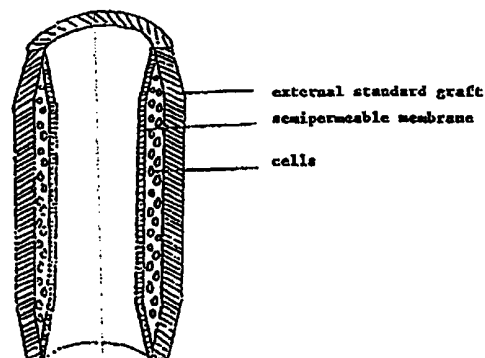
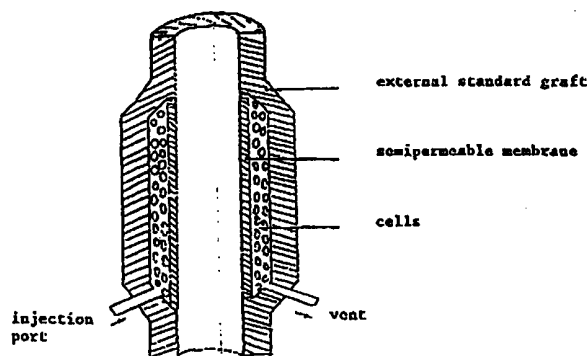


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(54) Title: GENETIC ENGINEERING OF PROSTHETIC BYPASS GRAFTS**(57) Abstract**

A prosthetic graft is disclosed comprised of two coaxial prosthetic tubes confining a space that contains cells that are genetically engineered to overproduce a thrombolytic enzyme. The cells may comprise endothelial cells, smooth muscle cells, fibroblasts, and stem cells. The outer tube is a standard graft. The inner tube is a semipermeable membrane to protect the genetically engineered cells from the shear stress of blood flow and from exposure to inflammatory cells and platelets, while allowing free diffusion and exchange of the thrombolytic enzyme, essential nutrients from the circulation, and waste products of metabolism.

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GENETIC ENGINEERING OF PROSTHETIC BYPASS GRAFTS

1. FIELD OF THE INVENTION

This invention relates to prosthetic bypass grafts, particularly to grafts with improved patency containing genetically engineered cells expressing selected genetic materials of interest and to methods for the use of such prosthetic grafts.

2. DESCRIPTION OF RELATED ART

Many important disease states involve stenosis or occlusion of arteries that supply vital organs. The pathologic mechanism most commonly implicated in such disease states is atherosclerosis. Examples include angina pectoris and myocardial infarction due to coronary artery disease; transient ischemic attacks and strokes due to cerebral vascular disease; renal vascular hypertension, and ultimately, renal failure due to renal artery stenosis; and claudication of the lower extremities, which is caused by vascular disease of peripheral arteries and, in its most severe form, can result in amputation.

An accepted and widely used therapeutic approach to advanced atherosclerotic disease is to bypass the site of major stenosis or occlusion with a prosthetic vessel made of synthetic material, such as Dacron ® or Gore-Tex ®. More than 350,000 vascular grafts are implanted each year. One major problem with this approach is that the prosthetic vessel is thrombogenic (i.e., it has the propensity to develop clots), which leads to a very high rate of graft failure.

During the past decade, a variety of approaches have been used by a small number of investigators to develop ways to improve the patency of bypass grafts. Seeding of endothelial cells (ECs) on prosthetic grafts was used to improve patency rate of small diameter prosthetic grafts (Eickhoff, J.H. et al. (1987) *J. Vasc. Surg.* 6:506-11; Herring, M. et al. (1978) *Surgery* 84:498-504): Though animal studies have shown that EC seeding increases graft patency, similar studies in humans have not shown benefit (Jensen, N. et al. (1994) *J. Cardiovasc. Surg.* 35:425-429). The

major problem encountered is that ECs adhere poorly to prosthetic graft material, and are easily stripped when exposed to flowing blood. This problem is one of several addressed by the design of the new graft of this invention.

Gene transfer to overexpress thrombolytic enzymes has been reported to
5 enhance the anti-thrombotic activity of vascular ECs and smooth muscle cells (SMCs)
(Dichek, D.A. (1993) *Thromb. Haemost.* 70:198-201; Dichek, D.A. et al. (1989)
Circulation. 80:1347-53; Ekhterae, D. and Stanley, J.C. (1995) *J. Vasc. Surg.* 21:953-
82; Podrazik, R.M. et al. (1992) *Ann Surg.* 216:446-52; Wilson, J.M. et al. (1989)
Science 244:1344-6). Tissue plasminogen activator, a thrombolytic proteinase, can
10 be overexpressed by ECs and SMCs following intracellular transfer of the tPA gene
(Ekhterae, D. and J.C. Stanley (1995) *J. Vasc. Surg.* 21:953-82; Shayani, V. et al.
(1994) *J. Surg. Res.* 57:495-504). This enzyme converts inactive plasminogen into
active plasmin, which then degrades fibrin complexes, a major component of
thrombus.

15 Another problem encountered with the seeding of tPA excreting cells is that
overexpression of tPA has been shown to further decrease seeded ECs retention on
prosthetic grafts. Increased tPA-induced nonspecific proteolysis of the supporting
extracellular matrix has been held responsible for this (Dunn, P.F. et al. (1996)
Circulation 93:1439-46; Huber, T.S. et al. (1995) *J. Vasc. Surg.* 22:795-803).

20 Semi-permeable membranes have been reported as effective immunoisolation
devices protecting allografts and xenografts from the host cellular immune response
(Brauker, J. et al. (1996) *Transportation* 67:1671-7; Lanza, R.P. and Chick, W.L.
(1997) *Immunol. Today* 18:135-9; Pollok, J.M.C. et al. (1997) *Transplant*
Proceedings 29:909-11) as well as a physical barrier to platelets. These
25 immunoisolators, implanted in various locations, have been used to deliver
therapeutic agents in animal studies (Cotton, C.K. (1996) *Trends Biotechnol.* 14: 158-
62; Winn, S.R. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:2324-8).

3. SUMMARY OF THE INVENTION

The present invention relates to novel prosthetic bypass grafts with improved patency rates. In particular, the present invention provides a graft comprised of two concentric cylindrical prosthetic tubes confining a space capable of containing
5 genetically engineered cells expressing one or more selected genetic materials of interest or other therapeutic agents. The outer prosthetic tube is a standard graft, whereas the inner prosthetic tube is a semi-permeable membrane.

Preferred prosthetic materials for use within the invention include a broad range of biologically compatible polymers known to have medically useful structure
10 and surface characteristics. Useful polymers within this context include, e.g., polyethyleneterephthalate, polytetrafluoroethylene, expanded polytetrafluoroethylene, polymers of lactide-glycolide, polyglactin, polydioxanone, polyurethanes, polypropylenes and polyesters.

In preferred embodiments, the porosity and the thickness of the semi-
15 permeable membrane are such that (i) the genetically engineered cells are protected from the shear stress of flowing blood as well as from exposure to inflammatory cells and platelets, and (ii) free diffusion and exchange of the thrombolytic enzyme, essential nutrients from the circulation, and waste product of metabolism is accomplished.

20 Cells of this invention include, but are not limited to, endothelial cells, smooth muscle cells, fibroblasts, and stem cells. These cells have stably incorporated in them genetic material of interest (e.g., that which encodes a protein of therapeutic value) and provide constitutive synthesis and delivery thereof useful in the prevention or treatment of diseases. In a preferred embodiment, cells are genetically engineered to
25 overproduce a thrombolytic enzyme or mutant forms thereof which are resistant to degradation. The genetically altered cells secrete the thrombolytic enzyme which diffuses onto the luminal surface of the graft to keep the graft free of thrombus.

Another aspect of the invention relates to grafts capable of releasing a second therapeutic drug. This drug may be housed within the graft with its release controlled by the semipermeable membrane. In a specific embodiment, the genetically engineered cells in the thromboresistant graft produce a second therapeutically active protein. A sustained release of the protein into the circulation is thereby produced.

Within yet another aspect of the invention, methods of treatment are provided which involve implanting a prosthetic graft of the invention. Such methods are useful for treating stenotic or occluded arteries.

4. BRIEF DESCRIPTION OF DRAWINGS

Figures 1a and 1b are perspective views illustrating two variations of the bypass graft.

Figure 2 illustrates the antigen concentration (A) and enzyme activity (B) in the supernatants of cultured EC and tPA transduced EC (EC/tPA).

Figure 3 shows cell growth in a semipermeable membrane graft. Endothelial cells were stained with X-gal one day (A) and one month (B) after placement in "tea bag" of semipermeable membrane. Dark staining represents cells expressing β -Gal enzyme.

5. DETAILED DESCRIPTION OF THE INVENTION

Definitions

The term "cells" refers to cells of the present invention including, but not limited to, endothelial cells, smooth muscle cells, fibroblasts, and stem cells, that express incorporated genetic material encoding a polypeptide or a protein of interest or a gene encoding a selectable marker. Cells which express incorporated genetic material are referred to herein as transduced cells.

The term “genetic material” refers to genetic material of interest that has been incorporated into cells and expressed in the resulting genetically engineered cells. Genetic material includes any nucleic acid molecule.

The term “nucleic acid molecule” encompasses single-stranded and double-stranded DNA molecules, including genomic DNA, cDNA, DNA produced by
5 amplification reaction (such as polymerase chain reaction [“PCR”]), and DNA produced by oligonucleotide synthesis, as well as RNA molecules, such as mRNA. Genomic DNA can include non-transcribed and transcribed regions (such as 5’ and 3’ non-coding regions, introns, and coding regions of the genetic material of interest).
10 cDNA and mRNA molecules contain sequences corresponding to transcribed regions.

The term “genetically engineered” relates to ways of introducing genetic material of interest into cells.

The term “standard graft” refers to tubular grafts of biocompatible materials suitable for use as prosthetic bypass grafts.

15 The term “tissue plasminogen activator” or “tPA” refers to a protein with the general property of activating the plasminogen protein. In particular, tPA refers to the human tissue plasminogen activator.

The term “tPA variant” encompasses proteins and protein fragments substantially homologous to human tissue plasminogen activator that retain the
20 property of activating tissue plasminogen. “Substantially homologous” refers to proteins possessing at least 70% amino acid identity when best aligned with tPA. “Best alignment” is an alignment of two or more sequences, with appropriate gaps introduced, that maximizes identity of identity of corresponding amino acid residues.

The term “reagent” refers to any genetically encoded secreted product of a cell
25 that has biological activity. Reagents include but are not limited to both linear and cyclic peptides, lipids, carbohydrates, nucleic acids, polyketides and antibiotics.

Prosthetic Bypass Grafts

As described above, the present invention relates to prosthetic grafts that are able to deliver therapeutic enzymes directly into the circulation. Well known obstacles encountered in the design of such grafts include (i) platelet adherence, (ii) fibrin crosslinking, (iii) host cell immune responses, (iv) flow induced shear injury of the cells seeded onto the graft surface, and (v) chemically induced detachment of engineered cells (for example by tPA).

These problems are addressed by the present invention, in which a semipermeable membrane is incorporated into the graft design as a physical barrier to protect the genetically engineered cells from the shear stress of blood flow, as well as inhibit immune responses. The use of genetically engineered cells overexpressing a thrombolytic enzyme and the ability to control the amount to be delivered into the circulation will inhibit fibrin crosslinking. Furthermore, platelet adherence can be inhibited by both systemic and/or local therapy (examples include cyclooxygenase inhibitors and hirudin).

The novel graft consists of two concentric cylindrical prosthetic tubes enclosing a space that contains genetically engineered cells which express selected genetic material of interest. The outer prosthetic tube is a standard graft, whereas the inner prosthetic tube is a semi-permeable membrane (Figs. 1a, b). Preferred prosthetic materials for use within the invention include a broad range of biologically compatible polymers known to have medically useful structure and surface characteristics. Useful polymers within this context include, but are not limited to, polyethyleneterephthalate (e.g., Dacron ®), polytetrafluoroethylene, expanded polytetrafluoroethylene (PTFE, e.g., Gore-Tex ®, W. L. Gore, Flagstaff, Ariz.), polyolefins, 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; polyvinylidene 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, and ethylene-vinyl acetate copolymers; polyamides, such as Nylon 66 and polycaprolactam; alkyd resins; polycarbonates; polyoxymethylenes; polyimides; 5 polyethers; epoxy resins; polyurethanes; polyesters; polymers of lactide-glycolide, and polygalactin. In a preferred embodiment, the standard graft is manufactured of PTFE.

The graft can be constructed in a variety of sizes to accommodate a particular vascular application. The reservoir of such an improved graft containing the 10 genetically engineered cells will prevent or treat complications associated with conventional grafts including, but not limited to, protection of the cells from: the shear stress of blood flow; exposure to inflammatory cells; platelet deposition; coagulation; thrombosis; neointimal hyperplasia; and fibrosis. Furthermore, the short half lives of drugs such as hormone polypeptides will not be a limitation in such a 15 continuous delivery system.

The porosity (internodal distance) of the semipermeable membrane can be varied to affect the rate of drug release. Preferably, the porosity and thickness of the semipermeable membrane will permit free diffusion and exchange of the thrombolytic enzyme, essential nutrients from the circulation, and waste products of metabolism, 20 but will prevent cell and platelet trans-membrane passage. Also, the tested semipermeable membrane will be manufactured as a cylinder and placed concentrically into a compatible standard graft.

The genetically engineered cells can be introduced into the graft at a time determined by one with skill in the art. The genetically altered cells preferably will be 25 injected between the two materials at the time of implantation. In one embodiment, the graft comprises an injection port means integral with the external standard graft to facilitate this injection and a vent means to prevent undue pressure during the injection process. The cells can be introduced as a suspension or in any other appropriate form known to one with skill in the art. For example, cells could be

microencapsulated in polymeric materials. In another embodiment, the cells can be introduced to the internal surface of the tubular external graft before putting the internal semipermeable membrane in place.

This prosthesis can be utilized to provide local drug delivery by utilizing
5 arterial blood flow for prevention or treatment of any disease or condition distal to the site of arterial implantation of the device. As contemplated by the present invention, the device described herein may contain a substance in its reservoir that inhibits platelet deposition and thrombus formation or promotes thrombolysis and thrombus dissolution. Examples of such substances (in addition to tPA) include, but are not
10 limited to, plasmin, urokinase (UK), single chain prourokinase (scuPA), streptokinase, prostaglandins, cyclooxygenase inhibitors, phosphodiesterase inhibitors, thromboxane synthetase inhibitors; antagonists of glycoprotein receptors including GP Ib, GP IIb/IIIa, antagonists of collagen receptors, and antagonists of platelet thrombin receptors.

15 In addition, the substance in the reservoir can be any substance, including any drug, and the device can be used for local delivery of such substances to prevent or treat a variety of disease syndromes or to promote or enhance desired activity within the body. For example, the substance can be an anticoagulant, including but not limited to, heparin, hirudin, hirulog, hirugen, activated and non-activated protein C;
20 synthetic or naturally occurring antagonists of: thrombin, Factor Xa, or other activated or non-activated coagulation proteases, coagulation factors, e.g., FbI, FIX, FVIII, FV, FVII, or tissue factor. Since the semi-permeable membrane of the present invention serves as a barrier to cellular immunity, it is possible to use allogenic cells in the graft.

Also, the substance in the reservoir may affect platelet metabolic function.
25 Examples of such substances include, but are not limited to, prostaglandins, cyclooxygenase inhibitors, phosphodiesterase or thromboxane synthetase inhibitors, inhibitors of calcium transport, or elevators of cyclic adenosine monophosphate (cyclic AMP) such as forskolin, or cyclic AMP agonists.

Furthermore, particular examples where the devices of the present invention can be utilized include, but are not limited to, local drug delivery to treat cancer or to maintain perfusion of tissue or organ transplants while the body establishes revascularization of the subject tissue, or as a dialysis access graft. For dialysis access applications, the reservoir can be positioned proximal to the dialysis access site on the graft and utilized to deliver drugs which prevent hyperplasia, thrombosis and occlusion at the distal end of the graft. In the case of diseases such as diabetes release of appropriate amounts of insulin can be envisioned.

Utilizing the disclosed device, one skilled in the art can determine suitable dosage requirements and treatment regimens for any substance placed in the reservoir of the device. Dosages and regimens will vary, of course, depending upon the tissue targeted for therapy and upon the particular drug utilized and can be determined by optimization procedures known in the art. The device described herein is able to achieve very high drug concentrations locally while minimizing total drug requirements and circulating drug levels, therefore allowing for the efficient use of agents which are available in limited amounts or which could produce side effects.

Genetically Engineered Cells

In general, cells are engineered to produce one or more desired proteins or reagents. As the semipermeable membrane protects the cells from cellular immunity, a number of cell types can be incorporated. The preferred embodiments of the invention use mammalian cells including smooth muscle cells, epithelial cells and fibroblasts. Allogenic cells may also be used including, but not limited to, bacterial cells, yeast or other fungal cells, or the cells of higher organisms.

A gene encoding a protein product of interest can be introduced into said cells by the methods of genetic engineering. Methods chosen are within the knowledge of one skilled in the art, and will vary according to the cell type used. Genetic material of interest incorporated into cells according to the method described can be any selected DNA of interest (e.g., all or a portion of a gene encoding a product of interest) or any selected RNA of interest. For example, it can be DNA or RNA which

is present and expressed in normal cells; DNA or RNA which does not normally occur in cells; DNA or RNA which normally occurs in cells but is not expressed in them at levels which are biologically significant (levels sufficient to produce the normal physiological effect of the protein or polypeptide it encodes); DNA or RNA which occurs in cells and has been modified in such a manner that it can be expressed in such cells; and any DNA or RNA which can be modified to be expressed in cells, alone or in any combination thereof. This genetic material of interest is referred to herein as incorporated genetic material. Genetic material of interest incorporated into cells will enable the transduced cells to overproduce the desired therapeutic gene and protein.

A number of methods are available to one with skill in the art for introducing genetic material of interest into cells. Genetic information of interest can be introduced into cells by means of any virus which can express the genetic material of interest in such cells, including, but not limited to, retroviruses, SV40, herpes virus, adenovirus and human papilloma virus. Retroviruses refer to RNA viruses; that is, the viral genome is RNA. This genomic RNA is, however, reverse transcribed into a DNA copy which is integrated stably and efficiently into the chromosomal DNA of transduced cells. This stably integrated DNA copy is referred to as a provirus and is inherited by daughter cells as any other gene.

Genetic material of interest can also be introduced into cells in such a manner that it is not incorporated stably into the recipient cells, but is expressed episomally (remains distinct or separate from the recipient cell genome), for example by using liposomes. Furthermore, chemical or physical means can be used to introduce genetic material of interest into cells. An example of a chemical means is the commonly used calcium phosphate transfection procedure and an example of a physical means is electroporation whereby cells are exposed to an electric current which enables the entry into the cell of genetic material of interest.

The protein product of interest may be in itself a therapeutic protein. In particular the genetic material is chosen such that it encodes one of the desired protein

products discussed above or a variant thereof. The genetic material of interest is chosen such that the protein product is expressed in therapeutically useful amount by the genetically engineered cells.

Alternatively, or in addition, the protein product chosen may not be in itself therapeutic, but may catalyze or help to catalyze the formation of a therapeutic reagent that is secreted by the genetically engineered cells..

EXAMPLES

Without intending to limit it in any manner, the present invention will be more fully described by the following examples. The materials and methods which are common to the examples are as follows.

Methods

Retroviral Vectors Production and Cell Transduction. Both amphotropic and vesicular stomatitis virus G glycoprotein (VSV-G) pseudotyped MuLV vectors were generated from a transient three-plasmid transfection system (Soneoka, 1995). Briefly, 293T cells (American Type Culture Collection, CRL-1573) (1×10^6 cells in a 100 mm-diameter dish) were transfected by calcium phosphate precipitation with plasmids: vector plasmid, gag-pol genes, and env gene of amphotropic or VSV-G gene. The vector plasmid is LtSN for vector delivering tPA gene (Dichek, D.A. et al. (1994) *Blood* 84:504-516). Sixteen hours posttransfection, the cells were cultured with 10 ml of medium containing 10 mM sodium butyrate for 12 h. Then the culture was replaced with 6 ml of fresh medium without butyrate to allow production of retroviral vectors. The viral supernatants were harvested after a further 12 h of incubation at 37 °C, filtered through 0.4 µm filter and titered by infection on NIH 3T3 cells.

Transductions of EC with retroviral vectors were accomplished by placing 5 ml of the LtSN viral supernatants with polybrene (8 µg/ml) onto the cultured EC in a 100 mm plate ($\sim 1 \times 10^6$ cells). One day after the transduction, the cells were cultured in the medium containing 0.6 mg/ml neomycin analog G418 (Gibco/BRL) for 10-

14 days to select the transduced cells. The pool of the selected cells were named as EC/tPA and assayed for their tPA production.

Determination of tPA concentration EC/tPA and EC were plated on 24-well plates with 2×10^5 cells/well and were cultured until 90% confluent. The culture medium was then replaced with 1 ml of serum-free William medium. The cells were
5 continually cultured at 37 °C for 24, 48, 72, and 96 hrs and the supernatants at each time point were collected and stored at -70 °C for different assays described below.

The fibrinolytic enzyme activity of the cell culture supernatant was determined by chromogenic assay using Chromolize tPA assay kit from Biopool (Ventura, CA).
10 The concentration of tPA in the supernatant was determined by measuring the tPA antigen concentration with TintElize tPA ELISA assay kit from Biopool according to the manufacturer's directions.

Endothelial and Smooth Muscle Cell Harvest and Transduction. Endothelial cells can be obtained by standard procedures from umbilical vein, saphenous vein or
15 other sources (e.g., Balconi *et al.* (1986) *Med. Biol.* 64: 231-245; Ryan *et al.* (1985) *Tissue Cell* 17:171-176; Budd *et al.* (1989) *Br. J. Surg.* 76: 1259-1261). Cells can be harvested by mechanical or, preferably, enzymatic methods. In specific, excreta human vein samples can be obtained from patients undergoing bypass procedures. The vein samples are flushed with RPMI 1640 media containing 20 mM HEPES,
20 2 mM L-glutamine, 50 units/ml penicillin, 50 µg/ml gentamicin and 2.5 µg/ml amphotericin B, clamped at one end, filled with 0.1% collagenase I in Dulbecco's phosphate buffered saline (DPBS); and incubated 15 min at 37 °C to dislodge the endothelial cells. The dissociated ECs are pelleted by centrifugation at room temperature, 1200 rpm (200xg) for 5 min. Cells are resuspended and plated on
25 fibronectin (0.1% in PBS) pre-coated dishes in M199 medium (Gibco/BRL, Gaithersburg, MD) with the addition of 20% fetal bovine serum (FBS), 17.5 units/ml porcine intestinal mucosa-derived heparin (Sigma, St. Louis, MO), 50 µg/ml EC growth supplement (Becton Dickinson), 2 mM L-glutamine, and the antibiotics mentioned above.

Following the ECs dissociation, the remainder of the vein samples are used for SMC harvesting. The vein is incised longitudinally and the intimal layer is scraped off with a scalpel. The cellular composition of the underlying media is primarily vascular smooth muscle. The opened vein is placed into tissue culture flasks. These tissues are agitated in DPBS containing collagenase I (1.8 mg/ml) and elastase (0.2 mg/ml) for 1 hour at 37 °C. Single-cell suspension supernatants are removed and pelleted by centrifugation at 1200 rpm for 5 min. These SMCs are resuspended and cultured in M199 medium containing 20% FBS, L-glutamine (2 mM), and the antibiotics mentioned above.

Confirmation of EC and SMC identity of each culture is performed. ECs are characterized by cellular morphological observation through microscopy as well as fluorescent staining for von Willebrand factor and low-density lipoprotein uptake. SMCs are identified by positive reaction in a smooth muscle α -actin assay. Both ECs and SMCs are passaged at a 1:3 ratio and be used for experiments between passages 2 and 15.

The cultured cells are transduced with retroviral vectors by mixing the cultured cells with the viral supernatants. Transduced cells are selected with neomycin analog G418 to produce a population of cells secreting the targeted gene product.

Gene Expression Measurements. The enzyme activity and antigen concentration of tPA in medium conditioned with transduced cells is measured using Chromolize tPA and TintElize tPA assay kits from Biopool (Ventura, CA). Northern blot analysis of transduced ECs and SMCs is performed to identify the persistence and stability of gene expression in the transduced cells. The total cellular RNA is extracted from the ECs and SMCs (Chomczynski, P. and Sacchi, N. (1987) *Anal. Biochem.* 162:156-9). Electrophoresis of 7-10 μ g of each sample through 1.2% formaldehyde agarose gels is performed. The RNA is then blotted onto nitrocellulose membranes (Schleicher and Schuell, Inc., Keene, NH), vacuum dried, and hybridized with a radiolabeled fragment of plasminogen activator cDNA. Membranes are then

washed in 0.2 N saline citrate and 0.1% sodium dodecyl sulfate at 55 °C and subsequently exposed to phosphor screens for quantification on a Phosphorimager (Molecular Dynamics, Sunnyvale, CA).

Graft manipulation. The semi-permeable membranes are tested for their permeability by sealing and culturing transduced cells in 1-2 cm “tea bags” made from the membrane. The activity of the specific genetic material (e.g., polypeptide, protein, and the like) is measured on both sides of the membrane.

Grafts are implanted into a porcine animal model using standard surgery and/or stent graft technology. Patency rates, tissue response (e.g. myointimal hyperplasia), and serological evidence of thrombolysis are compared to control animals undergoing similar procedures using untransduced cells. In specific, cells are harvested from the animal, then amplified and transduced with tPA vectors. These transduced cells are injected into a space created between a 6 mm internal diameter cylindrical semi-permeable membrane mounted in a 7 mm outer diameter tubular PTFE graft. The length of the graft is 3-4 cm. A transverse aortotomy is performed above the iliac bifurcation. The graft is entirely premounted on a short modified balloon expandable stent (Johnson and Johnson, Warren, NJ). The assembly is deployed over a balloon catheter into the infrarenal aorta just below the kidneys.

Example 1: Enzyme Activity of tPA Transduced ECs.

Analysis of tPA enzyme activity from stable tPA transduced ECs indicated that the tPA enzyme activity increases over time, correlating to an increase of tPA antigen concentration in the supernatants of cultured tPA transduced ECs (EC/tPA) (Fig. 2).

Example 2: Semi-Permeable Membrane Design Parameters.

Different semi-permeable membranes have been provided by W.L. Gore & Associates Inc. (Flagstaff, AZ) and Atrium Medical Corporation (Natick, MA). The membrane design parameters include inertness, strength, durability, and pore size that is smaller than a platelet but large enough for protein and lipid diffusion. It was

shown that cells packaged within these membranes can survive for two weeks, and secreted tPA was detected on both sides of the membrane. No evidence of these cells being able to traverse the membranes has been obtained *in vitro*. Cells packaged within a “tea bag” made of these membranes can survive and grow for one month
5 (Fig. 3).

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

CLAIMS

What is claimed is:

- 1 1. A prosthetic bypass graft comprising:
 - 2 (a) a biocompatible tubular external graft member open at each end; and
 - 3 (b) a tubular semipermeable membrane attached internal to and coaxial with
 - 4 said external graft member such that a sealed cavity is formed between said membrane
 - 5 and said graft member;
 - 6 wherein said semipermeable membrane has pores smaller than the
 - 7 diameter of a platelet, but larger than that of a lipid or protein.
- 1 2. The prosthetic bypass graft of Claim 1 further comprising genetically
- 2 engineered cells disposed between said external graft member and said internal
- 3 semipermeable membrane.
- 1 3. The prosthetic bypass graft of Claim 2 wherein said genetically engineered
- 2 cells produce a therapeutic protein or reagent.
- 1 4. The prosthetic bypass graft of Claim 3 wherein said therapeutic protein or
- 2 reagent is an anticoagulant.
- 1 5. The prosthetic bypass graft of Claim 4 wherein said anticoagulant is heparin,
- 2 hirudin, hirulog, hirugen, activated protein C, non-activated protein C, an antagonist
- 3 of thrombin, an antagonist of Factor Xa, an antagonist of FII, an antagonist of FIX,
- 4 an antagonist of FIII, an antagonist of FV, an antagonist of FVII, or an antagonist of
- 5 tissue factor.
- 1 6. The prosthetic bypass graft of Claim 3 wherein said therapeutic protein or
- 2 reagent is a thrombolytic agent.

1 7. The prosthetic bypass graft of Claim 6 wherein the thrombolytic agent is tissue
2 plasminogen activator (tPA) or a tPA variant.

1 8. The prosthetic bypass graft of Claim 6 wherein the thrombolytic agent is
2 plasmin, urokinase, single chain prourokinase, streptokinase, a prostaglandin, a
3 cyclooxygenase inhibitor, a phosphodiesterase inhibitor, a thromboxane synthetase
4 inhibitor, an antagonist of glycoprotein receptors including GPIb, and GPIIb/IIIa, an
5 antagonist of collagen receptors, or an antagonist of platelet thrombin receptors.

1 9. The prosthetic bypass graft of Claim 3 wherein said therapeutic protein or
2 reagent alters platelet metabolism.

1 10. The prosthetic bypass graft of Claim 9 wherein said therapeutic protein or
2 reagent is a prostaglandin, a cyclooxygenase inhibitor, a phosphodiesterase inhibitor,
3 a thromboxane inhibitor, an inhibitor of calcium transport, an elevator of cyclic AMP,
4 or a cyclic AMP agonist.

1 11. The prosthetic bypass graft of Claim 2 wherein the genetically engineered
2 cells are derived from endothelial cells or smooth muscle cells.

1 12. The prosthetic bypass graft of Claim 2 wherein the genetically engineered
2 cells are derived from endothelial cells and smooth muscle cells.

1 13. The prosthetic bypass graft of Claim 1 further comprising injection port means
2 for introducing cells between external graft member and internal semipermeable
3 membrane and vent means to release pressure.

1 14. The prosthetic bypass graft of Claim 13 wherein the injection port means and
2 vent means are integral with said external graft member.

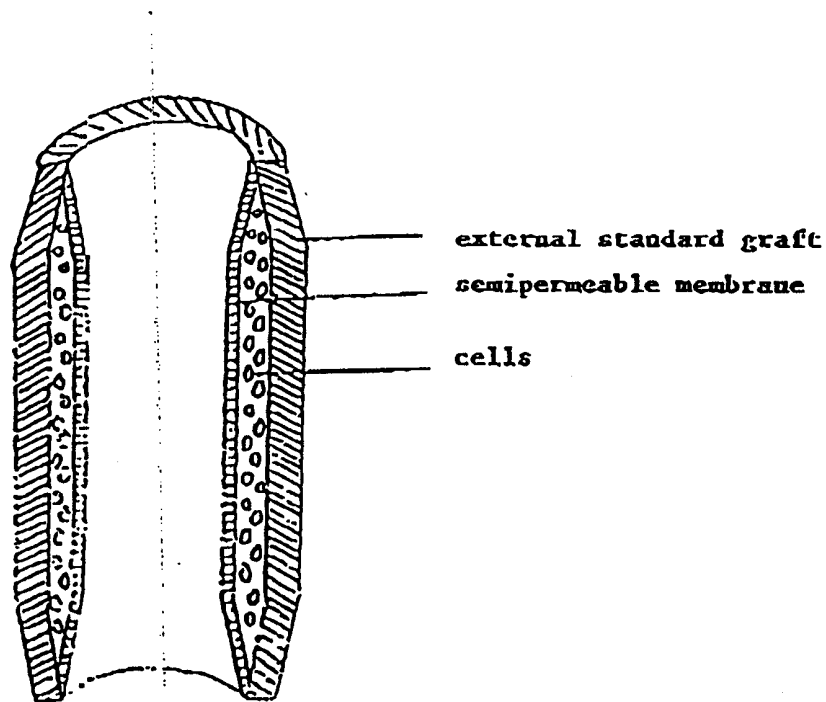
- 1 15. A method for vascular grafting comprising implantation in an artery the
2 prosthetic bypass graft of Claim 1.
- 1 16. The method of Claim 15 further comprising the step of placing genetically
2 engineered cells such that they are disposed between said external graft member and
3 said semi-permeable membrane.
- 1 17. The method of Claim 16 wherein the genetically engineered cells are derived
2 from endothelial cells.
- 1 18. The method of Claim 17 further comprising the step of seeding the external
2 graft member with smooth muscle cells.
- 1 19. The method of Claim 16 wherein said genetically engineered cells produce a
2 therapeutic protein or reagent in a therapeutically useful amount.
- 1 20. The method of Claim 19 wherein said therapeutic protein or reagent is a
2 thrombolytic agent.
- 1 21. The method of Claim 20 wherein said thrombolytic agent is tissue
2 plasminogen activator (tPA) or a tPA variant.
- 1 22. The method of Claim 20 wherein said thrombolytic agent is plasmin,
2 urokinase, single chain prourokinase, streptokinase, a prostaglandin, a cyclooxygenase
3 inhibitor, a phosphodiesterase inhibitor, a thromboxane synthetase inhibitor, an
4 antagonist of glycoprotein receptors including GPIb, and GPIIb/IIIa, an antagonist of
5 collagen receptors, or an antagonist of platelet thrombin receptors.
- 1 23. The method of Claim 19 wherein said therapeutic protein or reagent is an
2 anticoagulant.

1 24. The method of Claim 23 wherein said anticoagulant is heparin, hirudin,
2 hirulog, hirugen, activated protein C, non-activated protein C, an antagonist of
3 thrombin, , an antagonist of Factor Xa, an antagonist of FBI, an antagonist of FIX, an
4 antagonist of FIII, an antagonist of FV, an antagonist of FVII, or an antagonist of
5 tissue factor.

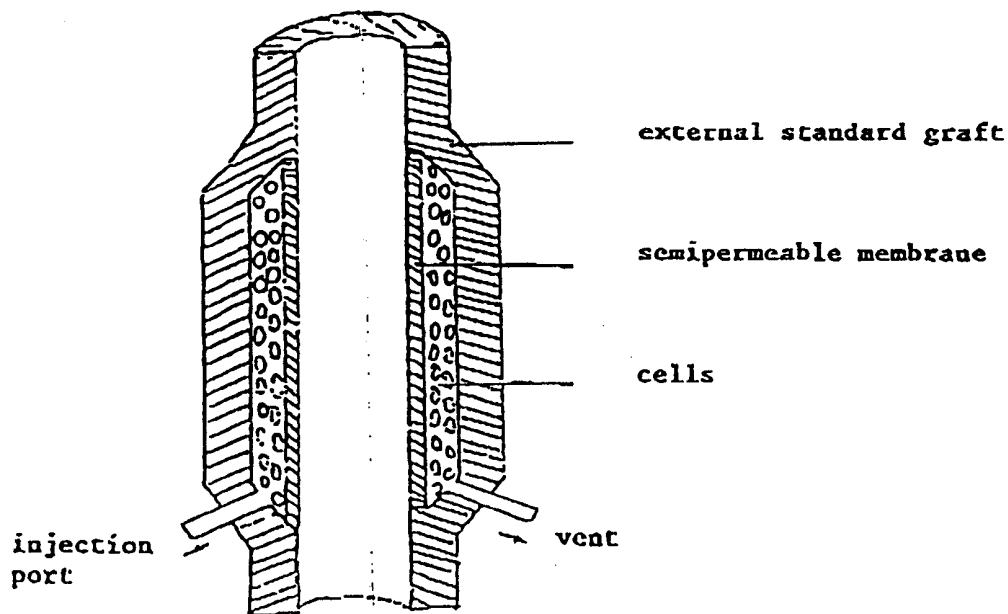
1 25. The method of Claim 19 wherein said therapeutic protein or reagent alters
2 platelet metabolism.

1 26. The method of Claim 25 wherein said therapeutic protein or reagent is a
2 prostaglandin, a cyclooxygenase inhibitor, a phosphodiesterase inhibitor, a
3 thromboxane inhibitor, an inhibitor of calcium transport, an elevator of cyclic AMP,
4 or a cyclic AMP agonist.

Figure 1



A



B

Figure 2

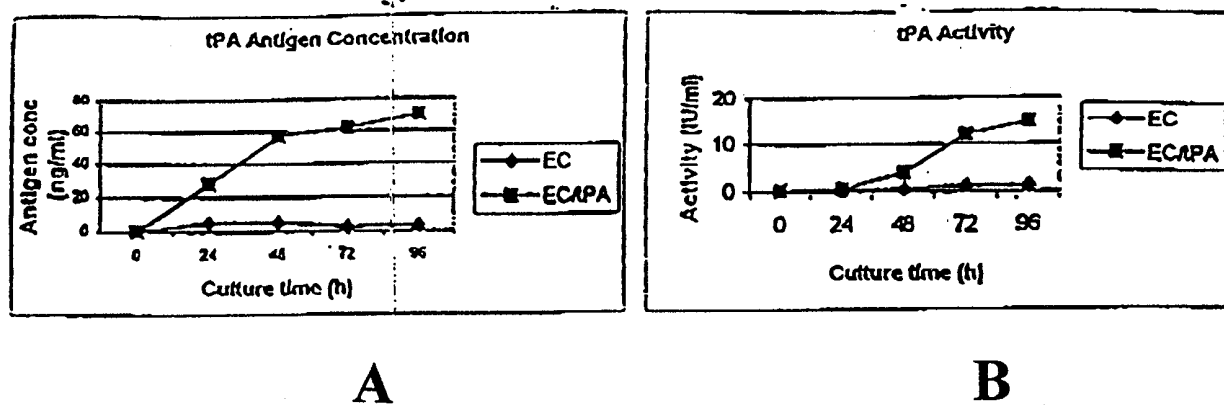
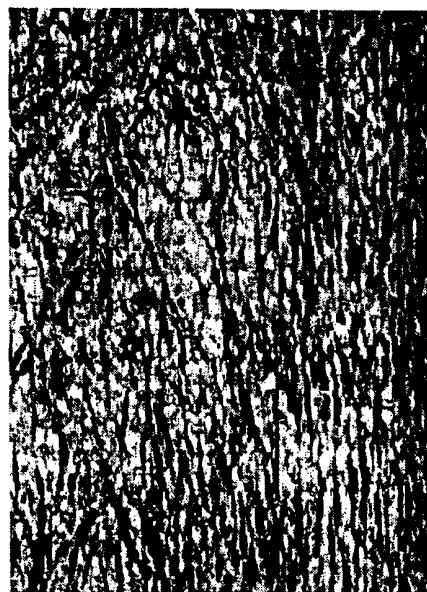


Figure 3



A



B