis, endothelial cell mitogens may also be administered to the patient in conjunction with, or subsequent to, the administration of the EC progenitor cells. Endothelial cell mitogens can be administered directly, e.g., intra-alterally, intramuscularly, or intravenously, or nucleic acid encoding the mitogen may be used. See, Balfour, et al., supra (bFGF); Fu, et al., Circulation, 88:206-215 (1993) (FGF); Yamagawara, Miwa, et al., supra (bFGF); Ferrara, et al., Biochem. Phys. Res. Commun., 161:851-855 (1989) (VEGF); (Takashita, et al., Circulation, 90:228-234 (1994)).

[0104] Additionally, in another embodiment, tubularized tissue can be used to induce re-endothelialization of an
injured blood vessel, and thus reduce restenosis by indirectly inhibiting smooth muscle cell proliferation.

[0105] In another embodiment the tubularized tissue can be used to regulate vasculogenesis. For example, the tubularized tissue can be used to enhance vasculogenesis or to deliver a vasculogenesis modulator, e.g. anti- or pro-vasculogenic agents, respectively to sites of pathologic or utilitarian vasculogenesis.

[0106] In another embodiment the tubularized tissue can be used in a method of treating hypoxia, avascular necrosis, foot ulcer or gangrene result from diabetes, atherosclerosis, stroke, wound, fracture of bone, comprising administering to the patient host an effective amount of the tubularized tissue of the invention so as to increasing the vascularity of a tissue thereby treating hypoxia, avascular necrosis, foot ulcer or gangrene result from diabetes, atherosclerosis, stroke, wound, fracture of bone.

[0107] In one embodiment, the present invention may be used to enhance blood vessel formation in ischemic tissue, i.e., a tissue having a deficiency in blood as the result of an ischemic disease. Such tissues can include, for example, muscle, brain, kidney and lung. Ischemic diseases include, for example, cerebrovascular ischemia, renal ischemia, pulmonary ischemia, limb ischemia, ischemic cardiomyopathy and myocardial ischemia.

[0108] The tubularized tissue can be used for transplantation into organ/tissue in need for increased vascularity such as regenerating tissue, tissue suffering from avascular necrosis or hypoxia. For example ischemic cardiac tissue can be improved by transplantation of engineered capillary network. In another example such a “patch” of engineered capillaries can be implanted into a regenerating wound site as skin wounds or bone fractures etc.

[0109] In another embodiment single vessels can be used for bypassing occluded vessels as in cardiac coronary artery bypasses. In this particular situation, the present concept present a specific advantage by “protecting” the lumen of the vessel by the polymer and therefore preventing collapse of the bypass engineered vessel.

[0110] One of the major problems of bioartificial implants is the need for a well-vascularized network, which can provide the engineered tissue with continuous supply of oxygen and nutrients at the transplantation site. To date all attempts to address this issue have resulted in poor vascularization of engrafted tissues.

[0111] The tubularised tissue can be used also for the promoting the integration of grafts, tissues and organs transplantation. Adding the vascular tissue to the graft will promote angiogenesis and vascularization of the graft and can increase the integration. For example transplantation of a “patch” of engineered capillary networks followed by skin tissue grafts can promote the successful integration of the skin grafts. In another embodiment, a network of blood vessels can be formed in order to ex-vivo growing other cell culture or tissues which will be supplied with gases and nutrients from the blood network.

[0112] Promotion of vascularization is achieved in this concept by the integration of the engineered tissue in vivo with the host vascular tissue. This process is achieved by angiogenesis invoked from the engrafted vascular tissue and from the host vascular tissue enabling both integration of the engineered vascular tissue to the host vascular system and promoting increased collateral vessels and anastomoses (see diagram). In order to effectively influence this process to promote angiogenesis, integration and stabilisation of new vessels growth factor delivery can be used. Time dependent delivery of VEGF (Angiogenesis) and Ang-1 (stabilisation) by means of protein or gene delivery can be used for this purpose (see diagram).

[0113] In another embodiment, a nucleic acid encoding the EC mitogen can be administered to a blood vessel perfusing the ischemic tissue or to a site of vascular injury via a catheter, for example, a hydrogel catheter, as described by U.S. Ser. No. 08/675,523, the disclosure of which is herein incorporated by reference. The nucleic acid also can be delivered by injection directly into the ischemic tissue using the method described in U.S. Ser. No. 08/545,998.

[0114] As used herein the term “endothelial cell mitogen” means any protein, polypeptide, mitogen or portion that is capable of, directly or indirectly, inducing endothelial cell growth. Such proteins include, for example, acidic and basic fibroblast growth factors (aFGF and bFGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), transforming growth factor alpha and beta. (TGF-alpha. and TGF-beta.) platelet-derived endothelial growth factor (PD-ECGF), platelet-derived growth factor (PDGF), tumor necrosis factor a (TNF-alpha), hepatocyte growth factor (HGF), insulin like growth factor (IGF), erythropoietin, colony stimulating factor (CSF), macrophage-CSF (M-CSF), granulocyte/macrophage CSF (GM-CSF) and nitric oxidesynthes (NOS). See, Klagsbrun, et al., Annu. Rev. Physiol., 53:217-239 (1991); Folkman, et al., J. Biol., Chem., 267:10931-10934 (1992) and Symes, et al., Current Opinion in Lipidology, 5:305-312 (1994). Mitogens or fragments of a nitrogen may be used as long as they induce or promote EC cell growth.

[0115] In one embodiment the endothelial cell mitogen contains a secretory signal sequence that facilitates secretion of the protein. Proteins having native signal sequences, e.g., VEGF can be used. Proteins that do not have native signal sequences, e.g., bFGF, can be modified to contain such sequences using routine genetic manipulation techniques. See, Nabel et al., Nature, 362:844 (1993).

[0116] The nucleotide sequence of numerous endothelial cell mitogens, are readily available through a number of computer databases, for example, GenBank, EMBI, and Swiss-Prot. Using this information, a DNA segment encoding the desired may be chemically synthesized or, alternatively, such a DNA segment may be obtained using routine procedures in the art, e.g., PCR amplification. A DNA encoding VEGF is disclosed in U.S. Pat. No. 5,332,671, the disclosure of which is herein incorporated by reference.

[0117] In certain situations, it may be desirable to use nucleic acids encoding two or more different proteins in order optimize the therapeutic outcome. For example, DNA encoding two proteins, e.g., VEGF and bFGF, call be used, and provides an improvement over the use of bFGF alone. In another embodiment an angiogenic factor can be combined with other genes or their encoded gene products to enhance the activity of targeted cells, while simultaneously inducing angiogenesis, including, for example, nitric oxide synthase, L-arginine, fibrocin, urokinase, plasminogen activator and heparin.
0118 The term "effective amount" means a sufficient amount of compound, e.g., nucleic acid delivered to produce an adequate level of the endothelial cell mitogen, i.e., levels capable of inducing endothelial cell growth and/or inducing angiogenesis. Thus, the important aspect is the level of mitogen expressed. Accordingly, one can use multiple transcripts or one can have the gene under the control of a promoter that will result in high levels of expression. In an alternative embodiment, the gene would be under the control of a factor that results in extremely high levels of expression, e.g., tat and the corresponding tar element.

0119 The tubularized tissue obtained in the invention can be served for determining cellular or tissue function of an agent such as its effect on processes such as proliferation, apoptosis, toxicity angiogenesis, vasculogenesis or differentiation comprising the following steps: obtaining the tubularized tissue described in the invention; contacting the tubularized tissue with an agent, so as to obtain a tubularized tissue contacted with an agent; comparing cellular or tissue function of the tubularized tissue contacted with an agent, to a tubularized tissue which was not contacted with the agent, thereby determining cellular or tissue function of the agent. The agent can be a protein, a hormone, a RNA molecule, a DNA molecule, an antibody an antagonist and the like.

0120 In another embodiment, the tubularized tissue can be served for screening candidate genes which are involved in cellular or tissue function such as proliferation, apoptosis, toxicity angiogenesis, vasculogenesis or differentiation, the method comprising the steps of: obtaining the tubularized tissue; obtaining mRNA from the tubularized tissue and the cell; synthesizing cDNA from them RNA; amplifying the cDNA-hybrid, so as to obtain an amplified product; detecting the amplified product; and comparing the amplified products from the tubularized tissue and the cell, thereby identifying candidate nucleic acid sequence which is involved in the cellular or tissue function.

0121 This Ex vivo system can be utilized for evaluating the effects of physical/biomechanical effects on tissue formation and maturation ex vivo and to replace the controversial animal models.

EXAMPLES

Experimental Procedures

0122 Tissue Culture:

0123 cells were cultured in low glucose, low bicarbonate DMEM medium (Biet Haemek)+10% fetal calf serum (Biet Haemek), the environmental conditions were of 5% CO2 and 370 C.

0124 Seeding of Filament-Like Polymeric Scaffold with Vascular Endothelial Cells:

0125 The filamentous scaffold designed for the vascular tissue was seeded with to endothelial cells, b-End-2 (Jia, G Q et. al., 1999 and Hahme M et. al., 1993), genetically engineered to express the reporter gene, green fluorescent protein (GFP), 2 million cells per scaffold (calculated cells per cm2 polymeric scaffold surface) in a stirring flask for 24 hrs (Zipori 1989, Jia et al. 1999, Vunjak-Novakovic et al. 2000). Culture medium contains DMBM supplemented with 10% FCS, high glucose.

0126 Complete Endothelialization of the Filament-Like Polymeric Scaffold:

0127 In order to achieve a spatially uniform distribution of the endothelial cells, to promote rapid, homogenous tissue development, and a confluent cell monolayer covering the scaffold's surface, the seeded scaffold was cultured in a rotary bioreactor for 7 days.

0128 Ex vivo Analysis of Endothelialized Filament-Like Polymeric Scaffold Cultured in a Rotary Bioreactor:

0129 Following 7 days of culturing, scaffold was harvested and fixed using three different methods each for a different analysis:

0130 Protein Localization:

0131 PECAM (CD31): Serial sections (5-6 μm) of PFA-fixed, paraffin-embedded tissue were deparaffinized in xylene and rehydrated through graded ethanol. Following antigen retrieval (incubation in citrate buffer and heating) and inhibition of endogenous peroxidase (incubation in 1% hydrogen peroxide in TBS) immunostaining was performed using mouse monoclonal antibody directed against PECAM which was used as an endothelial cell marker (Pharmingen, San Diego, Calif.). Sections were incubated with avidin-biotin-peroxidase complex. Antibody binding was detected by DAB (Shevski D et al. 1992).

0132 GFP: polyclonal rabbit anti-GFP (Clontech Laboratories, Palo Alto, Calif.) was used for GFP immunostaining as a gene reporter marker for b-End-2 endothelial cell line (FIG. 1e). A second method for GFP localization study was used included PFA-fixed OCT embedded tissue using fluorescence microscopy (FIGS. 6b & 6c). Reticulum staining (Wildler-silver nitrate stain) specific histochemical staining for basement membrane.

0133 In vivo Transplantation:

0134 Mice were anesthetized using 30 ml of ketamine-Xylazine mixture (85%, 15% respectively) injected intra peritonial per mouse. Skin was shaved in the low back area of the mouse, and wandered with chlorhexidine solution 0.5%. Skin was cut with scissors, and subcutaneous pocket was formed in the sacral area. Endothelialized filament-like polymeric scaffold was placed in the subcutaneous pocket, covered with the skin, which was sutured with clips. The skin and the clips were swabbed with chlorhexidine.

0135 In vivo Analysis of Engrafted Vascular Tissue:

0136 MRI Analysis of Blood Vessel Density, Functionality and Maturation:

0137 MRI experiments were performed on a horizontal 4.7 T Bruker Biospec spectrometer, using an actively RF decoupled surface coil, 2 cm in diameter, and a birdcage transmission coil (Abramovitch et al., 1999). Mice were anesthetized (75 mg/kg Ketamine+3 mg/kg Xylazine, i.p.) and placed supine with the tumor located at the center of the surface coil. Transplanted primitive micro-capsillary network tissue’s vascularity was reflected by reduction of the mean signal intensity inside the tumor in gradient echo T2* weighted images (TR-repetition time=100 ms; TE-echo time=10 ms). Data is reported as the apparent vessel density [AVD=−ln(s(0))/s(0))], in which s(0) is the mean intensity in the transplanted primitive micro-capsillary network tissue, and s(0) is the mean intensity of a distant muscle, as
described (Abramovitch et al., 1998). Functionality and maturation of the neovasculature were determined from gradient echo images acquired during the inhalation of air—CO2 (95% air and 5% CO2) and oxygen—CO2 (95% oxygen and 5% CO2; carbogen), as described (Abramovitch et al., 1999). Four images were acquired at each gas mixture (65 s/image; slice thickness=0.6 mm; TR=100 ms; TE=10 ms; spectral width=25,000 Hz; field of view=3 cm; 256x128 pixels; in plane resolution=110 μm; four averages). Other experimental details were as reported previously (Abramovitch et al., 1999).

[0138] Data Analysis. MRI data were analyzed on a PC computer using IDL software (Research Systems Inc.). Vascular function (VF) was derived from images acquired during inhalation of carbogen (95% oxygen 5% CO2) and air—CO2 (95% air 5% CO2) as follows:

\[
VF = \frac{\ln(\text{carbogen}/\text{air-CO})}{TE + C_{599}}
\]

Where TE is the echo time, Y is the fraction of oxy-hemoglobin, b is the volume fraction of blood and CMRI=599 s=1 at 4.7 T. This parameter measures the capacity of oxygen delivery from the lungs to each pixel in the image (Abramovitch et al., 1998). Vasodilatation (VD) was derived from air and air-CO2 images, as described (Abramovitch et al., 1999; Abramovitch et al., 1998). Positive VD corresponds to increased signal intensity by hypercapnia, due to elevated blood oxygenation and/or increased blood flow (Abramovitch et al., 1998). Data are presented in color maps overlayed on the original baseline image for absolute values of VD and VF>0.005 (Abramovitch et al., 1999).

Experimental Results

[0139] Ex-Vivo Formation of Primitive Vascular Tissue:

[0140] Endothelial b-end-2 cells were cultured on polymeric filamentous scaffolds for 7 and 14 days in a rotary bioreactor. It was observed that endothelial monolayer has 5 coated all the surface area of the polymeric filament after 7 days of culturing (FIG. 4a). The coated cells had the morphology of flat endothelium lining the surface of the scaffold.

[0142] Primitive Vascular Tissue Formed ex vivo, Mature and Function in vivo:

[0143] Primitive capillary network formed ex vivo was transplanted subcutaneous into nude mice. 28, 42, 56, and 70 days after transplantation mice were sacrificed and samples were analysed microscopically. Analysis revealed the beginning of maturation of the primitive vessels allowing the flow of red blood cells inside the forming lumens of the vessels as the polymer degrades (FIG. 7b). Moreover, Reticulum staining has showed the formation of basement membrane, which is typical for blood vessels, surrounding the engineered vessels (FIGS. 7c & 7d). Analysis of the cells revealed that the engineered vessels were surrounded by GFP positive cells (FIGS. 1e, 6b & 6c) having the morphology of endothelial cells and expressing PECAM (FIG. 10). This observation indicates that engrafted endothelial cells differentiated into mature endothelium and that the engineered vessels were undergoing maturation in vivo and acquired functionality.

What is claimed is:

1. A tubularized tissue made according to the steps of:
   - seeding a cell on the exterior surface of scaffold so as to obtain a scaffold encircled by said cells;
   - incubating said scaffold encircled by said cells so as to form a tubularized tissue; thereby obtaining a tubularized tissue.

2. The tubularized tissue of claim 1, whereby in the step of obtaining a tubularized tissue, the tubularized tissue is a monolayer tubular tissue.

3. The tubularized tissue of claim 1, whereby in the step of obtaining a tubularized tissue, the tubularized tissue is a multi-layer tubular tissue.

4. The tubularized tissue of claim 1, whereby in the step of obtaining a tubularized tissue, the tubularized tissue is a multi-layer tubular tissue comprising more than one type of cells.

5. The tubularized tissue of claim 1, whereby in the step of obtaining a tubularized tissue, said tubularized tissue is a vascularized tissue.

6. The tubularized tissue of claim 1, whereby in the step of obtaining a tubularized tissue, said tubularized tissue is a vascularized tissue.

7. The tubularized tissue of claim 1, whereby in the step of obtaining a tubularized tissue, said scaffold is in the form of a string.

8. The tubularized tissue of claim 1, whereby in the step of obtaining a tubularized tissue, said scaffold is in the form of a string.

9. The tubularized tissue of claim 2, wherein the polymeric biodegradable scaffold comprises cross-linked dextran, arabino-galactan, chitosan, polyaclactide-glycolide, alginates, pullulan, gelatin, collagen, fibrin, fibrinogen or albumin.

10. The tubularized tissue of claim 1, whereby in the step of obtaining a scaffold, the diameter of said scaffold is 4-500 um.

11. The tubularized tissue of claim 1, whereby in the step of obtaining a scaffold, said scaffold is degradable upon exposure to predetermined environmental conditions.

12. The tubularized tissue of claim 1, whereby in the step of obtaining a scaffold, said scaffold is a non-biodegradable scaffold.

13. The tubularized tissue of claim 1, whereby in the step of obtaining a scaffold, said scaffold comprises macromolecules necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof.

14. The tubularized tissue of claim 13, wherein the macromolecules necessary for cell growth is a bone morphogenetic protein, a bone morphogenetic-like protein, an epidermal growth factor, a fibroblast growth factor, a platelet derived growth factor, an insulin like growth factor, a transforming growth factor, a vascular endothelial growth factor, Ang1, PIGF and combinations thereof.

15. The tubularized tissue of claim 1, whereby in the step of obtaining a scaffold, said scaffold contains also a therapeutic agent.

16. The tubularized tissue of claim 15, wherein said therapeutic agent is an anti-infective, a hormone, an anal-
gesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

17. The tubularized tissue of claim 15, wherein said therapeutic agent is a nucleic acid sequence which encodes an anti-infective, a hormone, an analgesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

18. The tubularized tissue of claim 1, wherein said cell is engineered to express a therapeutic agent and/or a macromolecule necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof.

19. The tubularized tissue of claim 18, wherein said therapeutic agent is anti-infective, a hormone, an analgesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

20. The tubularized tissue of claim 18, wherein said macromolecule necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof is a bone morphogenic protein, a bone morphogenic-like protein, an epidermal growth factor, a fibroblast growth factor, a platelet derived growth factor, an insulin like growth factor, a transforming growth factor, a vascular endothelial growth factor, AngI, PIGF and combinations thereof.

21. The tubularized tissue of claim 1, whereby in the step of obtaining a cell, said cell is a mesenchymal stem cell, mesodermal progenitor cell, endothelial precursor cell or neonatal dermal micro vascular endothelial cells.

22. The tubularized tissue of claim 1, whereby in the step of obtaining a cell, said cell is capable of differentiating into an endothelial cell.

23. The tubularized tissue of claim 1, whereby in the step of obtaining a cell, said cell is isolated from peripheral blood.

24. The tubularized tissue of claim 1, whereby in the step of obtaining a cell, said cell is isolated from a mammalian artery, vein or capillary.

25. The tubularized tissue of claim 1, whereby in the step of obtaining a cell said cell is genetically engineered to express a reporter gene.

26. The tubularized tissue of claim 25, wherein the reporter gene is a fluorescent protein, Luciferase or b-gal.

27. The tubularized tissue of claim 1, wherein said step of growing scaffold encircled by cells so as to form a tubularized tissue is by the use of a bioreactor.

28. The tubularized tissue of claim 1, wherein the tubularized tissue is a single vessel, composition of several vessel or a capillary network.

29. A method of maturing cells into a tubularized tissue comprising the following steps:

- seeding a cell on the exterior surface of scaffold so as to obtain a scaffold encircled by cells;
- incubating said scaffold encircled by said cells so as to grow the tissue; whereby maturing cells into a tubularized tissue.

30. The method of claim 29, wherein said tubularized tissue is a monolayer tubular tissue.

31. The method of claim 29, wherein said tubularized tissue is a multilayer tubular tissue.

32. The method of claim 29, wherein said tubularized tissue is a multi-layer tubularized tissue comprising more than one type of cells.

33. The method of claim 29, wherein said tubularized tissue is a vascular tissue.

34. The method of claim 29, wherein said scaffold is in the form of a string.

35. The method of claim 29, wherein said scaffold is a biodegradable scaffold.

36. The method of claim 35, wherein said biodegradable scaffold is a polymeric biodegradable scaffold.

37. The method of claim 36, wherein said polymeric biodegradable scaffold comprising cross-linked dextran, arabinogalactan, chitosan, polylactide-glycolide, algamines, pullulan, gelatin, collagen, fibrin, fibrinogen or alubmin.

38. The method of claim 29, wherein the diameter of said scaffold is 4-500 μm.

39. The method of claim 29, wherein said scaffold is degradable upon exposure to predetermined environmental conditions.

40. The method of claim 29, wherein said scaffold is a non-biodegradable scaffold.

41. The method of claim 29, wherein said scaffold comprises macromolecules necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof.

42. The method of claim 41, wherein said macromolecules necessary for cell growth is a bone morphogenic protein, a bone morphogenic-like protein, an epidermal growth factor, a fibroblast growth factor, a platelet derived growth factor, an insulin like growth factor, a transforming growth factor, a vascular endothelial growth factor, AngI, PIGF and combinations thereof.

43. The method of claim 29, wherein said scaffold contains also a therapeutic agent.

44. The method of claim 43, wherein said therapeutic agent is an anti-infective, a hormone, an analgesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

45. The method of claim 29, wherein said scaffold is engineered to express a therapeutic agent and/or a macromolecule necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof.

46. The method of claim 45, wherein said therapeutic agent is anti-infective, a hormone, an analgesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

47. The method of claim 45, wherein said macromolecule necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof is a bone morphogenic protein, a bone morphogenic-like protein, an epidermal growth factor, a fibroblast growth factor, a platelet derived growth factor, an insulin like growth factor, a transforming growth factor, a vascular endothelial growth factor, AngI, PIGF and combinations thereof.

48. The method of claim 29, wherein said cell is a mesenchymal stem cell, mesodermal progenitor cell, endothelial precursor cell or neonatal dermal micro vascular endothelial cells.

49. The method of claim 29, wherein said cell is capable of differentiating into an endothelial cell.
50. The method of claim 29, wherein said cell is isolated from peripheral blood.
51. The method of claim 29, wherein said cell is isolated from mammalian artery, vein or capillary.
52. The method of claim 29, wherein said cell is genetically engineered to express a reporter gene.
53. The method of claim 52, wherein the reporter gene is a fluorescent protein, Luciferase or b-gal.
54. The method of claim 29, wherein said step of growing scaffold encircled by cells so as to form a tubularized tissue is by the use of a bioreactor.
55. The method of claim 29, wherein tubularized tissue is a single vessel, composition of several vessels or a capillary network.
56. A method of enhancing blood vessel formation in a patient in need thereof, comprising the following steps:
   selecting the patient in need thereof;
   isolating a cell from the patient;
   seeding a cell on the exterior surface of scaffold so as to obtain a scaffold encircled by cells;
   incubating said scaffold encircled by said cells so as to form a tubularized tissue; and readministering said tubularized tissue to the patient in need thereof, thereby enhancing blood vessel formation in a patient in need thereof.
57. The method of claim 56, wherein said tubularized tissue is a monolayer tubular tissue.
58. The method of claim 56, wherein said tubularized tissue is a multi-layer tubular tissue.
59. The method of claim 56, wherein said tubularized tissue is a multi-layer tubularized tissue comprising more than one type of cells.
60. The method of claim 56, wherein said tubularized tissue is a vascular tissue.
61. The method of claim 56, wherein said scaffold is in the form of a string.
62. The method of claim 56, wherein said scaffold is a biodegradable scaffold.
63. The method of claim 62, wherein said biodegradable scaffold is a polymeric biodegradable scaffold.
64. The method of claim 63, wherein said polymeric biodegradable scaffold comprising cross-linked dextran, arabinosilactan, chitosan, polyacide-glycolide, alginites, pullulan, gelatine, collagen, fibrin, fibrinogen or albumin.
65. The method of claim 56, wherein the diameter of said scaffold is 4-10000 um.
66. The method of claim 56, wherein said scaffold is degradable upon exposure to predetermined environmental conditions.
67. The method of claim 56, wherein said scaffold is a non-biodegradable scaffold.
68. The method of claim 56, wherein said scaffold comprises macromolecules necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof.
69. The method of claim 68, wherein said macromolecules necessary for cell growth is a bone morphogenetic protein, a bone morphogenetic-like protein, an epidermal growth factor, a fibroblast growth factor, a platelet derived growth factor, an insulin like growth factor, a transforming growth factor, a vascular endothelial growth factor, AngI, PIGF and combinations thereof.
70. The method of claim 56, wherein said scaffold contains also a therapeutic agent.
71. The method of claim 70, wherein said therapeutic agent is an anti-infective, a hormone, an analgesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.
72. The method of claim 56, wherein said cell is engineered to express a therapeutic agent and/or a macromolecule necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof.
73. The method of claim 72, wherein said therapeutic agent is anti-infective, a hormone, an analgesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.
74. The method of claim 72, wherein said macromolecule necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof is a bone morphogenetic protein, a bone morphogenetic-like protein, an epidermal growth factor, a fibroblast growth factor, a platelet derived growth factor, an insulin like growth factor, a transforming growth factor, a vascular endothelial growth factor, AngI, PIGF and combinations thereof.
75. The method of claim 56, wherein said cell is a mesenchymal stem cell, mesodermal progenitor cell, endothelial precursor cell or neonatal dermal micro vascular endothelial cells (foreskin).
76. The method of claim 56, wherein said cell is capable of differentiating into an endotelial cell.
77. The method of claim 56, wherein said cell is isolated from peripheral blood.
78. The method of claim 56, wherein said cell is isolated from mammalian artery, vein or capillary.
79. The method of claim 56, wherein said cell is genetically engineered to express a reporter gene.
80. The method of claim 79, wherein the reporter gene is a fluorescent protein, Luciferase or b-gal.
81. The method of claim 56, wherein said step of growing scaffold encircled by cells so as to form a tubularized tissue is by the use of a bioreactor.
82. The method of claim 56, wherein the tubularized tissue is a single vessel, composition of several vessels or a capillary network.
83. A method of regulating angiogenesis comprising administering to said patient host an effective amount of the tubularized tissue of claim 1, so as to promoting angiogenesis.
84. A method of regulating vasculogenesis in a tissue comprising administering to said patient host an effective amount of the tubularized tissue of claim 1, so as to promoting vasculogenesis.
85. A method of treating hypoxia, avascular necrosis, foot ulcer or gangrene result from diabetes, atherosclerosis, stroke, wound, fracture of bone, comprising administering to said patient host an effective amount of the tubularized tissue of claim 1 so as to increasing the vascularity of a tissue thereby treating.
86. A method of replacing a damaged blood vessel of a patient comprising the step of administering to the patient an effective amount of the tubularized tissue, thereby replacing damaged blood vessels.
87. The method of claim 86, wherein the blood vessel is a thick blood vessels or a thin blood vessel.
88. A method for inducing the formation of new blood vessels in an ischemic tissue in a patient in need thereof, comprising:

administering to said patient host an effective amount of the tubularized tissue of claim 1, thereby inducing the formation of new blood vessels.

89. The method of claim 88, wherein said patient is in need of treatment for cerebrovascular ischemia, renal ischemia, pulmonary ischemia, limb ischemia, ischemic cardiomyopathy and myocardial ischemia.

90. A method of enabling ex vivo organ engineering by introducing the tubularized tissue of claim 1 to an engineered tissue so as to enable a constant oxygen supply to said engineered tissue.

91. A method of engrafting in a patient in need thereof, comprising administering to said patient host an effective amount of the tubularized tissue of claim 1, thereby engrafting in a patient in need thereof.

92. A method of determining cellular or tissue function of an agent comprising the following steps:

obtaining the tubularized tissue of claim 1;

contacting said tubularized tissue with an agent, so as to obtain a tubularized tissue contacted with an agent;

comparing cellular or tissue function of said tubularized tissue contacted with an agent, to a tubularized tissue, thereby determining cellular or tissue function of the agent.

93. The method of claim 92, wherein the cellular or tissue function is proliferation, apoptosis, toxicity and differentiation.

94. A method of screening candidate genes which are involved in cellular or tissue function, said method comprising the steps of:

obtaining the tubularized tissue of claim 1;

obtaining mRNA from said tubularized tissue and said cell;

synthesizing cDNA from said mRNA; amplifying said cDNA-hybrid, so as to obtain an amplified product;

detecting said amplified product; and comparing said amplified products from said tubularized tissue and said cell, thereby identifying candidate nucleic acid sequence which is involved in the cellular or tissue function.

95. An ex-vivo system for determining cellular or tissue function of an agent according to the method of claim 92.

96. An ex-vivo system for screening candidate genes, which are involved in cellular or tissue according to the method of claim 94.

97. A hybrid tissue made according to the steps of:

seeding a cell that will form a first tissue type on the exterior surface of a branched scaffold so as to obtain a scaffold encircled by cells with spaces in between;

adding into said spaces in between, a cell that will form a second tissue type; and

incubating so as to obtain a hybrid tissue.

98. The hybrid tissue of claim 97, whereby in the step of obtaining a hybrid tissue, the hybrid tissue is a monolayer tubular tissue.

99. The hybrid tissue of claim 97, whereby in the step of obtaining a hybrid tissue, the hybrid tissue is a multilayer tubular tissue.

100. The hybrid tissue of claim 97, whereby in the step of obtaining a hybrid tissue, the hybrid tissue is a multilayered hybrid tissue comprising more than one type of cells.

101. The hybrid tissue of claim 97, whereby in the step of obtaining a hybrid tissue, said hybrid tissue is a vascularized tissue.

102. The hybrid tissue of claim 97, whereby in the step of seeding a cell on the exterior surface of scaffold, said scaffold is in the form of a string.

103. The hybrid tissue of claim 97, whereby in the step of seeding a cell on the exterior surface of scaffold, said scaffold is a biodegradable scaffold.

104. The hybrid tissue of claim 103, wherein the biodegradable scaffold is a polymeric biodegradable scaffold.

105. The hybrid tissue of claim 104, wherein the polymeric biodegradable scaffold comprising cross-linked dextran, arabinogalactan, chitosan, polypeptide-glycolide, alginate, pullulan, gelatine, collagen, fibrin, fibrinogen or albumin.

106. The hybrid tissue of claim 97, whereby in the step of obtaining a scaffold, the diameter of said scaffold is 4-500 um.

107. The hybrid tissue of claim 97, whereby in the step of obtaining a scaffold, said scaffold is degradable upon exposure to predetermined environmental conditions.

108. The hybrid tissue of claim 97, whereby in the step of obtaining a scaffold, said scaffold is a non-biodegradable scaffold.

109. The hybrid tissue of claim 97, whereby in the step of obtaining a scaffold, said scaffold comprises macromolecules necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof.

110. The hybrid tissue of claim 109, wherein the macromolecules necessary for cell growth is a bone morphogenic protein, a bone morphogenic-like protein, an epidermal growth factor, a fibroblast growth factor, a platelet derived growth factor, an insulin like growth factor, a transforming growth factor, a vascular endothelial growth factor, Ang1, PIGF and combinations thereof.

111. The hybrid tissue of claim 97, whereby in the step of obtaining a scaffold, said scaffold contains also a therapeutic agent.

112. The hybrid tissue of claim 111, wherein said therapeutic agent is an anti-inflammatory, a hormone, an analgesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

113. The hybrid tissue of claim 111, wherein said therapeutic agent is an a nucleic acid sequence which encodes an anti-inflammatory, a hormone, an analgesic, an anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

114. The hybrid tissue of claim 97, wherein said cell is engineered to express a therapeutic agent and/or a macromolecule necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof.

115. The hybrid tissue of claim 114, wherein said therapeutic agent is anti-inflammatory, a hormone, an analgesic, an
anti-inflammatory agent, a chemotherapeutic agent, an anti-rejection agent, a prostaglandin, RGD peptide and combinations thereof.

116. The hybrid tissue of claim 114, wherein said macromolecule necessary for cell growth, morphogenesis, differentiation, or tissue building and combinations thereof is a bone morphogenic protein, a bone morphogenic-like protein, an epidermal growth factor, a fibroblast growth factor, a platelet derived growth factor, an insulin like growth factor, a transforming growth factor, a vascular endothelial growth factor, Ang1, PIGF and combinations thereof.

117. The hybrid tissue of claim 97, whereby in the step of obtaining a cell, said cell is a mesenchymal stem cell, mesodermal progenitor cell, endothelial precursor cell or neonatal dermal micro vascular endothelial cells.

118. The hybrid tissue of claim 97, whereby in the step of obtaining a cell, said cell is capable of differentiating into an endothelial cell.

119. The hybrid tissue of claim 97, whereby in the step of obtaining a cell, said cell is isolated from peripheral blood.

120. The hybrid tissue of claim 97, whereby in the step of obtaining a cell, said cell is isolated from mammalian artery, vein or capillary.

121. The hybrid tissue of claim 97, whereby in the step of obtaining a cell said cell is genetically engineered to express a reporter gene.

122. The hybrid tissue of claim 121, wherein the reporter gene is a fluorescent protein, Luciferase or β-gal.

123. The hybrid tissue of claim 97, wherein said step of growing scaffold encircled by cells so as to form a hybrid tissue is by the use of a bioreactor.

124. The hybrid tissue of claim 97, wherein the hybrid tissue is a single vessel, composition of several vessels or a capillary network.

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