NEW PRODUCT
New product

Cardiovascular diseases affect a large segment of the human population, and are a cause for significant morbidity, costs and mortality in the society. Surgical procedures are undertaken in several cardiovascular diseases to relieve obstructions, stenosis or dilations in vessels of heart or peripheral organs or to create an access to vascular system in order to be able to clean blood during the dialysis. During the surgery, the implanted vessels are used to bypass or replace the diseased part of the vessel. Autologous vascular grafts, i.e blood vessels harvested from the patients own body and used as vascular grafts, perform better in comparative studies than synthetic vascular grafts (Stone, J Biomed Mater Res 59: 160-165, 2002, Welch, Ann Vasc Surg 1992;6:473-484, Laube, J Thorac Cardiovasc Surg 2000;120:134-141). However, more than 350 000 synthetic vascular grafts are implanted annually during bypass or vascular access operation when no suitable vessel is available either because the potential autologous vessel grafts are of unsatisfactory quality or have been used in previous operations.

The superiority of autologous vessels compared to synthetic grafts is demonstrated in several clinical studies; in coronary artery surgery internal mammary artery patency rate is 90% at 10 years (Kirklin, Barratt Boyes, Cardiac Surgery, Second edition, page 331) and vein graft patency rate is 87% at 1 year, 80% at 5 years and 59% at 10 years (Sabiston, Surgery of Chest, page 1905), whereas the results with synthetic grafts in coronary position are poor (Farrar, Heart Surg Forum, 2000;3(1):36-40); in peripheral arterial surgery, one-year occlusion rate for infragenual bypasses vary between 15-75% depending on the site of specific patent characteristic conditions (Dorffler-Melly, Cochrane Database Syst Rev 2003;4:CD000536), however, vein grafts perform better than synthetic grafts both in below-knee popliteal position (70% vs. 37%) at 5 years (Veith J Vasc Surg 1986;3:1048-1049) and in the infrageniculate bypass (49% vs. 12%) (Veith J Vasc Surg 1986;3:1048). In vascular access surgery, the synthetic grafts have 41% greater risk of primary failure and 91% higher incidence of revision (Gibson J Vasc Surg 2001;34:694-700). The better graft performance translates to decreased suffering for the patient and reduced costs.

However, biocompatible and well functioning synthetic vascular grafts could offer the advantage of shorter operation time and smaller incision than autologous veins, which is crucial advantage in some patient groups and would save time and reduce costs for the surgical procedures. The porous fabric vascular grafts were first introduced by Voorhees et al 1952, polyethylene terephthalate (Dacron) was introduced 1958 by DeBakey, expanded polytetrafluoroethylene (ePTFE) 1973 by Matsumoto et al (Nojiri Artif Organs 1995 Jan;19(1):32-8) and polyurethane grafts have also been used (Farrar, Heart Surg Forum, 2000;3(1):36-40, Jensen, Eur Surg Res 1996; 28(1):49-54, Allen, Aust NZ J Surg 1996;66:738-742). Dacron, polyurethane and ePTFE can successfully be incorporated in both large and small caliber arteries in animals (Zdrahala, J

The foreign surface contact with blood activates coagulation. As a foreign material, synthetic graft surface is thrombogenic and more prone to clot than autologous vein or arterial grafts. Proteins of the contact system and their inhibitors, complement system, coagulation system and fibrinolysis areas well as neutrophils, monocytes and platelets are activated. The ongoing coagulation and fibrinolysis processes occur on graft surface where initial red thrombus containing numerous erythrocytes is alternating with white thrombus consisting of dense fibrin network due to parallel fibrinolysis suggests a perpetuated vicious circle (Noishiki Artif Organs 1995;19(1):7-16). In addition, the macrophage adherence to the graft material results in tissue factor production subsequently creating a more thrombogenic environment (Kalman, J Vasc Surg 1993;17(3):531-7). Treatment of the graft with patients own blood i.e. preclotting, enhances graft healing compared to collagen impregnation (Guidoin ASAIO J 1996;42(6)) and reduces the inflammatory reaction (Lyman J Biomat Res 2001;58(3):221-37) indicating that initial foreign surface and blood contact is unfavourable, however, pre-clotting has not resulted in better clinical outcome in human trials.

The synthetic vascular graft does not heal with endothelial surfaces in humans. Single layer of endothelium covers normally the inner surface of blood vessels, heart and lymphatics and serves as a barrier and a bioregulator. Autologous vein and arterial grafts have a healthy endothelial cell
lining, whereas endothelial cell lining on synthetic vascular graft surface becomes complete in
dogs, pigs, sheep, or calf within 2-12 weeks depending on species. Endothelialisation of a
synthetic vascular graft can occur either via longitudinal growth from anastomosis area
(transanastomotic), ingrowth of capillaries and/or capillary endothelial cells through the graft
wall and into porosity (transinterstitial) or seeding of circulating endothelial precursor cells
endothelium is limited to anastomosis and a pseudointima develops on the synthetic vascular
graft lumen comprising fibrin and underlying smooth muscle cell, fibroblasts, macrophages,
collagen, and other tissue components (Nojiri, Artif Organs 1995 Jan;19(1):32-8, Sauvage Arch
Surg 1974;109:698-705). Transanastomotic pannus growth is due to a hyperplastic reaction from
the ends of host artery and the growth is continuous, however, transanastomotic pannus ingrowth
does not extend more than 6-10 mm from the anastomotic line in human adults. In contrast,
transinterstitial growth comprises transinterstitial migration of the endothelial cells originating
from capillaries in the surrounding tissue through attachment, spreading, inward migration and
proliferation into and through the pores of the graft thereby covering the synthetic vascular graft
luminal surface. Human adult lack capacity for transinterstitial endothelialisation of synthetic
vascular graft surface when the graft is placed into vascular system despite capacity of capillaries
to grow halfway into porosities (Kohler, Surgery 1992;112(5):901-7).

Synthetic vascular grafts develop intimal hyperplasia. The intimal hyperplasia lesion is usually
found between the endothelium and the internal elastic lamina or on the inner surface of the graft
and consists of abnormal migration and proliferation of vascular smooth muscle cells and
deposition of extracellular matrix (Ao, Eur J Vasc Endovasc Surg 2000;20:241-249). Intimal
hyperplasia is primarily caused by injury to the endothelial cell layer in the blood vessel (Clowes
Am J Pathol 1985;118:43-54) and possibly also by the foreign body reaction. In addition, flow
dependent factors, such as shear stress (Zarin, J Vasc surg 1987;5:413-420), blood flow
(Mattson Thromb Vasc Bio 1997;17:2245-2249), flow velocities (Rittgers, Circ Res
1978;42:792-801) – as well as graft dependent factors - material (Ao, Eur J Vasc Endovasc Surg
Campbell Ann Surg 1975;182:138-43) and impregnation material (Guidon, Biomater
1989;10:156-165) may influence restenosis rate. As mentioned above, no convincing difference
between the various graft materials is observed in clinical trials and all synthetic grafts in the
market develop intimal hyperplasia.

Infection occurs up to 2-35% of implanted vascular grafts despite use of prophylactic antibiotic
and results in 30-50 % mortality in peripheral arterial surgery and limb loss in one third to one
half of survivors.
In summary, the current vascular grafts have serious drawbacks with unhealed thrombogenic surface and subsequent blood clots, development of intimal hyperplasia, inflammation and infections. The nonhealed thrombogenic synthetic surface creates a relatively more susceptible environment for development of blood clots and intimal hyperplasia and inflammation. Thus, by providing endothelialisation on the synthetic vascular graft surface and promoting healing, the synthetic vascular graft thrombogenicity and flow characteristics could be improved and by reduction of production of mitogenic substances the intimal hyperplasia in the anastomosis reduced.

Several approaches are executed in prior art to either increase synthetic vascular graft biocompatibility. First, synthetic graft porosity and material are manipulated to enhance endothelialisation. Second, cell seeding or sodding on synthetic vascular grafts is exploited. Third, in vitro tissue engineering is performed. Fourth, homografts and xenografts are used. Fifth, polymers or chemical treatment of the synthetic surfaces are utilised to reduce thrombogenicity. Sixth, growth factors or genes are used to stimulate endothelialisation of the synthetic graft surface.

Synthetic graft porosity is of importance for vascular graft endothelialisation in animal studies (Wesolowski, Thorac Cardiovasc Surgeon 1982;30:196-208, Hara, Am J Surg;1967;113:766-69, Tsuchida, J Invest Surg 1993 Nov-Dec;6(6):509-18, Clowes, Am J Path 1986;123:220-230, Hirabayash, J Biomater Res 1992;26:1433-47, Golden, J Vasc Surg 1990;11:838-45). Capillary diameter is about 5 microns and consequently the pores should be larger than 5 microns in minimum cross sectional dimension in order to allow transinterstitial growth. Thereto, the pores need to be continuous from the outer surface of the graft to the luminal surface in order to provide channels for capillaries to grow through. Porous ePTFE and Dacron vascular grafts are coated with various materials such as albumin, gelatin, and collagen or preclotted with blood in order to prevent leakage through the graft and mask the highly reactive prosthesis surface. Type of coating may influence endothelial cell attachment to the surface (Vohra Br J Surg 1991;78(4):417-20) and graft healing (Guidoin ASAIO J 1996;42(6)) but has not influenced the clinical outcome in studies (Becquemin J Mal Vasc 1996;21 Suppl A:41-7). Generally, ePTFE graft internodal distance less than 40 microns has not lead to endothelialization and internodal distance 60 microns and larger has shown in several animal experiments good results in animals (Tsuchida, J Invest Surg 1993 Nov-Dec;6(6):509-18, Clowes, Am J Path 1986;123:220-230, Hirabayashi, J Biomater Res 1992;26:1433-47, Golden, J Vasc Surg 1990;11:838-45). However, increase in internodal distance to 90 microns causes focal loss of endothelial cells (Golden, J Vasc surg 1990;11:838-45). Several flap techniques, such as omentum with uncompromised vasculature wrapped around a vascular graft inoperated to vessel system, improves transgraft growth in porous grafts (Hazama, J of Surg Res 1999;81:174-180, Nishibe Surg Today


engineered blood vessels (Miwa J Vasc Surg 1994;19:658-67) in which the vessel is formed from intact layers of human vascular cells grown in culture to overconfluence, producing a cell self-assembly model- smooth muscle cells and fibroblasts are grown first followed by endothelial seeding; grafts with biodegradable (Niklasson, Science 1999;284:489-93) or collagen scaffolds (US 20020115208) in which the smooth muscle cells are cultured on the polymer tube exposed to pulsatile conditions, then seeded with endothelial cells, then again cultured and implanted; grafts with decellularised matrix tubes (Noishiki Jpn J Surg 1980;10(1):77-83, Satoh ASAIO Trans 1988;34(3):655-60, Badylak, Biomaterials 1999;20:2257-63, Sacks J Biomed Mat Res 1999;46:1-10) in which, the matrix operated in to the anatomical location is of extracellular matrix, such as collagen, which then becomes colonised after implantation as the by-pass artery; and artificial arteries grown in vivo (6,626,823, Thomas Cardiovasc Path 2003;12:271-276) after placing a molding device in the peritoneum, it becomes covered by wound healing tissue granulation tissue, which then forms a tube with mesothelial cells around this molding device. This scaffold can then be used as a graft, when turned inside out having mesothelial cells on the inside. Generally, it is easier to perform three dimensional tissue engineering in vivo than in vitro (Noishiki 2001;25;(3):159-163). In addition there is a technique combining gene transfer with polymer scaffolds; the scaffold is treated with cells- smooth muscle cells, myofibroblasts, chondrocytes or extracellular basement membrane and then seeded with endothelial cells and cultured. The problem with tissue engineering has been that the cultured endothelial cells seeded onto the lumens have exhibited a pro-inflammatory and pro-coagulant phenotype contributing to graft thrombosis. Therefore, it is suggested that by transfecting endothelial cells with retroviral vector anti-thrombotic and anti-inflammatory proteins could be introduced (Fields et al).

However all of these procedures have serious drawbacks: tissue engineered grafts and grafts with biodegradable scaffold comprise the unpractical, difficult and expensive steps of cell culture. The durability and safety of decellularised collagen tube, when implanted into high pressure vascular system as well as the initially non-endothelialised surface are drawbacks of decellularised collagen tube. In the artificial artery model in vivo the inner surface of the graft is mesothelium and not by endothelium and because of the lack of outer scaffold the durability and safety of the graft is questionable.

Thromboresistance could be improved by chemical approaches including modification of implant materials or adding chemical compounds to the grafts. Heparin has been used most often and can be bound to the synthetic graft surface by several ways (Larm, Biomat dev Art Org 1983;11:2-3:161-173, Hoffman 1983;117:328-331, Ritter, Surgery 1997 Nov;122(5):888-92, Iwai ASAIO J 1996;42(5):M693-7, Noh J Biomater Res 2000;49:112-119, Begovac Eur J Endovasc Surg 2003;25:432-437). Also, hirudin, prostaglandins, anticoagulant peptide sequences and dextran derivatives have been used (Thomas Cardiovasc Path 2003;12:271-276). Heparin has improved in some animal studies both ePTFE and polyurethane graft patency in but not reduced intimal hyperplasia in the anastomosis area (Walpoth, Circulation 1998 Nov 10; 98(19 Suppl):II319-23, Ritter, Surgery 1997 Nov;122(5):888-92, Begovac Eur J Endovasc Surg 2003;25:432-437). However, heparin grafts have a serious drawback of sudden occlusions despite excellent initial patency due to pannus formation in the distal anastomosis area where the pannus flap was directed against the stream acting like a valve (Noishiki, Artif Organs 1998 Jan; 22(1):50-62). Furthermore, endothelium does not adhere to heparinized surfaces. The long term results from human trials have not so far been convincing even though in one promising recent study a heparin bonded Dacron graft was reported to have 70% femoropopliteal patency at 1 year (Devine J Vasc Surg 2001;33:533-539).

So far, gene approach has been used in order to enhance endothelialisation in to anatomic location implanted artificial vascular grafts (US 200300594463) and in combination with cell sodding (Yamamoto et al Circulation 1996;637:3721). Patent US 200300594463 describes a porous vascular graft in which endothelialisation is enhanced by administering angiogenic genes, such as genes encoding for VEGF, to the tissue surrounding the vascular graft. The graft is implanted to the anatomical location and the genes are administered to the surrounding tissue before or after implantation of the vascular graft. Similarly, patent WO02/087610 describes a medical device such as vascular graft in which restenosis is inhibited and endothelialisation enhanced by administering gene encoding for ecSOD. The graft is implanted to the anatomical location and the genes are administered to the surrounding tissue before or after the graft implantation (WO02/087610). In both cases the foreign surface is exposed to blood during the time the process of endothelialisation is ongoing. This takes 2-8 weeks in animals and supposedly longer time in humans. In summary, both protein and gene therapy in present art have serious drawbacks. A prerequisite for achieving an angiogenic effect with proteins is the need for repeated or long term delivery of the protein, which limits in clinical setting the utility of using these proteins to stimulate endothelial growth. Although genes are the natural slow release form of proteins, they and proteins leave the foreign graft surface unendothelialised and exposed to the blood for a longer time during, and subsequently unfavourable events of thrombosis and stenosis occur due to activation of coagulation and production of mitogenic stimuli.

Furthermore, ex vivo transfection of adipose tissue with VEGF\textsubscript{165} is described, whereafter cells are sodded into a standard ePTFE graft, grafts maintenance in organ culture and then implanted in order to increase endothelialisation (Yamamoto et al, Circulation; I-637;3721). In addition, endothelial cells have been transfected with tPA (tissue plasminogen activator) (Dunn, Circulation 1996;93:1439-46, Huber, J Vasc Surg 1995:22;795-803) in order to reduce thrombogenicity or transfected with noninducible retroviral markers (Sackman, Cell Transplant 1995;4:219-235) and seeded onto grafts, however endothelial cells transduced with tPA were retained at a significantly lower rate than untransduced or cells transfected with noninducible retroviral markers appeared altered in their ability to stable adhere, respectively. However, enhanced adherence is also described after transfection with pro-urokinase (Falk, J Vasc Surg 1998;27:902-8). In addition, a method to transduce endothelial progenitor cells and then re-administer them has been described ( ). Subsequently, results from the ex vivo transfections and subsequent seedings are not convincing and the method is costly, cumbersome and unpractical.
Summary of the invention

The object of the present invention is to provide a solution to the aforementioned problems. More specifically, one object of the invention is to provide a medical device, which solves the problems of lack of biocompatible surfaces of medical implants resulting in thrombosis, intimal hyperplasia, infections and other problems. Another object of the invention is to provide a medical device useful in vascular surgery, which entails less risks of being occluded and stenosed than hitherto known devices.

Another object of the present invention is to provide a medical device, which is an alternative to vascular grafts implanted directly in anatomical location and stimulated by genes and proteins but avoids the risks associated with a period of time with unendothelialised synthetic surfaces exposed to blood and subsequent initiation of coagulation and processes enhancing development of restenosis.

Another object of the present invention is to provide a medical device, which has less risk for aneurysm formation and is less cumbersome to use in practice than in prior art described tissue engineered vascular grafts for improving the biocompatibility between foreign materials and the recipient or host thereof.

A further object of the invention is to provide a medical device useful as an alternative to allografts and xenografts but which avoids the risks of antigenicity and risk of aneurysm development.

The present device is useful in a wide variety of contexts, and may e.g. be a cardiovascular implant, such as an artificial part of a blood vessel or as a tubular implant replacing parts of other tubular structures in the body such as trachea, bronchi, oesophagus, small or large intestines, ureters or uterine tubes. In general terms, the present device may be used as an implant used for replacement of part of a mammalian body, where said implant is adapted for an at least partial contact with tubular structures. Most preferably, the device is selected from the group consisting of vascular grafts.

The present invention also relates to a method of producing a medical device according to the invention and to use of such medical devices. Further, the invention relates to a method of improving a mammalian body’s biocompatibility with a synthetic surface, which method comprises introducing a device comprising a porous scaffold and an inner synthetic device in the body with at least partial contact with tissues, allowing tissue to grow into the said porosity of the outer scaffold while the inner device inhibits excessive growth to the scaffold lumen, then
removing the inner device and implanting the device in alignment with the desired tubular structure in the body, most preferably a human blood vessel.

In one embodiment the method and use comprise stimulation of tissue growth into the said scaffold with drugs, growth factors or nucleic acids present in a biologically compatible medium in the inner device, between the inner and outer device, on the outer device or administering the drug, growth factors or genes to the surroundings thereof. The method and use are characterised in that the drug, protein or nucleic acid encode a translation or transcription product capable of promoting endothelialisation *in vivo* at least partially on said synthetic surface, said administration of nucleic acid being performed before, simultaneously as or after the introduction of the device in the body.

Furthermore, isolated endothelial cells, isolated cells from vasculature, peripheral stem cells, bone marrow and embryonic stem cells can be used as therapeutic substance.

The method may include administering of the therapeutic substance at least once and various therapeutic substances may be combined, depending on the case in question.

Further details regarding the method of treatment are disclosed below.

**BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 presents a side view of two scaffold variants according to the present invention. Figure 1A shows a scaffold 100 in one piece comprising an outer surface 102 and end surfaces 104. The outer surface 102 comprises pores 106. Figure 1B discloses a side view of a scaffold 200 of a knitted material. The scaffold comprises an outer surface 202 and two end surfaces 204. The knitted scaffold is comprised of several fibres 206 and pores 208 are formed between the fibres.

Figure 2 discloses an end view of a circular scaffold 300. The scaffold encloses an inner device 304. An open space 302 is formed between the scaffold 300 and the inner device 304.

Figure 3 discloses an end view of a circular scaffold 400. The scaffold encloses an inner device 404. An open space 402 is formed between the scaffold 400 and the inner device 404.
Definitions

Below, explanations are provided as to the meaning of some of the terms used in the present specification. Terms that are not specifically defined herein are to be interpreted by the general understanding thereof within the relevant technical field.

A “medical device” is here referred to as an implant comprising both a scaffold and an inner device and is understood as an object that is fabricated for being implanted at least partly in a mammalian. It is intended to be in contact with bodily tissues and fluids and after separation of the inner device and scaffold, the scaffold connected to tubular structures.

A “vascular graft” and “scaffold” are used interchangeably and are here referred to as a tubular implant connected to blood vessels and connected with the blood-flow.

An “inner device” is here referred to as an implant being inside scaffold and inhibiting excessive tissue growth to the lumen of the scaffold and also regulating the luminal diameter of scaffold.

The term “attach” and its derivatives refer to adsorption, such as physisorption, or chemisorption, ligand/receptor interaction, covalent bonding, hydrogen bonding, or ionic bonding of a polymeric substance or nucleic acids to the implant.

An “attached therapeutic substance” referred to here, represent the wide variety of drugs, proteins or genetic material, which can be transferred to the tissue growing into the scaffold of the device. Suitable therapeutic substances may be in any form, such as naked DNA, purified protein or combinations thereof. The therapeutic substance segment may also be incorporated in other carriers. The attached transferable therapeutic agent is attached to the device in such a way, that it can be taken up by the tissue growing into the scaffold.

A “tissue growing into the graft” here refers to any or all cells, which grow to the scaffold in primary unanatomical location of the device or contribute to the formation of new endothelial lining or capillarisation of the scaffold surface in the unanatomical location. This includes various tissues, such as fat, omentum, peritoneum, but the particular type of surrounding tissue is not important as long as the cells ultimately gives rise to the endotelialisation or capillarisation of the scaffold.

An “endothelium” is a single layer of flattened endothelial cells, which are joined edge-to-edge forming a membrane covering the inner surface of blood vessels, heart and lymphatics.
"Endothelialisation" is here referred to the growth of endothelial cells on all mammalian tissue or fluid contacting surfaces of a biomaterial, that is used to form a porous scaffold. Endothelialisation of surfaces can occur via longitudinal growth, ingrowth of capillaries and/or capillary endothelial cells through the pores in the implants, or seeding of circulating endothelial precursor cells. In this disclosure, it will be used interchangeably with the phrase "capillary endothelialisation", to refer to the growth of endothelial cells on substantially all tissue contacting surfaces of a biomaterial, that is used to form a porous scaffold, unless otherwise specified.

The terms "capillarisation" and "vascularisation" are here understood as the formation of capillaries and microcirculation on the implant surface, and they will be used interchangeably with endothelialisation, unless otherwise specified.

"Angiogenesis" and reflections thereof, such as "angiogenic", are here referred to formation and growth of endothelial cells in the existing mammalian tissue, such as in the surrounding tissue.

A therapeutic substance having "the potential to promote endothelialisation" of the scaffold, is here understood as a chemical substance or biomolecule, preferably a hormone, a receptor or a protein, more preferably a growth factor or gene, which, as a result of its activity, can induce endothelialisation or capillarisation of the medical implant. Furthermore, isolated endothelial cells, isolated cells from vasculature, peripheral stem cells, bone marrow and embryonic stem cells can be used as therapeutic substance. "Porosity" and reflections thereof, such as "pores" and "porous", are here referred, if not otherwise specified, to a biomaterial having small channels or passages, which start at a first surface and extend substantially through to a second surface of the biomaterial.

"Surface" refers to the interface between the biomaterial and its environment. It is intended to include the use of the word in both its macroscopic sense (e.g. two major faces of a sheet of biomaterial), as well as in its microscopic sense (e.g. lining of pores traversing the material).

A "restenosis" or "stenosis" is here referred to as growth of connective tissue after implanting a medical device and connecting it to a tubular structure leading to connective tissue growth in the tubular structure with subsequent narrowing of the tubular structure. The connective tissue growth may occur at any site in the body leading to narrowing of the tubular structure but occurs preferably in the site for connection of the device to the tubular structure in the body. The connective tissue growth comprises of either increase in some cell type in the area or increase in the volume or the constituents of extracellular matrix.
“Hyperplastic connective tissue reaction” here defines the reaction leading to an increase of number of connective tissue cells and/or an increase in the volume of extracellular matrix in the tissue, excluding tumour formation, whereby the bulk of the connective tissue may be increased.

The term compartment refers to any suitable compartment, such as for example a vial or a package.

The references having seven digits (e.g. 4,654,321), that are used throughout this specification, refers to numbers of US patent applications, if nothing else is specified.

Detailed description of the invention

The present invention relates to a kit, useful in vascular surgery, said kit comprising:

a) a porous tubular scaffold; and

b) an inner device adapted for being inserted into said porous tubular scaffold, said inner device having essentially the same cross-sectional shape as the cross-sectional cavity of the tubular scaffold, the cross-sectional area of said inner device being from 10 to 99% of the cross-sectional area of the cavity of said porous tubular scaffold, said inner device having essentially the same length as said porous tubular scaffold.

The inner diameter of the porous tubular scaffold can be from 1.0 mm to 50 mm, and preferably from 2 mm to 6 mm.

The thickness of the porous tubular scaffold can be from 0.1 – 2 mm, and preferably from 0.3 – 0.6 mm.

The porosity of the porous tubular scaffold is normally from 30 μm to 2000 μm.

The cross-sectional diameter of the inner device is normally 40 μm to 2000 μm less than the cross-sectional inner diameter of the porous tubular scaffold.

The porous tubular scaffold can be produced from woven or knitted synthetic fibres.

At least one of the ends of the inner device can be equipped with a handling device.

The porous tubular scaffold can made of a material selected from the group of tetrafluoroethylene polymers, aromatic/aliphatic polyester resins, polyurethanes, silicone rubbers, polyglycolic acid, polylactic acid, polydioxone, polyglyconate, collagen and polyethylene terephthalate
The inner device can be made of a material selected from the group of metal, titanium, titanium alloys, tin-nickel alloys, shape memory alloys, aluminium oxide, platinum, platinum alloys, stainless steel, MP35N, elgiloy, stellite, pyrolytic carbon, silver carbon, glassy carbon, polymer, polyamide, polycarbonate, polyether, polyester, polyolefin, polyethylene, polypropylene, polystyrene, polyurethane, polyvinyl chloride, polyvinylpyrrolidone, silicone elastomer, fluoropolymer, polyacrylate, polyisoprene, polytetrafluoretylene, rubber, ceramic, hydroxyapatite, human protein, human tissue, animal protein, animal tissue, bone, skin, laminin, elastin, fibrin, wood, cellulose, compressed carbon, glass, polyglycolic acid, polylactic acid, polydioxone, and polyglyconate.

At least one of the porous tubular scaffold and the inner device may comprise a therapeutic substance attached to its surface, said therapeutic substance having the potential to promote endothelialisation. This therapeutic substance may be chosen from the group of VEGF proteins and nucleic acid molecules encoding VEGF proteins. Furthermore, isolated endothelial cells, isolated cells from vasculature, peripheral stem cells, bone marrow and embryonic stem cells can also attached as therapeutic substance.

The invention also provides a porous tubular scaffold, useful in vascular surgery, wherein said porous tubular scaffold encloses an inner device, said inner device having essentially the same cross-sectional shape as the cross-sectional cavity of the tubular scaffold, the cross-sectional area of said inner device being from 10 to 99% of the cross-sectional area of the cavity of said porous tubular scaffold, said inner device having essentially the same length as said porous tubular scaffold, said inner device being recoverably attached to an end of said porous tubular scaffold.

Finally, the invention relates to a method for endothelialisation or capillarisation of a tubular medical device, comprising the steps of:

a) providing
   i) a porous tubular scaffold enclosing a recoverably attached inner device; or
   ii) a kit comprising a scaffold and an inner device, wherein said scaffold encloses said inner device;

b) surgically implanting said scaffold or said kit consisting of a scaffold enclosing an inner device into a human body in an anatomic location that does not have a tubular structure;

c) allowing the tissue of said anatomic location to grow into said scaffold;

d) surgically removing said scaffold or said kit consisting of a scaffold enclosing an inner device from said anatomic location, and

e) removing said inner device, thereby obtaining an endothelialised or capillarised tubular medical device.
Furthermore, the invention provides a method for implanting a tubular medical device to an anatomic tubular structure, comprising the steps of:

a) endothelialising or capillarising a tubular medical device according to the above disclosed method, and

b) surgically implanting said endothelialised or capillarised tubular medical device to a suitable anatomic tubular structure.

In a first aspect, the present invention relates to a medical device with improved biological properties for an at least partial contact with bodily tissues when introduced in connection to a tubular structure in a mammalian body, which device comprises a porous scaffold with lumen and an inner device inside the scaffold. The device is implanted in bodily tissues and tissue growth is allowed to occur to the porosity meanwhile the inner device inhibits the excessive tissue growth to the scaffold lumen and thus regulates scaffolds future lumen.

Furthermore, the present innovation relates to the tissue growth to the scaffold stimulated by therapeutic substance, such as drugs, growth factors and genes encoding for growth factors. Preferably drugs, growth factors and genes enhancing growth of endothelium are used for vascular purposes whereas to other purposes drugs, growth factors or genes stimulating cells and tissues favourable for the respective tubular structures such as ureter, trachea, bronchi, oesophagus, ovarian tube are used. The therapeutic substance i.e. chemical substance, drug, growth factor or nucleic acid is provided in a way whereby the tissue growth to the scaffold is optimal. Furthermore, isolated endothelial cells, isolated cells from vasculature, peripheral stem cells, bone marrow and embryonic stem cells can be used as therapeutic substance.

Despite formation of endothelial surface in vascular grafts in animals, the endothelial layers have not been observed to form in humans if the vascular graft is directly implanted to vascular system according to the prior art. Furthermore, the tissue engineering approaches in prior art have serious drawbacks, are cumbersome or associated with risk for dilation of the implanted vascular graft.

The endothelium formed according to the invention on the synthetic surface offers many of the advantages of a native surface. Endothelium is a single layer of flattened cells, which are joined edge to edge forming a membrane of cells covering the inner surface of blood vessels, heart and lymphatics. In theory, endothelialisation of the graft can occur either via longitudinal growth from the anastomosis area (transanastomatic), ingrowth of capillaries and/or capillary endothelial cells through the synthetic surface, such as a graft wall, and into porosities (transinterstitial), or seeding of circulating endothelial precursor cells. In the transinterstitial migration through the pores, the endothelial cells originate from capillaries through attachment, spreading, inward migration and proliferation. Thus, even though efforts have been made in the prior art to avoid thrombogenicity and the resulting clotting on polymeric surfaces of vascular grafts, such efforts
have been cumbersome or not proved satisfactory with smaller vessels, wherein thrombosis and hyperplasia have caused substantial problems. The present invention provides for the first time a device comprising a synthetic luminal surface in its scaffold, which is capable of being accepted by the vascular system upon implantation due to the endothelial layer thereon. The present invention provides a versatile technology useful with a range of tubular implants, and surprisingly also is efficient with small size synthetic vessel sections that have previously been known to clot.

US patent 6,626,823 describes a molding device implanted to peritoneum whereby a scar tissue grows on the device. Then the peritoneal outside is turned inside. Although the tissue grows around a molding device in tissues in both inventions, the inventions are distinctly different. In 6,626,823 no scaffold around the device, all the device material is removed before implantation and no device is implanted to vascular system and, whereas in the present invention the scaffold is the crucial part for providing stability and durability for the device after implanted to vascular system. In addition, in 6,626,823 the tissue is not stimulated by any means and the device is not used to carry drugs, growth factors or genes.

WO 98/20027 describes the therapeutic use of vascular endothelial growth factor (VEGF) to stimulate NO or prostacyclin production in the treatment of intimal hyperplasia, hypertension and atherosclerosis. More specifically, VEGF is delivered to the exterior of a blood vessel using a delivery reservoir in the form of a collar placed around the vessel. The collar then directs said agent to the vessel while diffusion thereof into the surrounding tissue is avoided. However, both delivery device and the nature thereof are distinctively different compared to the present invention. The present invention provides an optimal device implantable to one location in body where growth of endothelium is stimulated to the scaffold of the device, whereafter the inner device is removed and scaffold implanted to its anatomic location. Furthermore, the present invention provides a drug, growth factor and nucleic acid encoding an angiogenic factor, such as a VEGF gene, to enhance tissue growth into the scaffold of the device, which is then introduced into the vascular system to replace or support a native organ, whereas the device in WO 98/20027 is used to surround the vessels to inhibit tissue growth. In the present invention, said substances enter the cells of ingrowing tissue and provide stimulation for endothelial growth on the synthetic surface, whereas the purpose of WO 98/20027 is the contrary, since the aim thereof is to deliver a gene, such as VEGF, to a specific site in the body, where said gene will be expressed and suppresses any cell growth at the delivery site, i.e. it prevents hyperplasia. The contrary effects are achieved since the mode of delivery is different; the present invention provides a local delivery of substances to the tissue growing into the scaffold porosity in order to obtain as high expression as possible, while the WO 98/20027 delivery is designed to provide a directed administration to the anastomotic site to inhibit tissue growth. Thus, even though WO
98/20027 also uses a synthetic device, contrary to the present invention, no endothelial layer is created thereon. In fact, should a novel endothelial layer be created on the synthetic WO 98/20027 collar, that in itself would be a sign of failure of the intended purpose, which clearly illustrates the differences between WO 98/20027 and the invention.

US 200300594463 describes a medical device preferably a vascular graft coated with genes encoding for VEGF to enhance endothelialisation of the device after implantation of to the vascular system. Although both approaches describe stimulation of tissue growth with VEGF there are distinct differences. In the present invention the device construction is completely different comprising of two parts in order to provide optimal conditions for in vivo tissue engineering purposes. Such a device could not be implanted directly to the vascular system and implantation of the vascular graft described in US 200300594463 to a tissue pouch would result in occlusion of the graft and making it unusable. In the present invention, the inner device inhibits excessive tissue growth, whereas in US 200300594463 tissue growth is not limited by any method. In US 200300594463 the vascular graft is implanted directly to the vascular system with foreign surface exposed to the blood, whereas in current invention the vascular graft is implanted first in other tissues, tissue allowed to grow into the scaffold, the inner device removed and scaffold implanted to the vascular system. In the present invention the endothelial lining is existent when implanting the scaffold of the device in to the vascular system, whereas in 200300594463 the graft has initially no endothelial surface and development thereof takes weeks despite stimulation with genes. Subsequently, the endothelial surface in the present invention provides prevention against clotting and harmful production of stenosis promoting factors from the moment of implantation.

WO02/087610 describes a method to inhibit restenosis on a medical device preferably a vascular graft or stent by administering gene encoding for ecSOD to enhance endothelialisation of the device after implantation of to the vascular system. In the present invention the device construction is completely different comprising of two parts in order to provide optimal conditions for in vivo tissue engineering purposes. Such a device could not be implanted directly to the vascular system and implantation of the vascular graft or stent described in WO02/087610 in tissue other than in connection with vascular system would result in occlusion of the device and making it unusable. In WO02/087610 the vascular graft is implanted directly to the vascular system with foreign surface exposed to the blood, whereas in the present invention the vascular graft is implanted first in other tissues, tissue allowed to grow into the scaffold, the inner device removed and scaffold implanted to the vascular system. In the present invention the endothelial lining is existent when implanting the scaffold of the device in to the vascular system, whereas in WO02/087610 the graft has initially no endothelial surface and development thereof takes weeks despite stimulation with genes. Subsequently, the endothelial surface in the present invention
provides from the moment of implantation prevention against clotting and harmful production of stenosis promoting factors.

Said device comprises of two parts: a porous circular scaffold and an inner device inhibiting excessive growth to the lumen of the scaffold. The scaffold is porous, in which case it allows capillary and endothelial cell growth through pores. The scaffold can be of any material but is preferably synthetic and most preferably of Dacron or porous ePTFE. The scaffold can be of any diameter but the inner diameter is preferably 2 mm-5cm in diameter. The most preferable diameter for vascular grafts is 2 mm-6mm. Preferably, the porosity is from about 30 μm to about 2000 μm. The inner device is either non-porous or porous preventing that the capillary, endothelial and other tissue growth occludes the scaffold lumen and thus securing an open lumen inside the scaffold and regulates the lumen inside the scaffold. The inner device can be of any material or combination of materials but is preferably of plastic. The inner device can be of any form but is preferably circular. The inner device can be of any diameter but preferably aligns with the inner surface of the scaffold and is of the same diameter as the tubular structure it is attached to, thus preferably having inner diameter of 2 mm - 5 cm and most preferably for vascular graft purposes an inner diameter of 2 mm – 6 mm. The above given objects and others are according to the present invention achieved by providing the said scaffold with improved biological properties for a contact with a tubular structure, preferably a human blood vessel. The most preferable blood vessel is a human artery or a new connection between an artery and vein. This is achieved by first introducing the device in a mammalian body in a suitable unanatomical tissue environment. The preferable locations are fat tissue or peritoneum. Then, after tissue ingrowth to the scaffold and development of endothelialised surface in the lumen of scaffold, the device is removed from this primary unanatomical location, inner device removed and the scaffold implanted in connection to a tubular structure in the body in the desired anatomical location.

Furthermore, the said device can be used to release therapeutic substances such as drugs, growth factors or nucleic acids to improve its biocompatibility i.e improve endothelialisation, reduce stenosis and prevent infections. Drugs, growth factors and nucleic acids are present in a biologically compatible medium and are characterised in that said drugs, growth factors or nucleic acids promote endothelialisation at least partially on a synthetic luminal surface of said scaffold, reduce stenosis or reduce infections in vivo. Promotion of endothelialisation can be accomplished by using inner device, space between the inner device or scaffold for the release of drugs, growth factors, or genes. In another embodiment, the biologically compatible medium is a biostable polymer, a bioabsorbable polymer, a biomolecule, a hydrogel polymer or fibrin. Furthermore, isolated endothelial cells, isolated cells from vasculature, peripheral stem cells, bone marrow and embryonic stem cells can be used as therapeutic substance.
In one embodiment, the growth factors are, or nucleic acids encode for, a protein or polypeptide selected from the group consisting of fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factor (TGF) and epidermal growth factor (EGF) families, placenta derived growth factor (PIGF), hepatocyte growth factor (HGF) and angiopoietin.

Preferably, the nucleic acid encodes vascular endothelial growth factor (VEGF), acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF), nitric oxide synthetase (NOS) or superoxide dismutase (SOD). Most preferably, growth factor and nucleic acid encodes for VEGF. Drugs can instead act directly to promote endothelialisation, reduce restenosis, reduce infections or induce production of said factors, act through receptors of said factors or induce production of other factors that have said effects.

In one advantageous embodiment, the therapeutic substance i.e. drug, growth factor or nucleic acid is present in a reservoir separate from said device enabling a successive delivery thereof to a mammalian body. In an alternative embodiment, the nucleic acid has been attached to the inner device by ionic or covalent bonding or is located in a hydrogel in the space between the inner device and scaffold.

The present device is useful in a wide variety of contexts in surgery, most preferably in vascular surgery, and depending on the intended use, both the scaffold and inner device may be made from a biomaterial selected from the group of non-soluble synthetic polymers, metals and ceramics with or without modification of the prosthesis surfaces. Thus, in one embodiment, the inner device and scaffold are made of a biocompatible material selected from the group consisting of metal, titanium, titanium alloys, tin-nickel alloys, shape memory alloys, aluminium oxide, platinum, platinum alloys, stainless steel, MP35N, elgiloy, stellite, pyrolytic carbon, silver carbon, glassy carbon, polymer, polyamide, polycarbonate, polyether, polyester, polyolefin, polyethylene, polyethylene terphtalate, polypropylene, polystyrene, polyurethane, polyvinyl chloride, polyvinylpyrrolidone, silicone elastomer, fluoropolymer, polyacrylate, polyisoprene, polytetrafluoretylene, rubber, ceramic, hydroxyapatite, human protein, human tissue, animal protein, animal tissue, bone, skin, lamminin, elastin, fibrin, wood, cellulose, compressed carbon and glass. Scaffolds and inner devices according to the invention may be of virtually any size or shape, so that their dimensions are adapted to fit the implantation site in the body.

The scaffold surface according to the invention is porous at least in some part of the scaffold. Most, preferably scaffold is porous in all parts. Thus, materials, which are porous, partly porous or combinations thereof, may be used to produce the vascular graft scaffold, depending on the implant embodiment. The purpose of the scaffold is to act as a structure to which the tissue can grow into as well as provide the required strength and durability after implantation to the vascular system. For example, graft porosity has been shown to be of importance in vascular graft endothelialisation in animals (Wesolowski, Thorac Cardiovasc Surgeon 1982;30:196-208,
Hara, Am J Surg;1967;113:766-69). The optimal internodal distance for ePTFE grafts has been approximately 60 μm, whereas knitted Dacron graft is more porous than woven Dacron and subsequently more suitable as a scaffold.

The inner device may be porous or nonporous. Thus, materials, which are nonporous, porous, partly porous or combinations thereof, may be used to produce the inner device, depending on the implant embodiment. Most preferably, the inner device is nonporous. The purpose of the inner device is dual; both inhibit excessive tissue ingrowth to the graft lumen and subsequent occlusion, while the device is in tissue site, and to regulate the inner diameter of the scaffold as well as the thickness of the inner layer in the scaffold when it is connected to vascular system.

The inner device may be aligned with the scaffold or a space can be left between the scaffold and inner device. Preferably, there is a space allowing formation of endothelial surface layer on the lumen side of the scaffold. The inner device can be of any form and be located anywhere within the scaffold but is preferably circular and located centrally in the scaffold. The inner device can be solid or have an inner hollow lumen, preferably it is solid. The inner device can be attached to the ends of the scaffold by any methods or be unattached. The preferable mechanical attachment of the inner device to the ends of the scaffolds provides stability for the inner device and subsequent centrally located lumen. However, in some embodiments only attachment of the scaffold in one end may be sufficient in order to allow maximal flexibility for the device in the body. In some embodiments, it may even be sufficient to only occlude the ends of the scaffold to prevent tissue growth longitudinally directly to the lumen but without, initially or not at all, inserting an inner device of same length as the scaffold. In addition, the inner device may also be only part of the length of the scaffold. The inner device is preferably attached to scaffold by using hook-like structures in the both ends of the inner device which in turn surround the ends of the scaffold. The hooks are removed in connection to removal of the inner device from the inside of the scaffold after the scaffold has become populated by tissue and endothelium.

Thus, the device comprising a scaffold and an inner device may be a surgical implant selected from the group consisting of medical implants connected to tubular structures in human body, such as a vascular graft, ureter graft, uterine tube graft, oesophagus graft, small bowel graft, large intestine graft, bronchial graft and tracheal graft. In one specific embodiment, the device is a vascular graft, such as an artificial part of a blood vessel. In general terms, the present device may be used as an implant used for replacement of a part of a tubular structure, where said implant is after first implantation to an unanatomical location, the relocated from anatomic location and adapted for an at least partial contact with blood or bodily fluids and tubular structures in correct anatomical location.

In order to improve the tissue ingrowth to the scaffold, scaffold, the inner device or the space between the inner device and scaffold may be treated in a variety of ways, in all or parts thereof,
e.g. by coating or administering growth stimulating substance. Most preferably the inner device is coated, as is discussed in more detail below in the experimental section in the general disclosure of materials and methods. In one embodiment, the nucleic acid and protein have been attached to the core by ionic or covalent bonding. In another embodiment the nucleic acid is mixed to the preclotting blood. In another embodiment, the biologically compatible medium is a biostable polymer, a bioabsorbable polymer, a biomolecule, or a hydrogel polymer is used.

An extremely wide variety of drugs, proteins or genetic materials can be used to enhance endothelialisation of the scaffold using the compositions and methods of invention. For example, the drug may directly or indirectly stimulate the growth of the endothelium, inhibit inhibitor of the endothelial growth, protein may bind to receptor and stimulate the receptor or inhibit binding of native protein to the receptor and the nucleic acid may be DNA (double or single stranded) or RNA (e.g. mRNA, tRNA, rRNA). A skilled artisan will easily recognise any such compounds. Angiogenic proteins and genes encoding for angiogenic proteins are used in animal and human trials to increase angiogenesis and provide biological by-pass i.e. collateral circulation without need for surgery (Pu et al., J Invest Surg, 1994;7:49-60, Ferrara & Alitalo, 1999;12:1359-64, Isner et al, Lancet, 1996;348:370-4, Baumgartner et al, Circulation, 1998;97:1114-23, (5,792,453), Yläherttuala & Alitalo Nat Med 2003, 6:694-701, Kastrup Curr Gene Ther 2003 Jun;3(3):197-206, Bekeredjian Am J Med Sci 2004;327(3):139-148).

endothelialisation *in vivo*, i.e. they are angiogenic factors. Thus, in one embodiment, a protein or polypeptide is selected from the group consisting of fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factor (TGF) and epidermal growth factor (EGF) families, placenta derived growth factor (PIGF), hepatocyte growth factor (HGF), and angiopoietin. In one specific embodiment, the protein is vascular endothelial growth factor (VEGF). It is easy for skilled artisan to foresee that there will be a plenty of angiogenic drugs in future and recognise such drugs.

The substance used to enhance endothelial formation in the scaffold may also be a coding nucleic acid, i.e. one that encodes a protein or a polypeptide, or it may be an anti-sense nucleic acid molecule, such as anti-sense RNA or DNA, that may function to disrupt gene expression. Alternatively, it may be an artificial chromosome. Thus, the nucleic acids may be genomic sequences, including exons or introns alone, or exons and introns, or coding DNA regions, or any construct that one desires to promote endothelialisation of the scaffold. Suitable nucleic acids may also be virtually any form, such as naked DNA or RNA, including linear nucleic acid molecules and plasmids, or a functional insert within the genomes of various recombinant viruses, including viruses with DNA genomes, and retroviruses. By a naked nucleic acid is meant a single or double stranded DNA or RNA molecule not incorporated into a virus or liposome. Antisense oligonucleotides bind specifically to complementary mRNA molecules, and thereby reduce or inhibit protein expression. The nucleic acid may also be incorporated in other carriers, such as liposomes and other viral structures. Most preferably, nucleic acid is naked DNA encoding for VEGF.

Several chemical, physical, and viral mediated mechanisms are used to improve gene transfer. Several different vehicles are employed in gene transfer. All of the methods have their advantages and drawbacks and are included in the present invention. Naked DNA as a double stranded DNA is minimally immunogenic. There are a number of viruses, live or inactive, including recombinant viruses, that can be used to deliver a nucleic acid to the tissues, such as retroviruses, lentivirus, adenoviruses (e.g. 5,882,887, 5,880,102) and hemagglutinating viruses of Japan (HVJ or Sendai virus) (5,833,651). Other examples of used viral vectors are adeno-associated viruses (AAV), herpes viruses, vaccinia viruses, lentivirus, poliovirus, other RNA viruses and influenza virus (Mulligan, Science 1993; 260: 926-32; Rowland, Ann Thorac Surgery 1995, 60:721-728). DNA can also be coupled to other types of ligands promoting its uptake and inhibiting its degradation (e.g. 5,972,900, 5,166,320, 5,354,844, 5,844,107, 5,972,707). It can also be coupled to a so called cre-lox system (Sauer&Henderson, Proc Natl Acad Sci; 1988, 85:5166). However, the limited duration of angiogenic protein expression associated with adenovirus and plasmid DNA transfection are sufficient for angiogenesis and development of cellular surface on the scaffold. The traditional chemical gene transfer methods are calcium phosphate co-precipitation, DEAE-dextran, polymers (5,972,707), and liposome-
mediated transfer (for example 5,855,910, 5,830,430, 5,770,220), and the traditional physical methods are microinjection, electroporation (5,304,120), iontophoresis, a combination of iontophoresis and electroporation (5,968,006), and pressure (5,922,687) Transfection efficiency can also be improved by pharmacological measures i.e. addition of PEI. Most preferably, naked DNA is used. With respect to the transfer and expression of therapeutic genes according to the present invention, the ordinary skilled artisan is aware that different genetic signals and processing events control levels of nucleic acids and proteins/peptides in a cell, such as transcription, mRNA translation, and post-translational processing. These steps are affected by various other components also present in the cells, such as other proteins, ribonucleotide concentrations and the like.

The invention may be employed to promote expression of a desired gene in the tissue ingrowing to the scaffold an implant, and to impart a certain phenotype, and thereby promote scaffold endothelialisation or vascularisation. This expression could be increased expression of a gene that is normally expressed (i.e. over-expression), or it could be the expression of a gene that is not normally associated with tissues surrounding the prosthesis in their natural environment. Alternatively, expression of a gene that is naturally expressed in such tissues could be suppressed, and again, to change or alter phenotype. Gene suppression may be a way of expressing a gene that encodes a protein that exerts a down-regulatory function. It may also utilise anti-sense technology.

Thus, the nucleic acids used with the inner device according to the present invention encode transcription or translation products capable of promoting or stimulating endothelialisation in vivo, i.e. they are angiogenic factors. Thus, in one embodiment, the nucleic acid encodes a protein or polypeptide selected from the group consisting of fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factor (TGF) and epidermal growth factor (EGF) families, placenta derived growth factor (PIGF), hepatocyte growth factor (HGF), and angiopoietin. In one specific embodiment, the nucleic acid encodes vascular endothelial growth factor (VEGF).

In one embodiment the therapeutic substance i.e. chemicals, drugs, proteins or genes enhancing endothelialisation of the scaffold are attached to the inner device. The cells growing into the scaffold are stimulated for angiogenesis and result in endothelialisation of the scaffold lumen meanwhile the inner device limits the magnitude of this process, a process that results in endothelialised or vascularised surface with the earlier described advantages of such a surface when the implant is then relocated to its anatomical location.

In one advantageous embodiment, the therapeutic substance is present in a reservoir separate from said core enabling a successive delivery thereof to a mammalian body. The device can be
initially implanted in any tissue unconnected to the vessel system to allow ingrowth of the tissue to the scaffold and development of the endothelial surface in the scaffold lumen. Most preferable initial tissues are peritoneal cavity, omentum or fat tissue.

5 In a second aspect, the invention provides a method for producing a device comprising a scaffold and inner device. Furthermore, the device can be formed either by the pretreating of the inner device with drugs, proteins or genes, and then fabricating the device from the treated inner device and scaffold, or by first fabricating the inner device and then treating the space between the inner device and scaffold, or first pretreating of the inner device lumen with drugs, proteins or genes and then fabricating the complete device by attaching inner device to it. Or the scaffold and inner device may be pretreated or nontreated separately and aligned to a device just before first implantation.

In a third aspect, in general terms, the present invention relates to methods for endothelialisation or capillarisation of tubular medical devices. More specifically, the method according to the invention for endothelialisation of vascular grafts by introducing a novel type of device disclosed as a method of improving a mammalian, e.g. a human, body’s acceptance of a synthetic surface, which method comprises introducing a device comprising a scaffold with synthetic surface and an inner device implantable in the body with an at least partial contact with bodily tissues in location other than anatomical location, allowing tissue grow into the scaffold, removing the device from primary location, removing the inner device and implanting the scaffold with a healed surface to an anatomical location. The methods of the invention thus generally comprise to contact any tissue other than the anatomical location for the tubular structure with the device, allow the tissue grow to the outer scaffold of the device while the inner device inhibits occlusion of the scaffold and thus regulates the size of the lumen, remove the device from its original location and remove the inner device, and implant the scaffold to the anatomical location in connection to a tubular structure in the body, most preferably a blood vessel. Alternatively, the tissue may be wrapped around the tubular graft, preferably vascular graft, before implantation to the body. The alternative methods are characterised in that a therapeutic substance i.e drug, protein, nucleic acids, isolated vascular cells or stem cells are promoting endothelialisation in vivo at least partially on said synthetic surface, said administration of therapeutic substance being performed in unanatomical location before, simultaneously as or after the introduction of the device in the body. The present innovation also relates to possibility to promote tissue growth into the scaffold with therapeutic substances and thus shorten the time needed for the initial maturation (growth of the tissue to the scaffold). In the promotion of endothelialisation with therapeutic substances, the preferred method involves to first add the therapeutic substance to the tissue compatible medium, to impregnate the inner device with the therapeutic substance-medium composition, and add the scaffold surrounding the inner device, then to use the impregnated device to contact an unanatomical tissue location,
remove the device, remove the inner device and contact the appropriate anatomical tubular structure with the scaffold. Alternatively, the tissue compatible medium can first be administered on the inner device, then the therapeutic substance added, scaffold administered around the inner device, whereafter the therapeutic substance-inner device-scaffold composition is applied to the primary unanatomical implantation site and tissue allowed to grow into it, the device removed from the primary location, inner device removed and scaffold implanted to anatomical location. Alternatively, the inner device can also be removed before removing the whole device from primary location. Alternatively, the unanatomical primary location may be in proximity to the anatomical location, in which case the inner device is removed and only ends of the scaffold mobilised, whereafter the scaffold is connected to the tubular structure. Also, a device containing therapeutic substance on the inner device can be used in combination with administration of therapeutic substance in the tissues surrounding the implant before or after implantation. The tubular scaffolds, such as vascular grafts, have a porosity that is high enough to allow growth of endothelial cells through the pores. As the skilled in this field will realise, combinations of such administrations are possible, such as a first administration of a certain amount, the introduction of the device, and thereafter one or more additional administration, either according to a predetermined scheme or depending on the body's acceptance thereof and the rate of growth of the new endothelial layer on the synthetic surface.

Depending on the nature of the situation, i.e., the condition of the patient who is to receive the device, the therapeutic substance may be drug stimulating endothelial cell growth, or protein, or nucleic acid encoding a protein or a polypeptide selected from the group consisting of fibroblast growth factor (FGF), platelet derived growth factor (PDGF), transforming growth factor (TGF) and epidermal growth factor (EGF) families, placenta derived growth factor (PIGF), hepatocyte growth factor (HGF) and angiopoietin, and specifically vascular endothelial growth factor (VEGF). As discussed above in relation to the device according to the invention, the nucleic acid can be administered in naked form, in a viral vector such as a retrovirus, a Sendai virus, an adeno associated virus or an adenovirus, or in a liposome. Furthermore, isolated endothelial cells, isolated cells from vasculature, peripheral stem cells, bone marrow and embryonic stem cells can be used as therapeutic substance.

Vascular graft materials

Materials used in the scaffolds and inner devices according to the invention

As used herein, the following terms and words shall have the following ascribed meanings. Tubular synthetic graft, will refer to an object that is fabricated, at least in part, from a biomaterial, and is intended for use in contact with tubular bodily tissues, including bodily fluids. Biomaterial shall refer to the composition of the material used to prepare a device, which
provides one or more of surfaces coming into contact with tissues. Porosity and inflections thereof (such as pores and porous), if not specified otherwise, shall refer to a biomaterial having small channels or passages which start at an external (e.g. first major) surface of the biomaterial and extend substantially through the biomaterial to an internal (e.g., second) surface. Rigid and inflections thereof, will, in case of a nonabsorbable biomaterial, when fabricated in the form of an medical device, refer to the ability to withstand the pressures encountered in the course of its use, e.g. to retain patency and pore structure in vivo. The surface shall refer to the interface between the biomaterial and its environment. The term is intended to include the use of the word in both its macroscopic sense (e.g. the two major faces of a sheet of biomaterial), as well as in its microscopic sense (e.g. the lining of pores traversing the material). Endothelialisation will, unless otherwise specified, be used interchangeably with the phrase capillary endothelialisation to refer to the growth of endothelial cells on substantially all tissue contacting surfaces of a biomaterial used to form a porous rigid or nonporous rigid implant.

Thus, the devices of the present invention include tubular medical devices, most preferably vascular grafts, intended for prolonged contact with tissues and blood, and in particular, those that can benefit from tissue growth to the surface, most preferably from the capillary endothelialisation, when used for in vivo applications. Tubular medical devices are also suitable for being placed in contact primary unanatomical tissue location. The latter requirement takes factors, such as the capacity of the said implants to provide a structure for the developing vascular endothelium, into consideration. Ingrown capillaries can provide endothelial cells to line the luminal surfaces of vascular grafts (scaffold) and when relocating the scaffold to anatomical location, protect vascular graft (scaffold) from thrombosis, infection, and development of secondary stenosis due to existence of endothelialised surface from the moment of connection to blood vessels. This means that the device has all the features commonly associated with biocompatibility, in that they are in a form that does not produce an adverse, an allergic, or any other untoward reaction when administered to a mammal.

Materials of the scaffold

Preferred biomaterials are those that provide sufficient rigidity for their intended purposes in vivo. For use in forming a vascular graft, for instance, the biomaterial will be of sufficient rigidity to allow the graft to retain graft patency and avoid dilatation in the course of its intended use. The choice of implant material will differ according to the particular circumstances and the site where the device is initially implanted and to where the scaffold is then relocated. Vascular grafts (scaffold) are made of biomaterials, selected from the group consisting of e.g. tetrafluoroethylene polymers, aromatic/aliphatic polyester resins, polyurethans, and silicone rubbers. However, any type of biocompatible microporous mesh may be used. The said biomaterials can be combined with each other or other substances, such as polyglycolic acid,
polylactic acid, polydioxone and polyglyconate. Preferred are expanded polytetrafluorethylene (ePTFE) and Dacron. Dacron may be with or without velour, or modified in some other way. Dacron is usually woven, braided or knitted and suitable yarns are between 10 and 400 deniers. It is preferably woven. The nodal regions of ePTFE are composed of nonporous PTFE that serves to provide tear resistance (e.g. for sutures and resistance to aneurysmal dilatation). The internodal regions are composed of fibers of PTFE, which serve to connect the nodes with the spaces between the fibers providing the porosity referred to herein. The nodal size can be expressed as the percentage of the tissue-contacting surface that is composed of nodal PTFE. The distance between nodes can be expressed as the average fibril length. In turn, the porosity is commonly expressed as the internodal distance (i.e. the average distance from the middle of one node to the middle of the adjacent node). Preferred ePTFE materials have nodes of sufficient size and frequency to provide adequate strength (e.g., with respect to aneurysmal dilatation) and internodal regions of sufficient frequency and fiber length to provide adequate porosity (to allow for capillary endothelialisation). Given the present specification, those skilled in the art will be able to identify and fabricate devices using biomaterials having a suitable combination of porosity and rigidity. Biomaterials are preferably porous to allow the attachment and migration of cells, which may be followed by the formation and growth of capillaries into the surface. Suitable pores can exist in the form of small channels or passages, which start at an external surface and extend partially or completely through the biomaterial. In such cases, the cross sectional dimensions of the pore capillary diameter are greater than 5 microns and typically less than 1 mm. The upper pore size value is not critical as long as the biomaterial retains sufficient rigidity, however it is unlikely that useful devices would have a pore size greater than about 1mm. Such pore dimensions can be quantified in microscope. As will be understood by those skilled in the art, several modifications of the graft materials and surfaces can be made, such as precoating with, for example, proteins (see e.g. 5,037,377, 4,319,363), non-heparinised whole blood and platelet rich plasma, glow-discharge modifications of surfaces, adding pluronic gel, fibrin glue, fibronectin, adhesion molecules, covalent bonding, influencing surface charges, with for example carbon (5,827,327, 4,164,045), and treating with a surfactant or cleaning agent, without excluding any other method. Moreover, the implant can be constructed as a hybrid of different internodal distances for the inner and outer surfaces, such as 60 microns as an outer value and 20 microns as an inner value, for the internodal distances (HYBRID PTFE). Also, more layers with different internodal distances may be used. They are all intended to fall within the scope of the present invention when not inhibiting endothelialisation. Potential biodegradable vascular implants may be used in connection with the compositions, devices and methods of this invention. For example, biodegradable and chemically defined polylactic acid, polylactic acid, matrices of purified proteins, semi-purified extracellular matrix compositions and also collagen can be employed. Also, naturally occurring autogenic, allogenic and xenogenic material, such as an umbilical vein, saphenous vein, native bovine artery or intestinal sub-mucosal tissue may be used as a vascular implant material. Examples of clinically used grafts are disclosed in
4,187,390, 5,474,824 and 5,827,327. Biodegradable or bioabsorbable materials, such as homopolymers e.g. poly-paradoxanone, polysyline or polyglycolic acid and copolymers; e.g., polylactic acid and polyglycolic acids or other bio materials, may be used either alone or in combination with other materials as the vascular graft material, as long as they provide the required rigidity. Also, other biological materials, such as intestinal submucosa, matrices of purified proteins and semi-purified extracellular matrix compositions may be used. Appropriate vascular grafts (scaffolds) will provide a surface for new endothelium growth, i.e., will act as an in situ scaffolding through which endothelial cells may migrate. It will be understood by a person skilled in the art that any material with biocompatibility, rigidity and porosity to allow transgraft growth will be acceptable.

Inner device
For use in forming an inner device, for instance, the biomaterial will be of sufficient rigidity to prevent excessive tissue growth into the scaffold lumen and regulate the inner diameter of the lumen in the course of its intended use. The choice of implant material will differ according to the particular circumstances and the site where the device is initially implanted and what kind of scaffold is chosen. Inner device are made of a biocompatible material selected from the group consisting of metal, titanium alloys, tin–nickel alloys, shape memory alloys, aluminium oxide, platinum, platinum alloys, stainless steel, MP35N, elgiloy, stellite, pyrolytic carbon, silver carbon, glassy carbon, polymer, polyamide, polycarbonate, polyether, polyester, polyolefin, polyethylene, polypropylene, polystyrene, polyurethane, polyvinyl chloride, polyvinylpyrrolidone, silicone elastomer, fluoropolymer, polycrlylate, polyisoprene, polytetrafluoretylene, rubber, ceramic, hydroxyapatite, human protein, human tissue, animal protein, animal tissue, bone, skin, laminin, elastin, fibrin, wood, cellulose, compressed carbon and glass. Inner device inhibits both excessive tissue growth to the scaffold lumen and regulates the inner diameter of the future lumen for vascular grafts but can also be used to deliver the drug, protein or gene composition to stimulate the tissue growing into the scaffold. The said biomaterials can be combined with each other or other substances, such as polyglycolic acid, polylactic acid, polydioxane and polyglyconate. Preferred are plastics. Given the present specification, those skilled in the art will be able to identify and fabricate suitable inner devices using biomaterials. Biomaterials may be porous or nonporous but are preferably nonporous to allow an even surface. However, if maximal release of the therapeutic substances is required the inner device may be porous. Suitable pores can exist in the form of small channels or passages, which start at an external surface and extend partially or completely through the biomaterial. In such cases, the cross sectional dimensions of the pores may be of any diameter. As will be understood by those skilled in the art, several modifications of the inner device surfaces or combinations thereof can be made, such as precoating with, for example, proteins (see e.g. 5,037,377, 4,319,363), non-heparinised whole blood and platelet rich plasma, glow-discharge modifications of surfaces, adding pluronic gel, fibrin glue, fibronectin, adhesion molecules,
covalent bonding, influencing surface charges, with for example carbon (5,827,327, 4,164,045), and treating with a surfactant or cleaning agent, without excluding any other method. They are all intended to fall within the scope of the present invention when not inhibiting endothelialisation. Potential biodegradable inner devices may be used in connection with the compositions, inner devices and methods of this invention. For example, biodegradable and chemically defined polylactic acid, polyglycolic acid, matrices of purified proteins, semi-purified extracellular matrix compositions and also collagen can be employed. Also, naturally occurring autogenic, allogenic and xenogenic material, such as collagen may be used as a vascular implant material. Biodegradable or bioabsorbable materials, such as homopolymers e.g. polyparadioxanone, polylysine or polyglycolic acid and copolymers; e.g., polylactic acid and polyglycolic acids or other bio materials, may be used either alone or in combination with other materials as the inner device material, as long as they provide the required rigidity. Also, other biological materials, such as matrices of purified proteins and semi-purified extracellular matrix compositions may be used. Appropriate inner devices will prevent excessive tissue growth into the scaffold lumen, regulate the inner diameter of the lumen in the course of its intended use and provide a surface for therapeutic substance delivery for cells growing through the scaffold, i.e., will act as an in situ drug delivery platform on to which endothelial cells and surface grows to. It will be understood by a person skilled in the art that any material with biocompatibility and rigidity will be acceptable.

Therapeutic substance composition

In another embodiment, the protein and nucleic acids are administered or attached to the device before introduction thereof in a mammalian body. In a specific embodiment, this is achieved by attaching the nucleic acid to the core by ionic or covalent bonding. This embodiment may if appropriate be combined with the last mentioned above, so as to provide a method wherein the device has been pretreated with nucleic acid, while the tissue surrounding the device is later supplemented with further additions of nucleic acid present in a suitable carrier. In one embodiment which is advantageous due to its simplicity, said carrier is sterile water or a sterile aqueous solution.

In alternative embodiments of the present method, the biologically compatible medium is a biostable polymer, a bioabsorbable polymer, a biomolecule, a hydrogel polymer or fibrin. In specific embodiments preclotted blood and pluronic gel are used,

The drugs, proteins and genes i.e therapeutic substances can be made into the compositions appropriate for contacting cells growing into the scaffold with appropriate (e.g. pharmaceutically acceptable) excipients, such as carriers, adjuvants, vehicles, or diluents. The means of making such a composition, and means of administration, have been described in the art. Where appropriate, the vectors can be formulated into preparations in solid, semisolid, liquid, or aerosol
forms, such as aerosol, spray, paste, ointment, gel, glue, powders, granules, solutions, injections, creme and drops, in the usual ways for their respective route of administration without excluding any other method. A pharmaceutically acceptable form, that does not ineffectuate the compositions of the present invention should be employed. In pharmaceutical dosage forms, the compositions can be used alone or in an appropriate association, as well as in combination with other pharmaceutically active compounds. For example, VEGF proteins and nucleic acids encoding for VEGF can be administered together with nucleic acids encoding for inhibiting platelet deposition or smooth muscle cell proliferation. Accordingly, the pharmaceutical composition of the present invention can be administered via various ways on the inner device. A person skilled in the art will recognise that although more than one way can be used for administration, a particular way can provide a more immediate and more effective reaction than the other way.

Local delivery can be accomplished by administration comprising topical application or instillation of the formulation on the inner device, inner side of the scaffold or in the space between the inner device and scaffold, or administration of the formulation directly, to the tissues surrounding the implant in vivo, or any other topical application method. Preferably, the local delivery is accomplished by application of the formulation on the inner device. Administration of the drug, protein or nucleic acid this way, enables the drug to be site-specific, in a way that release of high concentrations and/or highly potent drugs may be limited to direct application to the targeted tissue. Preferred methods is to deliver drugs, proteins or nucleic acids in an aqueous solution incorporated in fibrin, hydrogel, glycosaminoglycans, glycopolysaccharides, or any other biocompatible polymeric carrier matrix, such as alginate, collagen, hyaluronic acid, polyurethane, cellulose, polylactic acid which covers at least a portion of the implant (5,833,651). Drugs, proteins or nucleic acids can be added to the polymer-coated implant, either at the time of implant manufacture or by the physician prior to, during or after implantation. The polymer may also be either a biostable or a bioabsorbable polymer, depending on the desired rate of release or the desired degree of polymer stability. It may be naturally occurring or synthetic compound, also derivatives and salts of the compounds are included. A bioabsorbable polymer is more desirable, as it causes no chronic local response. Bioabsorbable polymers that may be used include, but are not limited to, poly(L-lactic acid), polycaprolactone, poly(lactide-coglycolide), poly(hydroxybutyrate), poly(hydroxybuturate-co-valerate), polydioxanone, polyorthoester, polyanhydride, poly(glycolic acid), poly(D,L-lactic acid), poly(lactic-co-glycolic acid), polyglactin, polydioxone, polygluconate, poly(glycolic acid-cotrimethylene carbonate), polyphosphoester, polyphosphoester urethane, poly(amino acids), cyanoacrylates, poly(trimethylene carbonate), poly(iminocarbonate), copoly(ether-esters)(e.g. PEO/PLA), polyalkylene oxalates, polyphosphazenes, and biomolecules, such as fibrin, fibrinogen, cellulose, starch, collagen, mucin, fibronectin, and hyaluronic acid. Also, biostable polymers with a relatively low chronic tissue response, such as polyurethanes, silicones, and
polyesters could be used if they can be dissolved and cured or polymerised on the implant, such as polyleolefins, polyisobutylene and ethylene-alphaolefin copolymers; acrylic polymers and copolymers, vinyl halide polymers and copolymers, such as polyvinyl chloride; polyvinyl ethers, such as polyvinyl methyl ether; polyvinylidine halides, such as polyvinylidene fluoride and polyvinylidene chloride; polyacrylonitrile, polyvinyl ketones; polyvinyl aromatics, such as polystyrene, polyvinyl esters, such as polyvinyl acetate; copolymers of vinyl monomers with each other and olefins, such as ethylene-methyl methacrylate copolymers, acrylonitrile-styrene copolymers, ABS resins, ethylene-vinyl acetate copolymers: polyamides, such as Nylon 66 and polycaprolactam; alkyd resins; polycarbonates; polyoxymethylene; polyimides; polyethers; epoxy resins; polyurethanes; rayon; rayon-triacetate; cellulose, cellulose acetate, cellulose butyrate; cellulose acetate butyrate; cellophane; cellulose nitrate; cellulose propionate; cellulose ethers; and carboxymethylcellulose (5,776,184). Also fibrin together with other biocompatible polymers, either natural or synthetic and their derivatives and salts, may be used. Fibrin has a number of features that make it particularly suited for sustained gene delivery. Fibrin has holes, gaps and spaces that support and provide room. After implantation, the drugs, proteins and nucleic acid moves from the fibrin mesh to the tissues surrounding the implant. Fibrin is capable of dehydration and rehydration, which makes a fibrin covered implant suitable for loading in a liquid suspension. Fibrin is also biodegradable and fibrin biodegradation on a fibrin/nucleic acid implant further facilitates nucleic acid contact with the surrounding tissue. Of the polymers glycopolysaccharides may be advantageous. In one aspect there is a solid/solid solution of polymer and drug. This means that the drug and the polymer both are soluble in the same solvent and have been intimately admixed in the presence of that solvent. The therapeutic substance and polymer can be applied in various ways, such as by simply immersing the implant into the solution or by spraying the solution onto the implant (5,776,184).

Various hydrogel polymers can be used, such as those selected from the group consisting of polycarboxylic acids, cellulose polymers, gelatin, alginate, poly 2-hydroxyethylmethacrylate (HEMA) polyvinylpyrrolidone, maleic anhydride polymers, polyamids, polyvinyl alcohols, polyethylene oxides, polyethylene glycol, polyacrylamide, polycarboxylic acids, e.g. polyacrylic acids, polysaccharide, e.g. a mucopolysaccharide such as hyaluronic acid (5,674,192)(5,843,089). The polymer can be porous or nonporous on the implant. Several layers of polymers can be utilised and several different polymers can be combined on the same implant. Different layers and different polymers can carry different pharmacological substances (5,833,651). Also, one or more surfaces of the inner device can be coated with one or more additional coats of polymer that is the same or different from the second polymer. The adhesion of the coating and the rate at which the therapeutic substance is delivered can be controlled by selection of an appropriate bioabsorbable or biostable polymer, and by the ratio of drug to polymer in the solution (5,776,184). The dosage applied to the inner device may also be controlled by regulating the time of presoaking therapeutic substance into the hydrogel coating to determine the amount of
absorption of the drug solution by the hydrogel coating. Other factors affecting the dosage are
the concentration of the therapeutic substance in the solution applied to the coating, and the
drug-releasability of the hydrogel coating, determined by, for example, the thickness of the
hydrogel coating, its resiliency, porosity and the ability of the hydrogel coating to retain the
drug, e.g. electrostatic binding or pore size, or the ionic strength of the coating, e.g. changed by
changing the pH. The release of the solid/solid solution of polymer and therapeutic substance can
further be controlled by varying the ratio of drug to polymer in the multiple layers. Coating need
not be solid/solid solution of polymeric and therapeutic substance, but may instead be provided
from any combination of therapeutic substance and polymer applied to implant. The ratio of
therapeutic substance to polymer in the solution will depend on the efficacy of the polymer in
securing the therapeutic substance onto the implant and the rate at which the coating is to release
the therapeutic substance to the tissues. More polymer may be needed if it has a relatively poor
efficacy in retaining the therapeutic substance on the implant, and more polymer may be needed
in order to provide an elution matrix that limits the elution of a very soluble therapeutic
substance. Therefore, a wide therapeutic substance-to-polymer rate could be appropriate, and it
could range from about 10:1 to 1:100 (5,776,184). Binding of the drug may also be
accomplished by electrostatic attraction of the drug to the coating or to a coating additive or a
mechanical binding, for example by employing a coating having a pore size that inhibits inward
flow of body fluids or outward flow of the drug itself, which might tend to release the drug.

Hydrogels are particularly advantageous in that the therapeutic substance is held within the
hydrogen-bond matrix formed by the gel (5,674,192). Examples of hydrogels are for example
HYDROPLUS ® (5,674,192), CARBOPOL ® (5,843,089), AQUAVENE ® (4,883,699),
HYPAN ® (4,480,642) or any of the pluronic gelsuch as pluronic gel F-127 (5,674,192). In
some cases, the hydrogel may be crosslinked prior to lining the implant, for example the
hydrogel coating on a vascular or endovascular graft may be contacted with a primer dip before
the hydrogel is deposited on the implant. If crosslinked it forms a relatively permanent lining on
the implant surface, and if left uncrosslinked it forms a relatively degradable lining on the
implant surface. For example, the longevity of a crosslinked form of a given hydrogel in the stent
lining, has been at least twice to that of its uncrosslinked form (5,843,089). Alternatively, the
hydrogel lining may be contacted with a crosslinking agent in situ (5,843,089). In general, when
dry, the hydrogel coating is preferably on the order of about 1 to 10 microns thick, and typically
of 2 to 5 microns. Very thin hydrogel coatings, e.g., of about 0.2-0.3 microns (dry) and much
thicker hydrogel coatings, e.g., more than 10 microns (dry) are also possible. Typically, the
hydrogel coating thickness may swell with a factor of about 6 to 10 or more, when hydrogel is
hydrated (5,674,192). Usually, the polymeric carrier will be biodegradable or bioeroding (taught
for example by 5,954,706, 5,914,182, 5,916,585, 5,928,916). The carrier can also be constructed
to be a biodegradable substance filling the pores, and release one or more substances into the
surrounding tissue by progressive dissolution of the matrix. Subsequently the pores will open.
The delivered therapeutic substances may be drugs, proteins or nucleic acids encoding for therapeutic protein, e.g. a naked nucleic acid or a nucleic acid incorporated into a viral vector or liposome.

Generally, attachment of the nucleic acid to the device, preferably to inner device, can also be done in several other ways, such as by using covalent or ionic attachment techniques. Typically, covalent attachment techniques require the use of coupling agents, such as glutaraldehyde, cyanogen bromide, p-benzoquinone, succinic anhydrides, carbodiimides, diisocyanates, ethyl chloroformate, dipyrirdyl disulphide, epichlorohydrin, azides, among others, without excluding any other agent, but any method that uses the described methods of this invention can be used and will be recognised by a person skilled in the art. Covalent coupling of a biomolecule to a surface may create undesirable crosslinks between biomolecules, and thereby destroying the biological properties of the biomolecule. Also, they may create bonds amongst surface functional sites and thereby inhibit attachment. Covalent coupling of a biomolecule to a surface may also destroy the biomolecules three-dimensional structure, and thereby reducing or destroying the biological properties (5,928,916). Ionic coupling techniques have the advantage of not altering the chemical composition of the attached biomolecule, and ionic coupling of biomolecules also has an advantage of releasing the biomolecule under appropriate conditions. One example is (4,442,133). The current techniques for immobilisation of biomolecules by an ionic bond have been achieved by introducing positive charges on the biomaterial surface utilising quaternary ammonium salts, polymers containing tertiary and quaternary amine groups, such as TDMAC, benzalconium chloride, cetylpyrridinium chloride, benzylidimethylstearylammonium chloride, benzylcetyldimethylammonium chloride, guanidine or biguanide moiety (5,928,916). The particular type of attachment method when practising the methods and compositions of the invention is not important, as long as the therapeutic substance on the inner device or released from the inner device stimulates the tissue growing into the scaffold in such a way that growing tissue is activated and, in the context of in vivo embodiments, ultimately give rise to endothelialisation of the scaffold without causing adverse reactions. The methods described herein are by no means all inclusive, and further methods to suit the specific application will be apparent to the skilled person of the art.

The composition of the present invention can be provided in unit dosage form, wherein each dosage unit, e.g. solution, gel, glue, drops and aerosol, contains a predetermined amount of the composition, alone or in appropriate combination with other active agents. The term unit dosage form, as used herein, refers to physically discrete units suitable as unitary dosages for human substance, whereby each unit contains a predetermined quantity of the compositions of the present invention, alone or in combination with other active agents, calculated in an amount sufficient to produce the desired effect, in association with a pharmaceutically acceptable diluent, carrier, or vehicle, where appropriate. The specifications for the unit dosage forms of the
present invention depend on the particular effect to be achieved, and the particular pharmacodynamics associated with the pharmaceutical composition in the particular host.

Accordingly, in some embodiments the present invention also provides a method of transferring a therapeutic substance to a host, which comprises administering the therapeutic of the present invention, preferably as a part of composition with the implant, using the aforementioned ways of administration or alternative ways known to those skilled in the art. The effective amount of the composition is such as to produce the desired effect in a host, which can be monitored using several end-points known to those skilled in the art. Effective stimulation of the endothelialisation, in accordance with the present invention, can be monitored in terms of a therapeutic effect (e.g. formation of capillaries, endothelialisation of surfaces and improved patency), or further by in case of genes evidence of the transferred gene or expression of the gene within the host (e.g. using the polymerase chain reaction in conjunction with sequencing, Northern or Southern hybridisations, or transscription assays to detect the nucleic acid in host cells, or using immunoblot analysis, antibody-mediated detection, mRNA or protein half-life studies, or particularised assays to detect protein or polypeptide encoded by the transferred nucleic acid, or impacted in level or function due to such transfer). One such particularised assay described in the examples includes Western immunoassay for detection of proteins encoded by the VEGF-gene. These methods are by no means all-inclusive, and further methods to suit the specific application will be apparent to a person skilled in the art. Moreover, the effective amount of the compositions can be further approximated through analogy to compounds known to exert the desired effect (e.g., compounds traditionally employed to stimulate angiogenesis can provide guidance in terms of the amount of a VEGF protein and nucleic acid to be administered to a host).

Furthermore, the preferred amounts of each active agent included in the compositions according to the invention, VEGF protein and gene is preferably included from about 0.1 micrograms to 10000 micrograms (although any suitable amount can be utilised either above, i.e. greater than about 10000 micrograms, or below, i.e. less than about 0.1 micrograms), provide general guidance of the range of each component to be utilised by the practitioner upon optimising the methods of the present invention for practice in vivo. Moreover, such ranges by no means preclude use of a higher or lower amount of a component, as might be warranted in a particular application. For instance, actual dose and schedule can vary depending on whether the compositions are administered in combination with other pharmaceutical compositions, or depending on interindividual differences in pharmacokinetics, drug disposition, and metabolism. A person skilled in the art can easily make any necessary adjustments in accordance with the exigencies of the particular situation. The amount of therapeutic substance that is applied to the surrounding tissue or the amount of gene composition that is applied on the implant or in the tissue, will be finally determined by the attending physician considering various biological and
medical factors. For example, one would wish to consider the particular therapeutic substance 
and vascular implant material, patient or animal size, age, sex, diet, time of administration, as 
well as any further clinical factors that may affect endothelialisation, such as serum levels of 
different factors and hormones. The suitable dosage regimen will therefore be readily 
determinable by a person skilled in the art in light of the coming disclosure, bearing the 
individual circumstances in mind.

Also, for these embodiments, when one or more different therapeutic substances (i.e. each 
encoding one or more different therapeutic genes) are employed in the methods described herein, 
the contacting of cells ingrowing to the scaffold with various components of the present 
invention can occur in any order or can occur simultaneously. Preferably it occurs 
simultaneously.

Experimental

I. 6 devices comprising knitted Dacron grafts (3 -5 mm inner diameter) as scaffolds and inner 
device with 1 mm smaller inner diameter are implanted to rat and rabbit peritoneum.

Six grafts are inoperated in same animal. First group of grafts is treated with 0,6 mL sterile water 
and airdrying for 1 hour. The second group is precoated with rat fibronectin 0,25 ug/ul (Sigma 
Chemical Co. St Louis, Mo) at a concentration of 10 ug/cm² and air dried for 1 hour. Thereafter 
dimeric plasma fibronectin’s heparin affinity is utilised by introducing heparin (Löwens, 
Ballerup, Denmark) at a concentration 2 U/cm² onto the Dacron graft surface for 1 hour. The 
third and fourth group receive in addition VEGF- plasmid (2 ug/uL; 300 ug/cm2) and VEGF 
protein solution on fibronectin-heparin coating, respectively. Grafts are let to airdry for one hour. 
Control animals receive on the right side of the abdominal wall plain control grafts and on the 
left side side control grafts with fibronectin and heparin. Treatment animals got on the right side 
of the abdominal wall grafts with the VEGF protein and on the left side grafts with VEGF 
plasmid fibronectin/heparin and plasmid. Animals are followed for 2 weeks (n=2).

Endothelialisation is measured with light and scanning electron microscopy.

II. 6 devices comprising knitted Dacron grafts (3 -5 mm inner diameter) as scaffolds and inner 
device with 1 mm smaller inner diameter is implanted to rat and rabbit peritoneum.

Six grafts are inoperated in same animal. Control group receives no treatment. The second group 
is precoated with preclotted blood, third group with preclotted blood-VEGF protein and fourth 
group with preclotted blood-VEGF-plasmid. Control animals receive on the right side of the
abdominal wall plain control grafts and on the left side side preclotted control grafts. Treatment animals get on the right side of the abdominal wall grafts with the preclotted blood-VEGF protein and on the left side grafts with preclotted blood-VEGF plasmid. Animals are followed for 2 weeks (n=2) and 4 weeks (n=2). Endothelialisation is measured with light and scanning electron microscopy.

III. 6 devices comprising knitted Dacron grafts (3-5 mm inner diameter) as scaffolds and inner device with 1 mm smaller inner diameter is implanted to rat and rabbit peritoneum.

Six grafts are inoperated in same animal. Control group receives no treatment. Inner device of the second group is treated with pluronic, Inner device of the third group with pluronic-VEGF protein and inner device of fourth group with pluronic-VEGF-plasmid. Control animals receive on the right side of the abdominal wall plain control grafts and on the left side side preclotted control grafts. Treatment animals get on the right side of the abdominal wall grafts with the preclotted blood-VEGF protein and on the left side grafts with preclotted blood-VEGF plasmid. Animals are followed for 2 weeks (n=2) and 4 weeks (n=2). Endothelialisation is measured with light and scanning electron microscopy.

IV. 6 devices comprising knitted Dacron grafts (3-5 mm inner diameter) as scaffolds and inner device with 1 mm smaller inner diameter is implanted to rat and rabbit peritoneum.

Six grafts are inoperated in same animal. Control group receives no treatment. Inner device of the second group is treated with fibrin, inner device of the third group with fibrin-VEGF protein and inner device of fourth group with fibrin-VEGF-plasmid. Control animals receive on the right side of the abdominal wall plain control grafts and on the left side side preclotted control grafts. Treatment animals get on the right side of the abdominal wall grafts with the preclotted blood-VEGF protein and on the left side grafts with preclotted blood-VEGF plasmid. Animals are followed for 2 weeks (n=2) and 4 weeks (n=2). Endothelialisation is measured with light and scanning electron microscopy.

V. 6 devices comprising knitted Dacron grafts (3-5 mm inner diameter) as scaffolds and inner device with 1 mm smaller inner diameter is implanted to rat and rabbit peritoneum.

Six grafts are inoperated in same animal. Control group receives no treatment. Inner device of the second group is treated with cationic coating, inner device of the third group with VEGF protein on cationic coating and inner device of fourth group with VEGF-plasmid and cationic coating. Control animals receive on the right side of the abdominal wall plain control grafts and on the left side side preclotted control grafts. Treatment animals get on the right side of the abdominal wall grafts with the preclotted blood-VEGF protein and on the left side grafts with...
preclotted blood-VEGF plasmid. Animals are followed for 2 weeks (n=2) and 4 weeks (n=2). Endothelialisation is measured with light and scanning electron microscopy.

Similar experiments can be performed with 60 microns ePTFE grafts as scaffold and inner devices. Furthermore, some of the animals will get FGF-2 protein and gene encoding for FGF-2 instead of VEGF.
Claims:

1. A kit, useful in vascular surgery, said kit comprising:
a) a porous tubular scaffold; and
b) an inner device adapted for being inserted into said porous tubular scaffold, said inner device
having essentially the same cross-sectional shape as the cross-sectional cavity of the tubular
scaffold, the cross-sectional area of said inner device being from 10 to 99 % of the cross-
sectional area of the cavity of said porous tubular scaffold, said inner device having essentially
the same length as said porous tubular scaffold.

2. A kit according to claim 1, characterised in that the inner diameter of the porous tubular
scaffold is from 1.0 mm to 50 mm, and preferably from 2 mm to 6 mm.

3. A kit according to anyone of claims 1 – 2, characterised in that the thickness of the porous
tubular scaffold is from 0.1 – 2 mm, and preferably from 0.3 – 0.6 mm.

4. A kit according to anyone of claims 1 – 3, characterised in that the porosity of the porous
tubular scaffold is from 30 μm to 2000 μm.

5. A kit according to anyone of claims 1 – 4, characterised in that the cross-sectional diameter
of the inner device is 40 μm to 2000 μm less than the cross-sectional inner diameter of the
porous tubular scaffold.

6. A kit according to anyone of claims 1 – 5, characterised in that the porous tubular scaffold
has been produced from woven or knitted synthetic fibres.

7. A kit according to anyone of claims 1 – 6, characterised in that at least one of the ends of the
inner device is equipped with a handling device.

8. A kit according to anyone of claims 1 – 7, characterised in that the porous tubular scaffold is
made of a material selected from the group of tetrafluoroethylene polymers, aromatic/aliphatic
polymer resins, polyurethanes, silicone rubbers, polyglycolic acid, polylactic acid, polydioxone,
polyglyconate, collagen and polyethylene terephthalate.

9. A kit according to anyone of claims 1 – 8, characterised in that the inner device is made of a
material selected from the group of metal, titanium, titanium alloys, tin-nickel alloys, shape
memory alloys, aluminium oxide, platinum, platinum alloys, stainless steel, MP35N, elgiloy,
stellite, pyrolytic carbon, silver carbon, glassy carbon, polymer, polyamide, polycarbonate,
polyether, polyester, polyolefin, polyethylene, polypropylene, polystyrene, polyurethane,
polyvinyl chloride, polyvinylpyrrolidone, silicone elastomer, fluoropolymer, polyacrylate, polyisoprene, polytetrafluoroethylene, rubber, ceramic, hydroxyapatite, human protein, human tissue, animal protein, animal tissue, bone, skin, laminin, elastin, fibrin, wood, cellulose, compressed carbon, glass, polyglycolic acid, polylactic acid, polydioxone, and polyglyconate.

10. A kit according to anyone of claims 1 – 9, characterised in that at least one of the porous tubular scaffold and the inner device comprises an attached therapeutic substance having the potential to promote endothelialisation.

11. A kit according to claim 10, characterised in that said therapeutic substance is chosen from the group of VEGF proteins and nucleic acid molecules encoding VEGF proteins, as well as isolated endothelial cells, isolated cells from vasculature, peripheral stem cells, bone marrow and embryonic stem cells.

12. A porous tubular scaffold, useful in vascular surgery, characterised in that said porous tubular scaffold encloses an inner device, said inner device having essentially the same cross-sectional shape as the cross-sectional cavity of the tubular scaffold, the cross-sectional area of said inner device being from 10 to 99 % of the cross-sectional area of the cavity of said porous tubular scaffold, said inner device having essentially the same length as said porous tubular scaffold, said inner device being recoverably attached to an end of said porous tubular scaffold.

13. A porous tubular scaffold enclosing a recoverably attached inner device according to claim 12, characterised in that the scaffold and/or the inner device fulfil the requirements of anyone of claims 2 – 11.

14. A method for endothelialisation or capillarisation of a tubular medical device, comprising the steps of:

a) providing

   i) a porous tubular scaffold according to anyone of claims 12 – 13; or

   ii) a kit according to anyone of claims 1 – 11, wherein said scaffold encloses said inner device;

b) surgically implanting said scaffold according to anyone of claims 12 – 13 or said kit consisting of a scaffold enclosing an inner device according to anyone of claims 1 – 11 into a human body in an anatomic location that does not have a tubular structure;

c) allowing the tissue of said anatomic location to grow into said scaffold;

d) surgically removing said scaffold according to anyone of claims 12 – 13 or said kit consisting of a scaffold enclosing an inner device according to anyone of claims 1 – 11, from said anatomic location, and
e) removing said inner device, thereby obtaining an endothelialised or capillarised tubular medical device.

15. A method for implanting a tubular medical device to an anatomic tubular structure, comprising the steps of:
   a) carrying out the method of claim 14; and
   b) surgically implanting said endothelialised or capillarised tubular medical device to a suitable anatomic tubular structure.
A. CLASSIFICATION OF SUBJECT MATTER

IPC7: A61F 2/06
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: A61F, A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE, DK, FI, NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-INTERNAL, WPI DATA, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>US 20030055494 A1 (DEDN BEUJDENHOUT ET AL), 20 March 2003 (20.03.2003)</td>
<td>1-15</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents
  "A" document defining the general state of the art which is not considered to be of particular relevance
  "B" earlier application or patent filed on or after the international filing date
  "L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another document or other special reason (as specified)
  "O" document referring to an oral disclaimer, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed
  "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  "&" document member of the same patent family

Date of the actual completion of the international search: 1 June 2005
Date of mailing of the international search report: 16-06-2005

Name and mailing address of the ISA/Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86
Authorized officer: Erika Stenroos/MP
Telephone No. +46 8 782 25 00

Form PCT/ISA/110 (second sheet) (January 2004)
**INTERNATIONAL SEARCH REPORT**

<table>
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<tr>
<th>Box No. II</th>
<th>Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)</th>
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<td></td>
<td>This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:</td>
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<tr>
<td>1. ☑ Claims Nos.: 14-15</td>
<td>because they relate to subject matter not required to be searched by this Authority, namely: Claims 14-15 relate to a method of treatment of the human or animal body by surgery or by therapy, as well as diagnostic</td>
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<td>2. ☐ Claims Nos.:</td>
<td>because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:</td>
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<tr>
<td>3. ☐ Claims Nos.:</td>
<td>because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</td>
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<th>Box No. III</th>
<th>Observations where unity of invention is lacking (Continuation of item 3 of first sheet)</th>
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<td>This International Searching Authority found multiple inventions in this international application, as follows:</td>
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1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. |
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. |
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.: |

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: |

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant’s protest. |
- ☐ No protest accompanied the payment of additional search fees. |

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2004)
Box II.1

methods /Rule 39.1(iv). Nevertheless, a search has been executed for these claims. The search has been based on the alleged effects of the device.
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