USE OF VEGF-C TO PREVENT RESTENOSIS

Inventors: Kari Alitalo, Espoo (FI); Seppo Yli-Herttuala, Kuopio (FI); Mikko O. Hiltunen, Kuopio (FI); Markku M. Jeltsch, Helsinki (FI); Marc G. Achen, North Melbourne (AU)

Assignees: Licentia Ltd, Helsinki (FI); Seppo Yli-Herttuala, Kuopio (FI); Ludwig Institute of Cancer Research, New York, NY (US)

Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

Appl. No.: 09/427,657
Filed: Oct. 26, 1999

Related U.S. Application Data

Provisional application No. 60/105,587, filed on Oct. 26, 1998.

Int. Cl.7 A01N 63/00; C12P 21/06; C12N 15/00; A61K 43/04; C07H 21/02

U.S. Cl. 424/93.2: 424/93.1; 424/93.21; 435/69.1; 435/320.1; 435/325; 435/455; 514/44; 536/23.1; 536/23.5

Field of Search 424/93.1, 93.2, 424/93.21, 93.6; 435/69.1, 320.1, 325, 455, 328; 514/44; 536/23.1, 23.5

References Cited

U.S. PATENT DOCUMENTS

5,087,244 A 2/1992 Wolinsky et al. ..... 604/33
5,631,237 A 5/1997 Dzu et al. ............. 514/44
5,653,689 A 8/1997 Buell et al. ........... 604/96
5,671,942 A 10/1997 Salahjade et al. ....... 604/28
5,679,400 A 10/1997 Tuch ......... 427/2.14
5,697,967 A 12/1997 Dinh et al. ........... 623/1
5,700,286 A 12/1997 Tartaglia et al. ........ 623/1
5,713,860 A 2/1998 Kaplan et al. ........... 604/96
5,749,848 A 5/1998 Jang et al. ........... 604/53
5,776,184 A 7/1998 Tuch ............. 623/1
5,776,755 A 7/1998 Alitalo et al. ........... 435/194
5,779,729 A 7/1998 Severini ........... 606/191
5,792,453 A 8/1998 Hammond et al. ....... 424/93.21
5,800,507 A 9/1998 Schwartz ........... 623/1
5,824,048 A 10/1998 Tuch ............. 623/1
5,830,879 A * 11/1998 Isner ............. 514/44
5,924,048 A 7/1999 McMarrack et al. ....... 702/13
5,932,540 A 8/1999 Hu et al. ........... 514/2
5,935,820 A 8/1999 Hu et al. ........... 435/69.4
5,994,300 A 11/1999 Bayne et al. ......... 514/12
6,121,246 A * 9/2000 Isner ............. 514/44

WO 96/24473 9/1995
WO 96/39515 12/1996
WO 97/02520 2/1997
WO 97/09427 3/1997
WO 97/17359 5/1997
WO 97/17442 5/1997
WO 99/08744 2/1999
WO 99/46364 9/1999

Other Publications

(Continued)

Primary Examiner—Jeffrey Fredman
Assistant Examiner—Sumesh Kaushal

Attorney, Agent, or Firm—Marshall, Gerstein & Borun LLP

ABSTRACT

The present invention provides materials and methods for preventing stenosis or restenosis of a blood vessel using Vascular Endothelial Growth Factor C (VEGF-C) and/or Vascular Endothelial Growth Factor D (VEGF-D) genes or proteins.
OTHER PUBLICATIONS


Achen, M.G. et al., “Vascular endothelial growth factor D (VEGF–D) is a dimer for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4),” Proc. Natl. Acad. Sci. USA 95(2):548–553 (Jan., 1998).


Dignam et al., “Balbiani ring 3 in Chironomus tentans encodes a 185–kDa secretory protein which is synthesized throughout the fourth larval instar,” Gene 88:133–140 (1990).


GenBank Accession No. AJ000185 Homo sapiens mRNA for vascular endothelial growth factor–D.

GenBank Accession No. AF014827 Rattus norvegicus vascular endothelial growth factor–D (VEGF–D) mRNA, complete cds.

GenBank Accession No. MMU73620 Mus musculus VEGF–C mRNA, complete cds.

GenBank Accession No. CCY15837 Coturnix coturnix mRNA for vascular endothelial growth factor C.

GenBank Accession No. D89628 Mus musculus mRNA for vascular endothelial growth factor D, complete cds.

GenBank Accession No. X94216 H. sapiens mRNA for VEGF–C protein.


* cited by examiner
FIG. 3A

FIG. 3B
USE OF VEGF-C TO PREVENT RESTENOSIS

This application claims priority benefit of U.S. Provisional Application No. 60/105,587, filed Oct. 26, 1998, incorporated herein by reference.

FIELD OF THE INVENTION

The present invention provides materials and methods to prevent stenosis and restenosis of blood vessels, and relates generally to the field of cardiovascular medicine.

BACKGROUND OF THE INVENTION

Coronary artery disease constitutes a major cause of morbidity and mortality throughout the world, especially in the United States and Europe. Percutaneous transluminal coronary angioplasty (e.g., balloon angioplasty, with or without intracoronary stenting) is now a common and successful therapy for such disease, performed hundreds of thousands of times per year in the United States alone. However, restenosis occurs in as many as one-third to one-half of such recanalization procedures, usually within six months of the angioplasty procedure. The economic cost of restenosis has been estimated at $2 billion annually in the United States alone. [Feldman et al., Cardiovascular Research, 32: 194–207 (1996), incorporated herein by reference.] Autopsy and atherectomy studies have identified intimal hyperplasia as the major histologic component of restenotic lesions. [Cerek et al., Am. J. Cardiol., 68: 24C–33C (1991).]

Restenosis also remains a clinical concern in angioplasty that is performed in peripheral blood vessels. Likewise, stenosis is a clinical concern following transplantation of blood vessels (e.g., grafts and gift vessels) for cardiac bypass surgery or for treatment of peripheral ischemia or intermittent claudication, for example (e.g., above-knee femoro-popliteal arterial bypass grafts).

Mazur et al., Texas Heart Institute Journal, 21: 104–111 (1994) state that restenosis is primarily a response of the artery to the injury caused by percutaneous coronary angioplasty, which disrupts the intimal layer of endothelial cells and underlying smooth muscle cells of the media. The authors state that multiple growth factors secreted by platelets, endothelial cells, macrophages, and smooth muscle cells are mechanistically involved in the restenosis process, and that proliferation of smooth muscle cells constitutes a critical pathogenic feature. According to the authors, this smooth muscle cell proliferation has proven refractory to mechanical and pharmacologic therapy. More recently, others have called into question whether smooth muscle cell proliferation is of penultimate importance in restenosis. See Libby, Circ. Res., 82: 404–406 (1998).


Chang & Leiden, Semin. Intervent. Cardiol., 1: 185–193 (1996), incorporated herein by reference, review somatic gene therapy approaches to treat restenosis. Chang and Leiden teach that replication-deficient adenoviruses comprise a promising and safe vector system for gene therapy directed toward prevention of restenosis, because such viruses can efficiently infect a wide variety of cell types, including vascular smooth muscle cells; such viruses can be produced in high titer (e.g., 10^9–10^12 plaque forming units per milliliter); such viruses can accommodate a transgene insert of, e.g., 7–9 kilobases (kb) in size; such viruses can be delivered percutaneously through standard catheters; and such viruses do not integrate into the host genome. Both Change & Leiden and Feldman et al., supra, also review cytotoxic and cytostatic gene therapy approaches, designed to kill or arrest proliferating vascular smooth muscle cells thought to be responsible for neointimal formations that characterize restenosis.


Cerek et al., Am. J. Cardiol., 68: 24C–33C (1991) suggest prevention of restenosis by inhibiting growth-factor-mediated healing of arterial injury. Potential roles of platelet-derived growth factor (PDGF), thrombospondin, insulin-like growth factor 1 (IGF-1), fibroblast growth factors (FGF's), transforming growth factor alpha (TGF-α) and beta (TGF-β), epidermal growth factor (EGF) are discussed.

Isner & Asahara, International Patent Publication No. WO 98/19712, incorporated herein by reference, suggest treating injured blood vessels and accelerating reendothelialization following angioplasty by isolating a patient’s endothelial progenitor cells and re-administering such cells to the patient. The authors suggest that the effectiveness of using an angiogenesis-promoting growth factor, such as vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF), may be limited by the lack of endothelial cells on which the VEGF or bFGF will exert its effect.

Martin et al., International Patent Publication No. WO 98/20027 suggest the use of VEGF gene or protein to treat or prevent stenosis or restenosis of a blood vessel. The authors suggest that any beneficial effect of VEGF arises from a different mechanism of action than the mechanism underlying an activity of VEGF related to stimulating re-endothelialization in cases where the endothelium has been damaged.

Callow et al., Growth Factors, 10: 223–228 (1994) state that intravenous injection of vascular permeability factor (a.k.a. VEGF) into rabbits that had been subjected to balloon angioplasty-induced endothelial denudation resulted in increased regeneration of endothelium compared to a control. The authors also stated that basic fibroblast growth factor (bFGF) is effective at promoting re-endothelialization, but that such re-endothelialization is accompanied by increases in neointimal lesion size.

Asahara et al., Circulation, 94: 3291–3302 (Dec. 15, 1996) state that local, percutaneous catheter delivery of a CMV-human-VEGF165 transgene achieved accelerated re-endothelialization in balloon-injured rabbits, and resulted in diminished intimal thickening. In a report by a related group of authors, Van Belle et al., J. Am. Coll. Cardiol., 29:1371–1379 (May, 1997) state that stent endothelialization was accelerated by delivery of a CMV-human-VEGF165 transgene and was accomplished by attenuation of intimal thickening.

Morishita et al., J. Atherosclerosis and Thrombosis, 4(3): 128–134 (1998) state that hepatocyte growth factor (HGF) has a mitogenic activity on human endothelial cells more potent than VEGF, and hypothesized that HGF gene therapy may have potential therapeutic value for the treatment of cardiovascular diseases such as restenosis after angioplasty. Morishita et al. also state that there is little knowledge about growth factors that stimulate only endothelial cells, but not vascular smooth muscle cells.

for application to human coronary restenosis, and that two independent studies suggest that VEGF delivery may actually worsen arterial intimal hyperplasia.

Brown et al., U.S. Pat. No. 5,795,898, suggest using an inhibitor of PDGF, FGF, EGF, or VEGF signaling to suppress accelerated atherogenesis involved in restenosis of coronary vessels or other arterial vessels following angioplasty.

The foregoing discussion demonstrates that a long-felt need continues to exist for improvements to angioplasty materials and/or methods, and/or for adjunct therapies, to reduce instances of restenosis.

**SUMMARY OF THE INVENTION**

The present invention addresses long-felt needs in the field of medicine by providing materials and methods for the prevention of stenosis or restenosis in mammalian blood vessels.

For example, the invention provides a method of treating a mammalian subject to prevent stenosis or restenosis of a blood vessel, comprising the step of administering to a mammalian subject in need of treatment to prevent stenosis or restenosis of a blood vessel a composition comprising a polynucleotide, the polynucleotide comprising a nucleotide sequence that encodes a vascular endothelial growth factor C (VEGF-C) polypeptide. In a preferred embodiment, the subject is a human subject.

While it is contemplated that the VEGF-C polynucleotide could be administered purely as a prophylactic treatment to prevent stenosis, it is contemplated in a preferred embodiment that the polynucleotide be administered shortly before, and/or concurrently with, and/or shortly after a percutaneous transluminal coronary angioplasty procedure, for the purpose of preventing restenosis of the subject vessel. In another preferred embodiment, the polynucleotide is administered before, during, and/or shortly after a bypass procedure (e.g., a coronary bypass procedure), to prevent stenosis or restenosis in or near the transplanted (grafted) vessel, especially stenosis at the location of the graft itself. In yet another embodiment, the polynucleotide is administered before, during, or after a vascular implantation in the vascular periphery that has been performed to treat peripheral ischemia or intermittent claudication. By prevention of stenosis or restenosis is meant prophylactic treatment to reduce the amount/severity of, and/or substantially eliminate the stenosis or restenosis that frequently occurs in such surgical procedures. The polynucleotide is included in the composition in an amount and in a form effective to promote expression of a VEGF-C polypeptide in a blood vessel of the mammalian subject, thereby preventing stenosis or restenosis of the blood vessel.

In a preferred embodiment, the mammalian subject is a human subject. For example, the subject is a person suffering from coronary artery disease that has been identified by a cardiologist as a candidate who could benefit from a therapeutic balloon angioplasty (with or without insertion of an intravascular stent) procedure or from a coronary bypass procedure. Practice of methods of the invention in other mammalian subjects, especially mammals that are conventionally used as models for demonstrating therapeutic efficacy in humans (e.g., primate, porcine, canine, or rabbit animals), also is contemplated.

For the practice of methods of the invention, the term “VEGF-C polypeptide” is intended to include any polypeptide that has a VEGF-C or VEGF-C analog amino acid sequence (as defined elsewhere herein in greater detail) and that possesses in vivo restenosis-reducing effects of human VEGF-C, which effects are demonstrated herein by way of example in a rabbit model. The term “VEGF-C polynucleotide” is intended to include any polynucleotide (e.g., DNA or RNA, single- or double-stranded) comprising a nucleotide sequence that encodes a VEGF-C polypeptide. Due to the well-known degeneracy of the genetic code, there exist multiple VEGF-C polynucleotide sequences that encode any selected VEGF-C polypeptide.

For treatment of humans, VEGF-C polypeptides with an amino acid sequence of a human VEGF-C are highly preferred, and polynucleotides comprising a nucleotide sequence of a human VEGF-C cDNA are highly preferred. By “human VEGF-C” is meant a polypeptide corresponding to a naturally occurring protein (prepro-protein, partially-protein, or fully-processed mature protein) encoded by any allele of the human VEGF-C gene, or a polypeptide comprising a biologically active fragment of a naturally-occurring mature protein. By way of example, a human VEGF-C comprises a continuous portion of the amino acid sequence set forth in SEQ ID NO: 2 sufficient to permit the polypeptide to bind and stimulate VEGFR-2 and/or VEGFR-3 phosphorylation in cells that express such receptors. A polypeptide comprising amino acids 131–211 of SEQ ID NO: 2 is specifically contemplated. For example, polypeptides having an amino acid sequence comprising a continuous portion of SEQ ID NO: 2, the continuous portion having, as its amino terminus, an amino acid selected from the group consisting of positions 30–131 of SEQ ID NO: 2, and having, as its carboxyl terminus, an amino acid selected from the group consisting of positions 211–419 of SEQ ID NO: 2 are contemplated. As explained elsewhere herein in greater detail, VEGF-C biological activities, especially those mediated through VEGFR-2, increase upon processing of both an amino-terminal and carboxyl-terminal propeptide. Thus, an amino terminus selected from the group consisting of positions 102–131 of SEQ ID NO: 2 is preferred, and an amino terminus selected from the group consisting of positions 103–113 of SEQ ID NO: 2 is highly preferred. Likewise, a carboxyl terminus selected from the group consisting of positions 211–227 of SEQ ID NO: 2 is preferred. As stated above, the term “human VEGF-C” also is intended to encompass polypeptides encoded by allelic variants of the human VEGF-C characterized by the sequences set forth in SEQ ID Nos. 1 & 2.

Moreover, since the therapeutic VEGF-C is to be administered as recombinant VEGF-C or indirectly via somatic gene therapy, it is within the skill in the art to make and use analogs of human VEGF-C (and polynucleotides that encode such analogs) wherein one or more amino acids have been added, deleted, or replaced with other amino acids, especially with conservative replacements, and wherein the anti-restenosis biological activity has been retained. Analogs that retain anti-restenosis VEGF-C biological activity are contemplated as VEGF-C polypeptides for use in the present invention. In a preferred embodiment, analogs having I, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 such modifications and that retain anti-restenosis VEGF-C biological activity are contemplated as VEGF-C polypeptides for use in the present invention. Polynucleotides encoding such analogs are generated using conventional PCR, site-directed mutagenesis, and chemical synthesis techniques.

Also contemplated as VEGF-C polypeptides are non-human mammalian or avian VEGF-C polypeptides and polynucleotides. By “mammalian VEGF-C” is meant a polypeptide corresponding to a naturally occurring protein
(prepro-protein, partially-processed protein, or fully-processed mature protein) encoded by any allele of a VEGF-C gene of any mammal, or a polypeptide comprising a biologically active fragment of a mature protein. The term “mammalian VEGF-C polypeptide” is intended to include analogs of mammalian VEGF-C’s that possess the in vivo restenosis-reducing effects of the mammalian VEGF-C. The fact that gene therapy using a transgene encoding human VEGF-C is effective to prevent restenosis in a rabbit model is evidence of the inter-species therapeutic efficacy of VEGF-C proteins.

Irrespective of which VEGF-C polypeptide is chosen, the VEGF-C polynucleotide preferably comprises a nucleotide sequence encoding a secretory signal peptide fused in-frame with the VEGF-C polypeptide sequence. The secretory signal peptide directs secretion of the VEGF-C polypeptide by the cells that express the polynucleotide, and is cleaved by a post-translational degradation of the VEGF-C polypeptide. For example, the VEGF-C polynucleotide could encode the complete prepro-VEGF-C sequence set forth in SEQ ID NO: 2, or could encode the VEGF-C signal peptide fused in-frame to a sequence encoding a fully-processed VEGF-C (e.g., amino acids 103–227 of SEQ ID NO: 2) or VEGF-C analog. Moreover, there is no requirement that the signal peptide be derived from VEGF-C. The signal peptide sequence can be that of another secreted protein, or can be a completely synthetic signal sequence effective to direct secretion in cells of the mammalian subject.

In one embodiment, the VEGF-C polynucleotide of the invention comprises a nucleotide sequence that will hybridize to a polynucleotide that is complementary to the human VEGF cDNA sequence specified in SEQ ID NO: 1 under the following exemplary stringent hybridization conditions: hybridization at 42°C in 50% formamide, 5xSSC, 20 mM NaPO₄, pH 6.8, and washing in 1xSSC at 55°C for 30 minutes; and wherein the nucleotide sequence encodes a polypeptide that binds and stimulates human VEGFR-2 and/or VEGFR-3. It is understood that variation in these exemplary conditions occur based on the length and GC nucleotide content of the sequences to be hybridized. Formulas standard in the art are appropriate for determining appropriate hybridization conditions. See Sambrook et al., Molecular Cloning: A Laboratory Manual (Second ed., Cold Spring Harbor Laboratory Press, 1989) §§ 9.47–9.51.

In preferred embodiments, the VEGF-C polynucleotide further comprises additional sequences to facilitate the VEGF-C gene therapy. In one embodiment, a “naked” VEGF-C transgene (i.e., a transgene without a viral, liposomal, or other vector to facilitate transfection) is employed for gene therapy. In this embodiment, the VEGF-C polynucleotide preferably comprises a suitable promoter and/or enhancer sequence (e.g., cytomegalovirus promoter/enhancer [Lehner et al., J. Clin. Microbiol., 29:2494–2502 (1991); Boshart et al., Cell, 41:521–530 (1985)]; Rous sarcoma virus promoter [Davis et al., Hum. Gene Ther., 4:151 (1993)]; TIE promoter [Korhonen et al., Blood, 86(5): 1828–1835 (1995)] or simian virus 40 promoter) for expression in the target mammalian cells, the promoter being operatively linked upstream (i.e., 5’) of the VEGF-C coding sequence. The VEGF-C polynucleotide also preferably further includes a suitable polyadenylation sequence (e.g., the SV40 or human growth hormone gene polyadenylation sequence) operably linked downstream (i.e., 3’) of the VEGF-C coding sequence. The polynucleotide may further optionally comprise sequences whose only intended function is to facilitate large-scale production of the vector, e.g., in bacteria, such as a bacterial origin of replication and a sequence encoding a selectable marker. However, in a preferred embodiment, such extraneous sequences are at least partially cleaved off prior to administration to humans according to methods of the invention. One can manufacture and administer such polynucleotides to achieve successful gene therapy using procedures that have been described in the literature for other transgens. See, e.g., Isner et al., Circulation, 91: 2697–2692 (1995); and Isner et al., Human Gene Therapy, 7: 989–1011 (1996); incorporated herein by reference in the entirety.


In embodiments employing a viral vector, preferred polynucleotides still include a suitable promoter and polyadenylation sequence as described above. Moreover, it will be readily apparent that, in these embodiments, the polynucleotide further includes vector polynucleotide sequences (e.g., adenoviral polynucleotide sequences) operably connected to the sequence encoding a VEGF-C polypeptide.

Thus, in one embodiment the composition to be administered comprises a vector, wherein the vector comprises the VEGF-C polynucleotide. In a preferred embodiment, the vector is an adenovirus vector. In a highly preferred embodiment, the adenovirus vector is replication-deficient, i.e., it cannot replicate in the mammalian subject due to deletion of essential viral-replication sequences from the adenoviral genome. For example, the inventors contemplate a method wherein the vector comprises a replication-deficient adenovirus, the adenovirus comprising the VEGF-C polynucleotide operably connected to a promoter and flanked on either end by adenoviral polynucleotide sequences.

The composition to be administered according to methods of the invention preferably comprises (in addition to the polynucleotide or vector) a pharmaceutically-acceptable carrier solution such as water, saline, phosphate-buffered saline, glucose, or other carriers conventionally used to deliver therapeutics intravascularly. Multi-gene therapy is also contemplated, in which case the composition optionally comprises both the VEGF-C polynucleotide/vector and another polynucleotide/vector selected to prevent restenosis. Exemplary candidate genes/vectors for co-transfection with VEGF-C transgenes are described in the literature cited above, including genes encoding cytokotoxic factors, cytostatic factors, endothelial growth factors, and smooth muscle cell growth/migration inhibitors. As described in greater detail below, a VEGF-D transgene is a preferred candidate for co-administration with the VEGF-C transgene. Co-administration of a VEGF transgene also is specifically contemplated.

The “administering” that is performed according to the present method may be performed using any medically-
accepted means for introducing a therapeutic directly or indirectly into the vasculature of a mammalian subject, including but not limited to injections; oral ingestion; intranasal or topical administration; and the like. In a preferred embodiment, administration of the composition comprising the VEGF-C polynucleotide is performed intravascularly, such as by intravenous, intra-arterial, or intracoronary arterial injection.

In a highly preferred embodiment, the composition is administered locally, e.g., to the site of angioplasty or bypass. For example, the administering comprises a catheter-mediated transfer of the transgene-containing composition into a blood vessel of the mammalian subject, especially into a coronary artery of the mammalian subject. Exemplary materials and methods for local delivery are reviewed in Lincoff et al., Circulation, 90: 2070–2084 (1994); and Wilensky et al., Trends Cardiovasc. Med., 3:163–170 (1993), both incorporated herein by reference. For example, the composition is administered using infusion-perfusion balloon catheters (preferably microporous balloon catheters) such as those that have been described in the literature for intracoronary drug infusions. See, e.g., U.S. Pat. No. 5,713,860 (Intravascular Catheter with Infusion Array); U.S. Pat. No. 5,087,244; U.S. Pat. No. 5,653,089; and Wolinsky et al., J. Am. Coll. Cardiol., 15: 475–481 (1990) (Wolinsky Infusion Catheter); and Lambert et al., Coron. Artery Dis., 4: 469–475 (1993), all of which are incorporated herein by reference in their entirety. Use of such catheters for site-directed somatic cell gene therapy is described, e.g., in Mazar et al., Texas Heart Institute Journal, 21: 104–111 (1994), incorporated herein by reference. In an embodiment where the VEGF-C transgene is administered in an adenovirus vector, the vector is preferably administered in a pharmaceutically acceptable carrier at a titer of $10^{7}–10^{12}$ viral particles, and more preferably at a titer of $10^{9}–10^{11}$ viral particles. The adenoviral vector composition preferably is infused over a period of 15 seconds to 30 minutes, more preferably 1 to 10 minutes.

For example, in patients with angioplasty due to a single or multiple lesions in coronary arteries and for whom PTCA is prescribed on the basis of primary coronary angiogram findings, an exemplary protocol involves performing PTCA through a 7F guiding catheter according to standard clinical practice for the femoral approach. If an optimal result is not achieved with PTCA alone, then an endovascular stent also is implanted. (A nonoptimal result is defined as residual stenosis of >30% of the luminal diameter according to a visual estimate, and B or C type dissection.) Arterial gene transfer at the site of balloon dilatation is performed with a replication-deficient adenoviral VEGF-C vector immediately after the angioplasty, but before stent implantation, using an infusion-perfusion balloon catheter. The size of the catheter will be selected to match the diameter of the artery as measured from the angiogram, varying, e.g., from 3.0 to 3.5F in diameter. The balloon is inflated to the optimal pressure and gene transfer is performed for a duration of 10 minutes inflation at the rate of 0.5 ml/min with virus titer of 1.15×$10^{10}$.

In another embodiment, intravascular administration with a gel-coated catheter is contemplated, as has been described in the literature to introduce other transgenes. See, e.g., U.S. Pat. No. 5,674,192 (Catheter coated with tenaciously-adhered swellable hydrogel polymer); Riessen et al., Human Gene Therapy, 4: 749–758 (1993); and Steg et al., Circulation, 96: 408–411 (1997) and 90: 1648–1656 (1994); all incorporated herein by reference. Briefly, DNA in solution (e.g., the VEGF-C polynucleotide) is applied one or more times ex vivo to the surface of an inflated angioplasty catheter balloon coated with a hydrogel polymer (e.g., Sider with Hydroplus, Mansfield Boston Scientific Corp., Watertown, Mass.). The Hydroplus coating is a hydrophilic polyacrylic acid polymer that is cross-linked to the balloon to form a high molecular weight hydrogel tightly adhered to the balloon. The DNA covered hydrogel is permitted to dry before deflating the balloon. Re-inflation of the balloon intravascularly, during an angioplasty procedure, causes the transfer of the DNA to the vessel wall.

In yet another embodiment, an expandable elastic membrane or similar structure mounted to or integral with a balloon angioplasty catheter or stent is employed to deliver the VEGF-C transgene. See, e.g., U.S. Pat. Nos. 5,707,385, 5,697,967, 5,700,286, 5,800,507, and 5,776,184, all incorporated by reference herein.

In another variation, the composition containing the VEGF-C transgene is administered extravascularly, e.g., using a device to surround or encapsulate a portion of vessel. See, e.g., International Patent Publication WO 98/20027, incorporated herein by reference, describing a collar that is placed around the outside of an artery (e.g., during a bypass procedure) to deliver a transgene to the arterial wall via a plasmid or liposome vector.

In still another variation, endothelial cells or endothelial progenitor cells are transfected ex vivo with the VEGF-C transgene, and the transfected cells are administered to the mammalian subject. Exemplary procedures for seeding a vascular graft with genetically modified endothelial cells are described in U.S. Pat. No. 5,785,965, incorporated herein by reference.

If the mammalian subject is receiving a vascular graft, the VEGF-C transgene-containing composition may be directly applied to the isolated vessel segment prior to its being grafted in vivo.

In another aspect, the invention provides a method of treating a mammalian subject to prevent stenosis or restenosis of a blood vessel, comprising the step of administering to a mammalian subject in need of treatment to prevent stenosis or restenosis of a blood vessel a composition comprising a VEGF-C polypeptide, in an amount effective to prevent stenosis or restenosis of the blood vessel. In a preferred embodiment, the administering comprises implanting an intravascular stent in the mammalian subject, where the stent is coated or impregnated with the composition. Exemplary materials for constructing a drug-coated or drug-impregnated stent are described in literature cited above and reviewed in Lincoff et al., Circulation, 90: 2070–2084 (1994). In another preferred embodiment, the composition comprises microparticles composed of biodegradable polymers such as PGLA, non-degradable polymers, or biological polymers (e.g., starch) which particles encapsulate or are impregnated by the VEGF-C polypeptide. Such particles are delivered to the intravascular wall using, e.g., an infusion angioplasty catheter. Other techniques for achieving locally sustained drug delivery are reviewed in Wilensky et al., Trends Cardiovasc. Med., 3:163–170 (1993), incorporated herein by reference.

Administration via one or more intravenous injections subsequent to the angioplasty or bypass procedure also is contemplated. Localization of the VEGF-C polypeptides to the site of the procedure occurs due to expression of VEGF-C receptors on proliferating endothelial cells. Localization is further facilitated by recombantly expressing the VEGF-C as a fusion polypeptide (e.g., fused to an apolipoprotein B-100 oligopeptide as described in Shih et al., Proc.
et al. (1990). Co-administration of VEGF-C polynucleotides and VEGF-C polypeptides also is contemplated.

In yet another embodiment, the invention provides the use of a VEGF-C polynucleotide or VEGF-C polypeptide for the manufacture of a medicament for the treatment or prevention of stenosis or restenosis of a blood vessel.

In still another embodiment, the invention provides a method of treating a mammalian subject to prevent stenosis or restenosis of a blood vessel, comprising the step of administering to a mammalian subject in need of treatment to prevent stenosis or restenosis of a blood vessel a composition comprising a polynucleotide, the polynucleotide comprising a nucleotide sequence that encodes a vascular endothelial growth factor D (VEGF-D) polypeptide. Such methods are practiced essentially as described herein with respect to VEGF-C-encoding polynucleotides, except that polynucleotides encoding VEGF-D are employed. A detailed description of the human VEGF-D gene and protein is provided in Achen, et al., Proc. Natl Acad. Sci. U.S.A., 95(2): 548–553 (1998); International Patent Publication No.WO 98/07832, published 26 Feb. 1998; and in Genbank Accession No. AJ000185, all incorporated herein by reference. A cDNA and deduced amino acid sequence for prepro-VEGF-D is set forth herein in SEQ ID NOs: 3 and 4. Of course, due to the well-known degeneracy of the genetic code, there exist multiple VEGF-D encoding polynucleotide sequences, any of which may be employed according to the methods taught herein.

As described herein in detail with respect to VEGF-C, the use of polynucleotides that encode VEGF-D fragments, VEGF-D analogs, VEGF-D allelic and interspecies variants, and the like which possess in vivo anti-restenosis effects of human VEGF-D are all contemplated as being encompassed by the present invention.

In yet another embodiment, the invention provides a method of treating a mammalian subject to prevent stenosis or restenosis of a blood vessel, comprising the step of administering to a mammalian subject in need of treatment to prevent stenosis or restenosis of a blood vessel a composition comprising a VEGF-D polypeptide, in an amount effective to prevent stenosis or restenosis of the blood vessel. Such methods are practiced essentially as described herein with respect to VEGF-C polypeptides.

In a related aspect, the invention provides materials and devices for practice of the above-described methods.

For example, the polynucleotides, polypeptides, vectors, compositions, and the like that are described for use in methods of the invention are themselves intended as aspects of the invention.

Likewise, the invention also provides surgical devices that are used to treat circulatory disorders, such as intravascular (endovascular) stents, balloon catheters, infusion-perfusion catheters, extravascular collars, elastomeric membranes, and the like, which have been improved by coating with, impregnating with, adhering to, or encapsulating within the device a composition comprising a VEGF-C polynucleotide, a VEGF-C polypeptide, a VEGF-D polynucleotide, and/or a VEGF-D polypeptide.

For example, in one embodiment, the invention provides an endovascular stent characterized by an improvement wherein the stent is coated or impregnated with a composition, the comprising at least one anti-restenosis agent selected from the group consisting of VEGF-C polynucleotides, VEGF-C polypeptides, VEGF-D polynucleotides, and VEGF-D polypeptides. Exemplary stents that may be improved in this manner are described and depicted in U.S. Pat. Nos. 5,800,507 and 5,697,967 (Medtronic, Inc., describing an intraluminal stent comprising fibrin and an elutable drug capable of providing a treatment of restenosis); U.S. Pat. No. 5,776,184 (Medtronic, Inc., describing a stent with a porous coating comprising a polymer and a therapeutic substance in a solid or solution with the polymer); U.S. Pat. No. 5,799,384 (Medtronic, Inc., describing a flexible, cylindrical, metal stent having a biocompatible polymeric surface to contact a body lumen); U.S. Pat. Nos. 5,824,048 and 5,679,400; and U.S. Pat. No. 5,779,729; all of which are specifically incorporated herein by reference in their entirety. Implantation of such stents during conventional angioplasty techniques will result in less restenosis than implantation of conventional stents. In this sense, the biocompatibility of the stent is improved.

In another embodiment, the invention provides an extravascular collar for delivery of a therapeutic agent to a blood vessel, characterized by an improvement wherein the collar is coated with or impregnated with or encapsulates a composition, the comprising at least one anti-restenosis agent selected from the group consisting of VEGF-C polynucleotides, VEGF-C polypeptides, VEGF-D polynucleotides, and VEGF-D polypeptides. An exemplary collar to be improved in this manner is described and depicted in International Patent Publication WO 98/02007 (Eurogene, Ltd.,ollar comprising a body adopted to provide a seal around a vessel and to define a reservoir for holding an anti-restenosis pharmaceutical formulation), incorporated herein by reference.

In yet another embodiment, the invention provides a polymer film for wrapping a stent, characterized by an improvement wherein the film is coated with or impregnated with a composition, the comprising at least one anti-restenosis agent selected from the group consisting of VEGF-C polynucleotides, VEGF-C polypeptides, VEGF-D polynucleotides, and VEGF-D polypeptides. An exemplary film to be improved in this manner is described and depicted in U.S. Pat. Nos. 5,700,286 and 5,707,365 (Advanced Cardiovascular Systems, Inc., sheaths of bioabsorbable polymeric material coated or impregnated with a restenosis-preventing therapeutic agent and attachable to an endovascular stent).

Similarly, the invention includes kits which comprise compounds or compositions of the invention packaged in a manner which facilitates their use to practice methods of the invention. In a simplest embodiment, such a kit includes a compound or composition described herein as useful for practice of the invention (e.g., VEGF-C or VEGF-D polynucleotides or polypeptides), packaged in a container such as a sealed bottle or vial, with a label affixed to the container or included in the package that describes use of the compound or composition to practice the method of the invention. Preferably, the compound or composition is packaged in a unit dosage form. In another embodiment, a kit of the invention includes both a VEGF-C or VEGF-D polynucleotide or polypeptide composition packaged together with a physical device useful for implementing methods of the invention, such as a stent, a catheter, an extravascular collar, a polymer film, or the like. In another embodiment, a kit of the invention includes both a VEGF-C or VEGF-D polynucleotide or polypeptide composition packaged together with a hydrogel polymer, or microparticle polymers, or other carriers described herein as useful for delivery of the VEGF-C/VEGF-D to the patient.

Additional features and variations of the invention will be apparent to those skilled in the art from the entirety of this application, and all such features are intended as aspects of the invention.
Likewise, features of the invention described herein can be re-combined into additional embodiments that also are intended as aspects of the invention, irrespective of whether the combination of features is specifically mentioned above as an aspect or embodiment of the invention. Also, only such limitations which are described herein as critical to the invention should be viewed as such; variations of the invention lacking limitations which have not been described herein as critical are intended as aspects of the invention.

In addition to the foregoing, the invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations specifically mentioned above. Although the applicant(s) invented the full scope of the claims appended hereto, the claims appended hereto are not intended to encompass within their scope the prior art work of others. Therefore, in the event that statutory prior art within the scope of a claim is brought to the attention of the applicants by a Patent Office or other entity or individual, the applicant(s) reserve the right to exercise amendment rights under applicable patent laws to redefine the subject matter of such a claim to specifically exclude such statutory prior art or obvious variations of statutory prior art from the scope of such a claim. Variations of the invention defined by such amended claims also are intended as aspects of the invention.

**BRIEF DESCRIPTION OF THE DRAWING**

**FIG. 1** depicts a cross-section of a blood vessel into which a drug delivery balloon catheter including a protective sheath has been inserted, the protective sheath serving to cover the balloon during insertion and positioning.

**FIG. 2A** depicts a perspective view of an expandable membrane having two layers that are spaced apart, prior to joining edges of the layers to each other.

**FIG. 2B** depicts a perspective view of the membrane of **FIG. 2A** that has been rolled into a tube and had opposite edges adjoined.

**FIGS. 3A** and **3B** depict, in perspective (**3A**) and longitudinal cross-section (**3B**), schematic views of an extravascular collar surrounding a portion of a blood vessel.

**FIG. 4A** depicts in cross-section a wire coated with a polymer or gel that can include (e.g., be impregnated with) a therapeutic composition.

**FIG. 4B** depicts a perspective view of an intravascular stent formed from the wire of **FIG. 4A**.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is based on the discovery that when a gene encoding human Vascular Endothelial Growth Factor C (VEGF-C) is administered to a mammal that has suffered a vascular trauma, such as the trauma that can occur during conventional balloon angioplasty procedures, restenosis of the injured vessel is reduced or eliminated. An in vivo controlled experiment demonstrating the efficacy of a VEGF-C transgene to prevent restenosis is described in detail in Example 1. Example 2 provides a side-by-side comparative study demonstrating that the anti-restenosis effects of VEGF-C appear superior to the anti-restenosis effects of VEGF administered in a comparable manner.

The growth factor named Vascular Endothelial Growth Factor C (VEGF-C), as well as native human, non-human mammalian, and avian polynucleotide sequences encoding VEGF-C, and VEGF-C variants and analogs, have been described in detail in International Patent Application Number PCT/US98/01973, filed 2 Feb. 1998 and published on 6 Aug. 1998 as International Publication Number WO 98/33917; in Joukov et al., *J. Biol. Chem.*, 273(12): 6693-6602 (1998); and in Joukov et al., *EMBO J.* 16(3): 3898-3911 (1997), all of which are incorporated herein by reference in their entirety. As explained therein in detail, human VEGF-C is initially produced in human cells as a prepro-VEGF-C polypeptide of 419 amino acids. A cDNA and deduced amino acid sequence for human prepro-VEGF-C are set forth in SEQ ID NO: 1 and 2, respectively, and a cDNA encoding human VEGF-C has been deposited with the American Type Culture Collection (ATCC), 10801 University Blvd., Manassas, Va. 20110-2209 (USA), pursuant to the provisions of the Budapest Treaty (Deposit date of 24 Jul. 1995 and ATCC Accession Number 97231). VEGF-C sequences from other species also have been reported. See Genbank Accession Nos. MMU735620 (Mus musculus); and CCY15837 (Coturnix coticus) for example, incorporated herein by reference.

The prepro-VEGF-C polypeptide is processed in multiple stages to produce a mature and most active VEGF-C polypeptide of about 21–23 kDa (as assessed by SDS-PAGE under reducing conditions). Such processing includes cleavage of a signal peptide (SEQ ID NO: 2, residues 1–31); cleavage of a carboxyl-terminal peptide (corresponding approximately to amino acids 228–419 of SEQ ID NO: 2 and having a pattern of spaced cysteine residues reminiscent of a Balbiani ring 3 protein (BR3P) sequence [Dignam et al., *Gene*, 88:133–40 (1990); Paulsson et al., *J. Mol. Biol.*, 211:331–49 (1990)]) to produce a partially-processed form of about 29 kDa; and cleavage (apparently extracellularly) of an amino-terminal peptide (corresponding approximately to amino acids 32–103 of SEQ ID NO: 2) to produce a fully-processed mature form of about 21–23 kDa. Experimental evidence demonstrates that partially-processed forms of VEGF-C (e.g., the 29 kDa form) are able to bind the Flt4 (VEGFR-3) receptor, whereas high affinity binding to VEGFR-2 occurs only with the fully processed forms of VEGF-C. It appears that VEGF-C polypeptides naturally associate as non-disulfide linked dimers.

Moreover, it has been demonstrated that amino acids 103–227 of SEQ ID NO: 2 are not all critical for maintaining VEGF-C functions. A polypeptide consisting of amino acids 113–213 (and lacking residues 103–112 and 214–227) of SEQ ID NO: 2 retains the ability to bind and stimulate VEGF-C receptors, and it is expected that a polypeptide spanning from about residue 131 to about residue 211 will retain VEGF-C biological activity. The cysteine residue at position 156 has been shown to be important for VEGFR-2 binding ability. However, VEGF-C/Cys156 polypeptides (i.e., analogs that lack this cysteine due to deletion or substitution) remain potent activators of VEGFR-3. If the anti-restenosis effects of VEGF-C are mediated through VEGFR-3, then use of VEGF-C/Cys156 polypeptides (and polynucleotides encoding them) is expected to provide anti-restenosis efficacy while minimizing VEGFR-2 mediated side-effects. The cysteine at position 165 of SEQ ID NO: 2 is essential for binding either receptor, whereas analogs lacking the cysteines at positions 83 or 137 compete with native VEGF-C for binding with both receptors and stimulate both receptors.

An alignment of human VEGF-C with VEGF-C from other species (performed using any generally accepted alignment algorithm) suggests additional residues wherein modifications can be introduced (e.g., insertions, substitutions, and/or deletions) without destroying VEGF-C biological activity. Any position at which aligned VEGF-C polypep-
tides of two or more species have different amino acids, especially different amino acids with side chains of different chemical character, is a likely position susceptible to modi-
...and quail VEGF-C is set forth in FIG. 5 of PCT/US98/01973.

Apart from the foregoing considerations, it will be under-
stood that innumerable conservative amino acid substitu-
tions can be performed to a wildtype VEGF-C sequence which are likely to result in a polypeptide that retains
VEGF-C biological activities, especially if the number of such substitutions is small. By “conservative amino acid substitu-
tion” is meant substitution of an amino acid with an
amino acid having a side chain of a similar chemical character. Similar amino acids for making conservative substitu-
tions include those having an acidic side chain (glutamic acid, aspartic acid); a basic side chain (arginine, lysine, histidine); a polar amide side chain (glutamine, asparagine); a hydrophobic, aliphatic side chain (leucine, isoleucine, valine, alanine, glycine); an aromatic side chain
(phenylalanine, tryptophan, tyrosine); a small side chain (glycine, alanine, serine, threonine, methionine); or an alip-
phatic hydroxyl side chain (serine, threonine). Addition or deletion of one or a few internal amino acids without destroying VEGF-C biological activities also is contempla-
ated.

Without intending to be limited to a particular theory, the mechanism behind the efficacy of VEGF-C in preventing restenosis is believed to relate to the ability of VEGF-C to stimulate re-endothelialization of the injured vessel (and/or of the intravascular stent) without significant concomitant stimulation of smooth muscle proliferation in the vessel. VEGF-C also may inhibit smooth muscle cell proliferation. Accordingly, candidate VEGF-C analog polypeptides can be
rapidly screened first for their ability to bind and stimulate autophosphorylation of known VEGF-C receptors
(VEGFR-2 and VEGFR-3). Polypeptides that stimulate one or both known receptors are rapidly re-screened in vitro for their mitogenic and/or chemotactic activity against cultured capillary or arterial endothelial cells (e.g., as described in WO 98/33917). Polypeptides with mitogenic and/or chemotactic activity are then screened in vivo as described herein for the ability to prevent restenosis. In this way, variants (analogs) of naturally occurring VEGF-C proteins are rapidly screened to determine whether or not the variants have the requisite biological activity to constitute “VEGF-C polypeptides” for use in the present invention.

The growth factor named Vascular Endothelial Growth Factor D (VEGF-D), as well as human sequences encoding VEGF-D, and VEGF-D variants and analogs, have been described in detail in International Patent Application Number PCT/US97/14696, filed 21 Aug. 1997 and published on 26 Feb. 1998 as International Publication Number WO
95(2): 548–553 (1998), both incorporated herein by refer-
ence in their entirety. As explained therein in detail, human
VEGF-D is initially produced in human cells as a prepro-
VEGF-D polypeptide of 354 amino acids. A cDNA and deduced amino acid sequence for human prepro-VEGF-D are set forth in SEQ ID NOs: 3 and 4, respectively. VEGF-D sequences from other species also have been reported. See Genbank Accession Nos. D89628 (Mus musculus); and AF014827 (Rattus norvegicus), for example, incorporated herein by reference.

The prepro-VEGF-D polypeptide has a putative signal peptide of 21 amino acids and is apparently proteolytically processed in a manner analogous to the processing of
prepro-VEGF-C. A “recombinantly matured” VEGF-D lacking residues 1–92 and 202–354 of SEQ ID NO: 4 retains the ability to activate receptors VEGFR-2 and VEGFR-3, and appears to associate as non-covalently linked dimers. Thus, preferred VEGF-D polynucleotides include those polynucle-
otides that comprise a nucleotide sequence encoding amino acids 93–201 of SEQ ID NO: 4. The guidance provided above for introducing function-preserving modifications into VEGF-C polypeptides is also suitable for introducing function-preserving modifications into VEGF-D polypep-
tides.

A therapeutic or prophylactic treatment of restenosis provided by the present invention involves administering to a mammalian subject such as a human a composition comprising a VEGF-C or VEGF-D polynucleotide or polypeptide or combination thereof (sometimes generically referred to herein as a “VEGF-C or VEGF-D therapeutic agent”).

The “administering” may be performed using any medically-accepted means for introducing a therapeutic directly or indirectly into the vasculature of a mammalian subject, including but not limited to injections; oral inges-
tion; intranasal or topical administration; and the like. In a preferred embodiment, administration of the composition comprising the VEGF-C or VEGF-D polynucleotide or polypeptide composition is performed intravascularly, such as by intravenous, intra-arterial, or intracoronary arterial injection.

In a highly preferred embodiment, the composition is administered locally, e.g., to the site of angioplasty or bypass. For example, the administering comprises a catheter-mediated transfer of the therapeutic composition into a blood vessel of the mammalian subject, especially into a coronary artery of the mammalian subject. Exemplary materials and methods for local delivery are reviewed in Lincoff et al., Circulation, 90: 2070–2084 (1994); and Wilensky et al., Trends Cardiovasc. Med., 3:163–170 (1993), both incorporated herein by reference. For example, the composition is administered using infusion-perfusion balloon catheters (preferably microporous balloon catheters) such as those that have been described in the literature for intracoronary drug infusions. See, e.g., U.S. Pat. No. 5,713, 860 (Intravascular Catheter with Infusion Array); U.S. Pat. No. 5,087,244; U.S. Pat. No. 5,653,689; and Wolinsky et al., J. Am. Coll. Cardiol., 15: 475–481 (1990) (Wolinsky Infu-
sion Catheter); and Lambert et al., Coron. Artery Dis., 4:
469–475 (1993), all of which are incorporated herein by reference in their entirety. Use of such catheters for site-
directed somatic cell gene therapy is described, e.g., in
Mazur et al., Texas Heart Institute Journal, 21: 104–111

For example, in patients with angina pectoris due to a single or multiple lesions in coronary arteries and for whom PTCAs is prescribed on the basis of primary coronary angiogram findings, an exemplary protocol involves performing PTCA through a 7F guiding catheter according to standard clinical practice using the femoral approach. If an optimal result is not achieved with PTCAs alone, then an endovascular stent also is implanted. (A nonoptimal result is defined as residual stenosis of >30% of the luminal diameter according to a visual estimate, and B or C type dissection.) Arterial gene transfer at the site of balloon dilatation is performed immediately after the angioplasty, but before stent implantation, using an infusion-perfusion balloon catheter. The size of the catheter will be selected to match the diameter of the artery as measured from the angiogram, varying, e.g., from 3.0 to 3.5F in diameter. The balloon is
inflated to the optimal pressure and gene transfer is performed during a 10 minute infusion at the rate of 0.5 ml/min with virus titer of 1.15x10^{10}

In another embodiment, intravascular administration with a gel-coated catheter is contemplated, as has been described in the literature to introduce other transgenes. See, e.g., U.S. Pat. No. 5,674,192 (Catheter coated with tenaciously-adhered swellable hydrogel polymer); Riessen et al., Human Gene Therapy, 4: 749–758 (1993); and Steg et al., Circulation, 96: 408–411 (1997) and 1997: 1648–1656 (1994); all incorporated herein by reference. As shown in FIG. 1, a catheter 10 is provided to which an inflatable balloon 12 is attached at a distal end. The balloon includes a swellable hydrogel polymer coating 14 capable of absorbing a solution comprising a therapeutic VEGF-C or VEGF-D therapeutic agent. Briefly, DNA is solution (e.g., the VEGF-C or VEGF-D polynucleotide) is applied one or more times ex vivo to the surface of an inflated angioplasty catheter balloon coated with a hydrogel polymer (e.g., Slider with Hydrolux, Mansfield Boston Scientific Corp., Watertown, Mass.). The Hydrolux coating is a hydrophilic polyacrylic acid polymer that is cross-linked to the balloon to form a high molecular weight hydrogel tightly adhered to the balloon. The DNA covered hydrogel is permitted to dry before deflating the balloon. Re-inflation of the balloon intravascularly, during an angioplasty procedure, causes the transfer of the DNA to the vessel wall. Thus, referring again to FIG. 1, the catheter with attached, coated balloon is inserted into the lumen 16 of a blood vessel 18 while covered by a protective sheath 20 to minimize exposure of the coated balloon to the blood prior to placement at the site of an occlusion 22. When the instrument has been positioned at the treatment region, the protective sheath is drawn back or the catheter is moved forward to expose the balloon, which is inflated to compress the balloon (and thus the coating) into the vessel wall, causing transfer of the VEGF-C or VEGF-D therapeutic agent to the tissue, in a manner analogous to squeezing liquid from a compressed sponge or transferring wet paint to a surface by contact.

In yet another embodiment, an expandable elastic membrane, film, or similar structure, mounted to or integral with a balloon angioplasty catheter or stent, is employed to deliver the VEGF-C or VEGF-D therapeutic agent. See, e.g., U.S. Pat. Nos. 5,707,385, 5,697,967, 5,700,286, 5,800,507, and 5,776,184, all incorporated by reference herein. As shown in FIGS. 2A–2B, a single layer 30 or multi-layer 30, 32 sheet of elastic membrane material (FIG. 2A) is formed into a tubular structure 34 (FIG. 2B), e.g., by bringing together and adhering opposite edges of the sheet(s), e.g., in an overlapping or a butting relationship. In this manner the elastomeric material may be wrapped around a catheter balloon or stent. A therapeutic VEGF-C or VEGF-D composition is combined with the membrane using any suitable means, including injection molding, coating, diffusion, and absorption techniques. In the multilayer embodiment depicted in the Figures, the edges of the two layers may be joined to form a fluid-tight seal. In a preferred embodiment, one layer of material is first processed by stretching the material and introducing a plurality of microscopic holes or slits 36. After the layers have been joined together, the sheet can be stretched and injected with the therapeutic VEGF-C/D composition through one of the holes or slits to fill the cavity that exists between the layers. The sheet is then relaxed, causing the holes to close and sealing the therapeutic composition between the layers until such time as the sheet is again stretched. This occurs, for example, at the time that an endovascular stent or balloon covered by the sheet is expanded within the lumen of a stenosed blood vessel. The expanding stent or balloon presses radially outward against the inner surface 38 of the tubular sheet covering, thus stretching the sheet, opening the holes, and delivering the therapeutic agent to the walls of the vessel.

In another variation, the composition containing the VEGF-C or VEGF-D therapeutic is administered extravascularly, e.g., using a device to surround or encapsulate a portion of vessel. See, e.g., International Patent Publication WO 98/20027, incorporated herein by reference, describing a collar that is placed around the outside of an artery (e.g., during a bypass procedure) to deliver a transgene to the arterial wall via a plasmid or liposome vector. As shown in FIGS. 3A and 3B, an extravascular collar 40 including a void space 42 defined by a wall 44 formed, e.g., of a biodegradable or biocompatible material. The collar touches the outer wall 46 of a blood vessel 48 at the collar’s outer extremities 50. Blood 52 flows through the lumen of the blood vessel. A longitudinal slit 54 in the flexible collar permits the collar to be deformed and placed around the vessel and then sealed using a conventional tissue glue, such as a thrombin glue.

In still another variation, endothelial cells or endothelial progenitor cells are transplanted ex vivo with the VEGF-C a VEGF-D transgene, and the transplanted cells as administered to the mammalian subject. Exemplary procedures for seeding a vascular graft with genetically modified endothelial cells are described in U.S. Pat. No. 5,785,965, incorporated herein by reference.

If the mammalian subject is receiving a vascular graft, the VEGF-C or VEGF-D therapeutic composition may be directly applied to the isolated vessel segment prior to its being grafted in vivo.

In another preferred embodiment, the administering comprises implanting an intravascular stent in the mammalian subject, where the stent is coated or impregnated with the therapeutic VEGF-C/D gene/protein composition. Exemplary materials for constructing a drug-coated or drug-impregnated stent are described in literature cited above and reviewed in Lincoff et al., Circulation, 90: 2070–2084 (1994). As shown in FIGS. 4A and 4B, a metal or polymeric wire 70 for forming a stent is coated with a composition 72 such as a porous biocompatible polymer or gel that is impregnated with (or can be dipped in or otherwise easily coated immediately prior to use with) a VEGF-C or VEGF-D therapeutic composition. The wire is coiled, woven, or otherwise formed into a stent 74 suitable for implantation into the lumen of a vessel using conventional materials and techniques, such as intravascular angioplasty catheterization. Exemplary stents that may be improved in this manner are described and depicted in U.S. Pat. Nos. 5,800,507 and 5,679,967 (Medtronic, Inc., describing an intraluminal stent comprising fibrin and an elutable drug capable of providing a treatment of restenosis); U.S. Pat. No. 5,776,184 (Medtronic, Inc., describing a stent with a porous coating comprising a polymer and a therapeutic substance in a solid or solid/solution with the polymer); U.S. Pat. No. 5,799,384 (Medtronic, Inc., describing a flexible, cylindrical, metal stent having a biocompatible polymeric surface to contact a body lumen); U.S. Pat. Nos. 5,824,048 and 5,679,400; and U.S. Pat. No. 5,779,729; all of which are specifically incorporated herein by reference in the entirety. Implantation of such stents during conventional angioplasty techniques will result in less restenosis than implantation of conventional stents. In this sense, the biocompatibility of the stent is improved.

In another preferred embodiment, the composition comprises microparticles composed of biodegradable polymers
such as PGLA, non-degradable polymers, or biological polymers (e.g., starch) which particles encapsulate or are impregnated by the VEGF-C or VEGF-C polypeptide/polymer. Such particles are delivered to the intra-vascular wall using, e.g., an infusion angioplasty catheter. Other techniques for achieving locally sustained drug delivery are reviewed in Wilensky et al., Trends Cardiovasc. Med., 3:163–170 (1993), incorporated herein by reference.

Administration via one or more intravenous injections subsequent to the angioplasty or bypass procedure also is contemplated. Localization of the VEGF-C or VEGF-D polypeptides to the site of the procedure occurs due to expression of VEGF-C/D receptors on proliferating endothelial cells. Localization is further facilitated by recombinantly expressing the VEGF-C or VEGF-D as a fusion polypeptide (e.g., fused to an apolipoprotein B-100 oligo-gopeptide as described in Shih et al., Proc. Nat’l Acad. Sci. USA, 87:1436–1440 (1990).

The pharmacological efficiency of VEGF-C polynucleotides, VEGF-C polypeptides, VEGF-D polynucleotides, and VEGF-D polypeptides to prevent stenosis or restenosis of a blood vessel is demonstrated in vivo, e.g., using procedures such as those described in the following examples, some of which are prophetic. The examples assist in further describing the invention, but are not intended in any way to limit the scope of the invention.

**EXAMPLE 1**

Use of Adenovirus-mediated VEGF-C Gene Transfer to Prevent Restenosis

The following experiments, performed in vivo in a rabbit restenosis model, demonstrate the efficacy of adenovirus-mediated intravascular VEGF-C gene transfer for the prevention of post-angioplasty restenosis.

**A. Materials and Methods**

1. Adenoviral constructs.

An adenovirus plasmid containing a cDNA encoding the complete human prepro-VEGF-C open reading frame operably linked to a cytomegalovirus (CMV) promoter and human growth hormone polyadenylation signal sequence was constructed as follows. A DNA fragment comprising a CMV promoter sequence was prepared by digesting the pcDNA3.1+ vector (Invitrogen) with SalI and filling-in the 5’ overhangs with the Klenow enzyme. The CMV promoter (nucleotides 5431–911) was excised from the vector with HindIII and isolated. A full-length human VEGF-C cDNA containing the 1997 bp sequence specified in SEQ ID NO: 1 (as well as less than 50 bases of additional non-coding and polylinker sequence) was excised from a VEGF-C pREP7 expression vector [described in WO 98/33917] with HindIII and Xho I and isolated. A human growth hormone polyadenylation signal (~860 bp) was excised from an eMHC vector with SalI and BamHI. The CMV promoter, VEGF-C cDNA, and hGH polyadenylation signal fragments were simultaneously ligated into a BamHI and EcoRV-digested pCR1 vector. The ligated CMV promoter and VEGF-C cDNA is shown in SEQ ID NO: 17. The resulting construct was opened with BglII and partially digested with BamHI. The full transcriptional unit was ligated into BglII-opened pAdBglII vector. This construct [designated pAdBglIII VEGF-C] was then used to create recombinant adenovirus containing the CMV-VEGF-C-hGH transcriptional unit, using standard homologous recombination techniques. [Barr et al., Gene Ther., 1:51–58 (1994).]

Replication-deficient E1–E3 deleted adenoviruses were produced in 293 cells and concentrated by ultracentrifugation using techniques known in the literature. [See, e.g., Barr et al. (1994).] A control plasmid comprising the lacZ gene operably linked to the same promoter was also used. [Laitinen M. et al., Hum. Gene Ther., 9: 1481–1486 (1998).] The lacZ adenovirus had a nuclear targeted signal, to direct the β-galactosidase expression to the nucleus. Replication-deficient E1–E3 deleted adenoviruses were produced in 293 cells and concentrated by ultracentrifugation (Barr et al., 1994). The adenoviral preparations were analyzed for the absence of helper viruses and bacteriological contaminants.

2. Animal model.

New Zealand White rabbits were employed for the gene transfer study. A first group of rabbits was fed a 0.25% cholesterol diet for two weeks, then subjected to balloon denudation of the aorta, then subjected three days later to the adenovirus-mediated gene transfer. A second group of rabbits was only subjected to the gene transfer. Animals were sacrificed 2 or 4 weeks after the gene transfer. The number of experimental (VEGF-C) and control (lacZ) animals in both study groups was 6.

In the first group of rabbits, the whole aorta, beginning from the tip of the arch, was denuded using a 4.0 F arterial embolectomy catheter (Sorin Biomedical, Irvine, Calif.). The catheter was introduced via the right common artery to the aortic arch and inflated, and the aorta was denuded twice.


The gene transfer was performed using a 3.0 F channel balloon local drug delivery catheter (Boston Scientific Corp., Maple Grove, Mass.). Using fluoroscopical control, the balloon catheter was positioned caudal to the left renal artery, in a segment free of side branches, via a 5 F percutaneous introducer sheath (Arrow International, Reading, Pa.) in the right carotid artery and inflated to 6 ATM with a mixture of contrast media and saline. The anatomical location of the balloon catheter was determined by measuring its distance from the aortic orifice of the left renal artery. Virus titer of 1.15×10^10 plaque forming units (pfu) was administered to each animal in a final volume of 2 ml (0.9% NaCl), and the gene transfer was performed at 6 ATM pressure for 10 minutes (0.2 ml/min). In the second study group the animals had only gene transfer and they were sacrificed 2 weeks after the gene transfer. The number of animals in each study group (0.9% NaCl only; lacZ gene transfer; and VEGF-C gene transfer) was 3. All studies were approved by Experimental Animal Committee of the University of Kuopio in Finland.

4. Histology.

Three hours before sacrifice, the animals were injected intravenously with 50 mg of BrdU dissolved in 40% ethanol. After the sacrifice, the aortic segment where the gene transfer had been performed was removed, flushed gently with saline, and divided into five equal segments. The proximal segment was snap frozen in liquid nitrogen and stored at −70°C. The next segment was immersion-fixed in 4% paraformaldehyde/15% sucrose (pH 7.4) for 4 hours, rinsed in 15% sucrose (pH 7.4) overnight, and embedded in paraffin. The medial segment was immersion-fixed in 4% paraformaldehyde/phosphate buffered saline (PBS) (pH 7.4) for 10 minutes, rinsed 2 hours in PBS, embedded in OCT compound (Miles), and stored at −70°C. The fourth segment was immersion-fixed in 70% ethanol overnight and embedded in paraffin. The distal segment was directly stained for β-galactosidase activity in X-GAL staining solution at +37°C. For 16 hours, immersion-fixed in 4% paraformaldehyde/15% sucrose (pH 7.4) for 4 hours, rinsed in 15% sucrose overnight, and embedded in paraffin. Paraffin sections were used for immunocytochemical detection of smooth muscle
cells (SMC), macrophages, and endothelium. Gene transfer efficiency was evaluated using X-GAL staining of OCT-embedded tissues. BrdU-positive cells were detected according to manufacturer’s instructions. Morphometry was performed using haematoxylin-and-eosin stained paraffin sections using image analysis software. Measurements were taken independently by two observers from multiple sections, without knowledge of the origin of the sections. Intima/media (I/M) ratio was used as a parameter for intimal thickening.

B. Results.

Histological analysis of the balloon-denuded mice revealed that the lacZ-transfected control group had an I/M ratio of 0.61 two weeks after the gene transfer, which represented a statistically significant difference (p<0.05) from the VEGF-C-transfected groups (I/M ratio 0.40). The tendency that VEGF-C group had a smaller I/M ratio persisted at 4 weeks time point after the gene transfer.

In the second group of rabbits that were subjected only to gene transfer to the vessel wall (without endothelial denudation), the I/M ratio in the lacZ group was 0.3, compared to 0.15 for the VEGF-C group. This difference, too, represented a statistically significant (p<0.05) inhibition in neo intima formation in VEGF-C group.

The BrdU labeling will permit analysis of smooth muscle cell proliferation in VEGF-C-transfected versus control lacZ animals. SMC proliferation is expected to be reduced in the VEGF-C-transfected population.

The foregoing data demonstrate that VEGF-C gene transfer significantly reduced intimal thickening at two weeks time point after aortic denudation and after vessel wall damage caused by the gene transfer catheter without balloon denudation. These data indicate a therapeutic utility for VEGF-C gene transfer for the prevention of post-angioplasty restenosis.

EXAMPLE 2

Comparative Example Demonstrating that Anti-Restenosis Effects of VEGF-C Appear Superior to Those of VEGF

The following experiments demonstrate the efficacy of adenovirus-mediated intravascular VEGF and VEGF-C gene transfer for the prevention of post-angioplasty restenosis, and demonstrates that VEGF-C appeared to provide a superior therapeutic efficacy compared to VEGF.

A. Material and Methods

1. Adenoviral constructs.

VEGF (murine VEGF-A164; SEQ ID NO: 18) adenovirus was constructed using the same promoter as the VEGF-C construct, and following similar procedure as described in Example 1. The VEGF-A164 adenoviral construct was produced in 293T cells and concentrated essentially as described in Example 1, and analyzed to be free of helper virus, lipopolysaccharides, and bacterial contaminants.

2. Animal model.

Sixty three New Zealand White rabbits were divided into two major groups, the first having 0.25% cholesterol diet for two weeks and balloon denudation of the aorta before gene transfer, and the second group having only the gene transfer. Gene transfer was performed in the first group of rabbits three days after denudation, and the animals were sacrificed 2 or 4 weeks after the gene transfer. Number of rabbits in each study group (lacZ, VEGF, and VEGF-C) was 6. In the second study group, the rabbits had only the gene transfer, without cholesterol diet or balloon denudation, and were sacrificed 2 or 4 weeks after the gene transfer. The number of rabbits in each study group (0.9% saline, lacZ, VEGF, and VEGF-C) was 3.


Gene transfer was performed according to the procedure described in Example 1.

4. Histology.

Histology was performed essentially as described in Example 1 with the following modifications: SMC were detected using HHF35 (DAKO, 1:50 dilution), macrophages were detected using RAM-11 (DAKO, 1:50 dilution), endothelium was detected using CD31 (DAKO, 1:50 dilution), and T cells were detected using MCA 805 (DAKO, 1:100 dilution). Controls for immunostainings included incubations with class- and species matched immunoglobulins and incubations where primary antibodies were omitted. Morphometry and image analysis were performed using Image-Pro Plus™ software and an Olympus AX70 microscope (Olympus Optical, Japan). Statistical analyses were performed using the ANOVA and modified t-test. P<0.05 was considered statistically significant.

B. Results.

Histological analysis of the balloon-denuded rabbit aorta shows intimal thickening and SMC proliferation. Two weeks after gene transfer, the lacZ control group had the highest I/M ratio (0.57±0.04) whereas VEGF-C (0.38±0.02) and VEGF (0.49±0.17) groups showed decreased intimal thickening. The difference in I/M ratios between lacZ and VEGF-C groups was significant (P<0.05), whereas those between lacZ and VEGF groups were not statistically significant, at the two-week time point. The tendency that both VEGF and VEGF-C groups had smaller I/M ratios persisted at the four week time point when the I/M ratio was 0.73±0.16, 0.44±0.14, and 0.63±0.21 for the lacZ, VEGF, and VEGF groups, respectively. Hematoxylin-and-eosin and immunostainings of the transfected arteries indicate that intimal thickening in all arteries was composed predominantly of SMC.

Use of adenoviral vectors can lead to immunoavailability and inflammatory responses, partly because high titer adenovirus induces expression of NFκB and activates a CD8 response. However, no signs of inflammation nor foam cell accumulation were detected as judged by macrophage and T-cell immunostainings. In addition, human clinical gene therapy grade viruses were used together with short exposure times in the transfected arteries, which may also help explain the absence of severe inflammatory reactions in this study.

The percentage of proliferating cells was analyzed using BrdU labeling. No significant differences were seen, although the VEGF-C group tended to have a lower proliferation rate, consistent with the observation that VEGF-C transduced arteries had smaller I/M ratios at both time points. Two weeks after balloon denudation, the percentage of proliferating cells was 1.8±0.4, 2.2±0.7, and 1.2±0.0 for the lacZ, VEGF, and VEGF-C groups, respectively, and after four weeks, the percentage of proliferating cells was 0.3±0.1, 1.2±0.5, and 0.3±0.1 for the lacZ, VEGF, and VEGF-C groups, respectively. Endothelial regeneration was analyzed by measuring the length of intact endothelium from histological sections. No significant differences were found between the study groups.

The potential of adenovirus to cause the damage to the vessel wall and neointima formation was tested by performing high-titer adenovirus gene transfer to intact abdominal aorta of rabbits without balloon-denudation. Control rabbits were treated in the same way with 0.9% saline. The positioning of the gene transfer catheter caused some internal elastic lamina damage and moderate induction of neointima
formation after the procedure. At the two-week time point, the I/M ratio in the lacZ group was 0.24±0.06, in the control group 0.28±0.05, in the VEGF-C group 0.18±0.07, and in the VEGF group 0.15±0.03. At the four-week time point the lacZ group had an I/M ratio of 0.22±0.13, the VEGF-C group 0.15±0.03, and the VEGF group 0.23±0.11.

This study shows a beneficial therapeutic effect of intravascular adenovirus-mediated VEGF-C gene transfer on the vessel wall after balloon injury, and also compares VEGF-C and VEGF adenovirus-mediated gene transfer for the prevention of neointima formation. Although different receptor binding profiles of VEGF-C and VEGF might have led to different biological effects in the vessel wall, both VEGFs reduced intimal thickening two weeks after gene transfer. Thus, both VEGFs are potential candidates for vascular gene therapy of ischemic atherosclerotic diseases. However, according to this experiment, VEGF-C appears to prevent restenosis more effectively than VEGF in this model system. The superior ability of VEGF-C to prevent restenosis, as compared to VEGF, could be due to expression or activity of VEGFR-3 which is a receptor for VEGF-C and VEGF-D, but not for VEGF. Alternatively, the apparent superiority may be attributable to a restenosis-promoting effect of VEGF mediated through VEGFR-1 or due to differential ligand effects (VEGF-C versus VEGF) mediated through the common receptor VEGFR-2, which is reportedly expressed in vascular smooth muscle cells. [See Grosskreutz et al., Microvasc. Res., 58(2): 128–136 (September, 1999)].

EXAMPLE 3

Expression of Transfected VEGFs in the Aortic Wall

Using the aortic segments from the same experimental animals described in Example 2, mRNA expression of lacZ, VEGF-C and VEGF (murine VEGF-A164) was analyzed in aortic tissue after gene transfer. Total RNA was extracted from transfected aortic segments using Trizol Reagent (Gibco-BRL), and 2 μg of RNA was used for cDNA synthesis. Primers for lacZ, VEGF-C and VEGF were designed to distinguish between endogenous and transduced genes by selecting the 5' primers from the CMV promoter and the 3' primers from the coding regions.

For lacZ amplification, primers were: 5' primer 5'-TGGAGGCTAGGTCTGGAC-3' (SEQ ID NO: 5) and 3' primer 5'-AACTCTGCTGTCCTCCCTCA-3' (SEQ ID NO: 6). The first PCR cycle was an initial incubation at 96°C for 4 minutes followed by 80°C for 3 minutes during which the DNA polymerase was added. This was followed by 30 cycles, each consisting of 94°C for 45 seconds, 58°C for 45 seconds, and 72°C for 50 seconds, followed by a final extension of 72°C for 5 minutes. 5 μl of the first PCR product was used for the second PCR with 5' primer 5'-GGTACCAACGTGCTGGAC-3' (SEQ ID NO: 11) and 3' primer 5'-CCGTGCATGGTGACG-3' (SEQ ID NO: 10). The first PCR cycle was an initial incubation at 96°C for 4 minutes followed by 80°C for 3 minutes during which the DNA polymerase was added. This was followed by 39 cycles each consisting of 94°C for 30 seconds, 56°C for 40 seconds, and 72°C for 90 seconds, followed by a final extension of 72°C for 5 minutes, followed by a final extension of 72°C for 5 minutes. 5 μl of the first PCR product was used for the second PCR with 5' primer 5'-TCTCCCAAAACCTACACCG-3' (SEQ ID NO: 11) and 3' primer 5'-CAAGTGCATGTGGAC-3' (SEQ ID NO: 12). The first PCR cycle was an initial incubation at 96°C for 3 minutes followed by 80°C for 3 minutes followed by 39 cycles each consisting of 94°C for 60 seconds, 57°C for 30 seconds, and 72°C for 90 seconds, followed by a final extension of 72°C for 5 minutes.

For VEGF amplification, primers were: 5' primer 5'-TCGTGGATACATACGCTGGCG-3' (SEQ ID NO: 13) and 3' primer 5'-TTCCAGGGTAACTCAAGAAGCTGC-3' (SEQ ID NO: 14). The first PCR cycle was an initial incubation at 96°C for 4 minutes followed by 80°C for 3 minutes, followed by 39 cycles each consisting of 94°C for 30 seconds, 53°C for 40 seconds, and 72°C for 90 seconds, followed by a final extension of 72°C for 5 minutes. 5 μl of the first PCR product was used for the second PCR with 5' primer 5'-GACCTGGTCTTTACTGCTG-3' (SEQ ID NO: 15) and 3' primer 5'-GGAAACATTTACGCTGCG-3' (SEQ ID NO: 16). The first PCR cycle was an initial incubation at 96°C for 3 minutes followed by 80°C for 3 minutes followed by 39 cycles each consisting of 94°C for 60 seconds, 54°C for 30 seconds, and 72°C for 90 seconds, followed by a final extension of 72°C for 5 minutes.

The mRNA of lacZ, VEGF-C and VEGF was detected in aortic wall tissue up to four weeks after gene transfer.

Gene transfer efficiency was evaluated by assaying lacZ expression, analyzed by X-Gal staining for β-galactosidase activity, in OCT embedded tissue sections. Transfection efficiency was 1.1%±0.5 and 0.3%±0.1, two and four weeks respectively, after intravascular catheter-mediated gene transfer.

EXAMPLE 4

Expression of VEGF Receptors in the Aortic Wall

Using the experimental animals described in Example 2, VEGFR-1, VEGFR-2, and VEGFR-3 expression in aortic tissue was analyzed by immunostainings and in situ hybridization. Immunohistochemistry was performed using clone sc-316 (Santa Cruz Biotechnology, 1:50 dilution) to detect VEGFR-1, clone sc-6251 (Santa Cruz Biotechnology, 1:500 dilution) to detect VEGFR-2, and clone sc-637 (Santa Cruz Biotechnology, 1:500 dilution) to detect VEGFR-3. Controls for immunostainings included incubations with class- and species matched immunoglobulins and incubations where primary antibodies were omitted. In situ hybridization of VEGF receptor mRNAs was carried out using 33P-UTP labeled riboprobes. Expression of all receptors was localized to endothelium. VEGFR-2 was also expressed in neointimal SMCs.

EXAMPLE 5

Use of Naked VEGF-C Transgene Therapy to Prevent Restenosis

The procedures described in Example 1 or 2 are repeated, with the following modifications. Instead of using an adenovirus vector for delivery of the VEGF-C transgene, a mammalian expression vector is constructed for direct gene transfer (of naked plasmid DNA). The VEGF-C coding sequence is operably linked to a suitable promoter, such as the CMV promoter, and preferably linked to a suitable
polyadenylation sequence, such as the human growth hormone polyadenylation sequence. Exemplary VEGF-C vectors can be modeled from vectors that have been described in the literature to perform vector-free gene transfer for other growth factors, by substituting a VEGF-C coding sequence for a VEGF coding sequence. [See, e.g., Issner et al., *Circulation*, 91: 2687–2692 (1995); and Issner et al., *Human Gene Therapy*, 7: 989–1011 (1996), incorporated herein by reference.] A similar construct comprising a lacZ gene is used as a control.

A hydrogel-coated balloon catheter (Boston Scientific) is used to deliver the VEGF-C transgene essentially as described in Asahara et al., *Circulation*, 94: 3291–3302 (Dec. 15, 1996), incorporated herein by reference. Briefly, an angioplasty balloon is prepared ex vivo by advancing the deflated balloon completely through a teflon protective sheath (Boston Scientific). The balloon is inflated and a conventional pipette is used to apply the transgene construct (e.g., 50–5000 µg transgene DNA in a saline solution) to the Hydrogel coating the external surface of the inflated balloon. After the transgene solution has dried, the balloon is deflated, withdrawn into the protective sheath, and re-inflated to minimize blood flow across the balloon surface until the balloon is properly positioned in the target artery.

Intima/media (I/M) ratio is again used as a parameter for intimal thickening. Reduced I/M ratio in animals treated with the VEGF-C transgene-coated balloon catheter is considered indicative of therapeutic efficacy. As described in Example 2, comparison of the therapeutic efficacy of VEGF-C gene transfer with other therapies, such as VEGF gene transfer, can be conducted in parallel.

EXAMPLE 6

Use of VEGF-C Gene Therapy to Prevent Restenosis Following Angioplasty with Stent

The procedures described in the preceding examples are repeated with the modification that initial balloon angioplasty is accompanied by implantation of a coronary stent using conventional procedures. The VEGF-C transgene is delivered concurrently or immediately before or after stent implantation essentially as described in the preceding examples. Increased quantities (e.g., two-fold to ten-fold) of the transgene (compared to angioplasty without stent) and increased transfection time may be desirable, as described in Van Belle et al., *J. Am. Coll. Cardiol.*, 29:1371–1379 (May, 1997), incorporated by reference herein. Decreased neointimal thickening and/or decreased thrombotic occlusion in the VEGF-C gene-treated animals versus control animals treated with a marker gene is considered evidence of the efficacy of the VEGF-C gene therapy.

EXAMPLE 7

Use of an Extravascular Collar to Reduce Vascular Stenosis

An inert silicon collar such as described in International Patent Publication No. WO 98/20027 is surgically implanted around the carotid arteries of New Zealand White Rabbits. The collar acts as an irritation agent that will induce intimal thickening, and contains a reservoir suitable for local delivery of a VEGF-C transgene or protein pharmaceutical formulation. Gene transfer, using the VEGF-C adenovirus construct or control construct described in Example 1 is initiated five days later by injecting 10^9–10^11 pfu into the collar. Animals are sacrificed 14 or 28 days later and histological examinations are performed as described in Example 1. Intima/media thickness ratio [Yla-Herttuala et al., *Arteriosclerosis*, 6: 230–236 (1986)] is used as an index of stenosis. Reduced I/M ratio in the VEGF-C-transfected rabbits, as compared to the lacZ control rabbits, indicates therapeutic efficacy of VEGF-C gene transfer for preventing arterial stenosis.

EXAMPLE 8

Use of VEGF-C Polypeptides to Reduce or Prevent Restenosis

The procedures described in Example 1 are repeated except, instead of treating the test animals with an adenovirus containing a VEGF-C transgene or lacZ control, the animals are treated with a composition comprising a VEGF-C polypeptide in a pharmaceutically acceptable carrier (e.g., isotonic saline with serum albumin), or with carrier solution alone as a control. Test animals receive either 10, 100, 250, 500, 1000, or 5000 µg of a VEGF-C polypeptide via intra-arterial infusion, e.g., as described in Example 1. A second group of animals additionally receive an injection of the VEGF-C polypeptide 7 days later. The animals were sacrificed and histological examination performed as described in Example 1. Reduced I/M ratio in the VEGF-C-treated animals versus control animals provides evidence of the therapeutic efficacy of VEGF-C polypeptide treatment. Reception of the experiment using various sustained-release VEGF-C formulations and materials as described above is expected to further enhance the therapeutic efficacy of the VEGF-C polypeptide. Moreover, a treatment regimen comprising the simultaneous administration of VEGF-C protein (to provide immediate therapy to the target vessel) with a VEGF-C transgene (to provide sustained therapy for several days or weeks) is specifically contemplated as a variation of the invention.

EXAMPLE 9

Anti-Stenosis/Anti-Restenosis Activity of VEGF-D

The procedures described in the preceding examples are repeated using a composition comprising a VEGF-D polynucleotide or VEGF-D polypeptide in lieu of the VEGF-C polynucleotide/polypeptide, to demonstrate the ability of VEGF-D to prevent stenosis or restenosis of a blood vessel.

While the present invention has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those in the art, all of which are intended as aspects of the present invention. Accordingly, only such limitations as appear in the claims should be placed on the invention.
SEQUENCE LISTING

ACGCGCGCG CTCGCCAAA AGCTACACG ACGCGACCG CACGCGCGTC CTCGGCGGC
60
CTCGCGCG CTCGCCGGC CGAGTACGA GTAACGTTT CTCGCGAAC
120
TTTACGTTA CCGCGCGGG CGTCGCCTGG GAGCGCGCG TGCAGGTTT
180
GGAAAGCGGA GGCGCGGCG CGTCGCGCT CGCAGCGGG GGAAGCGGG
240
GCGAGCGGG GGGAGGGG ACCAGGAGG CGCGCGCG GCAGCGGGG
300
CCACCCCTGC CGCGCGCGA GCAGCGTCC CCACCCCCGG GTCGAGCC AC
357

Net Hist
1

TTG CTG GGC TTC TTC TTT GTC GGC TGT TCT CTA GCC GTC GCC GTC
405
Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala Leu
5

CTC CCG GGT CCT GCC GAG GCC GCC GGC GCC GCC GCC TCC GGT GCC
453
Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe Glu Ser
20

GGT CTC GAC TCC GAG GCT GCC GAC GCC GCG GCC GGT GTC GTC GTC
501
Gly Leu Asp Leu Ser Ala Asp Ala Pro Ala Gly Ser Ser Ser Ser Thr
35

TAT GCA AGC AAA GAT CTC GAG GAG CAG TTA CCG TCT GTC ATT GCA
549
Tyr Ala Ser Lys Asp Leu Glu Glu Leu Arg Ser Val Ser Val
55

GAT GAA CTC ACT ACT GTA CTC TAC CCA GAA TAT TGG AAA ATG TAC AGT
597
Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met Tyr Lys
70

TGT CAG CGA AAA GGA GAA TGG CCC CAT AAG AAG GAA CGG GCC ACG
645
Cys Gly Leu Arg Lys Gly Gly Trp Glu His Asp Arg Glu Ala Asn
55

CTC ACC TCA AGG ACA GAA GAG ACT ATA AAA TTT GCT GCA GCA CAT TAT
693
Leu Aen Ser Thr Thr Thr Phe Phe Ala Ala His Tyr
190

AAT ACA GAG ATT TGG AAA AGT ATT ATT ATT ATT ATT ATT ATT ATT ATT ATT
741
Aen Thr Glu Ile Leu Lys Ser Ile Asp Aen Glu Trp Aen Thr Aen Thr
115

TGC ATG CCA CGG GAG GTG TGT ATA GAT GTG GGG AAG GAG TTT GGA GTG
789
Cys Met Pro Arg Glu Val Cys Ile Aep Gly Thr Val
135

GCG ACA AAC ACC TTC TTA AAA CTT CAA GTG TCT TCC TAC ACA TGT
837
Ala Thr Aen Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr Arg Cys
150

GGG GTG TGC AAT AGT GAG GGG CTT CAG TCC ATG AAC ACC ACG ACG
885
Gly Gly Cys Aen Ser Glu Leu Glu Cys Met Aen Thr Ser Thr
165

AGC TAC CTG CGA AAG AGT TTA TAT ACA GTG CCT CTC TCT CCA
933
Ser Tyr Leu Ser Leu Tyr Pro Phe Glu Ile Thr Val Pro Leu Ser Glu
180

GCG CCC AAA CCA GAA ACT AAG TTT GCG AAT CAC ACT TCC TGC CGA
981
Gly Pro Gly Pro Val Thr Ile Ser Phe Ala Aen His Thr Ser Cys Arg
---continued---

195 200 205 210

tgc atg tac cag gt gtt gac gaa cca gtt cag cag att atc cag
Cys Met Ser Lys Leu Asp Val Tyr Arg Gin Val Ser Ile Arg
215 220 225

1029

cat toc cgg cca aca cta cca cag tgt cag gca gcy aac aag acc
Arg Ser Leu Pro Ala Thr Leu Pro Glu Cys Gin Ala Ala Arg Lys Thr
230 235 240

1077

tg ccc acc att tac atg tgt aat cac gcc acc ctc tgc cag cagn
Cys Pro Thr Asn Tyr Met Thr Asn His Ile Cys Arg Cys Leu Ala
245 250 255

1125

cag gaa gat ttt aty ttc tcc gat gtt gaa gat gac tca aca gat
Gln Glu Aep Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Thr Aep
260 265 270

1173

gga ttc cac gat atc tgt gga cca aac aag gag cgt gat gaa gag acc
Gly Phe His Asp Ile Cys Gly Pro Aen Lys Gin Leu Aep Gin Glu Thr
275 280 285 290

1221

tgt cag tgt tgc cag aag cgg ggg gtt cgg cct gcc acc tgt gga ccc
Cys Gin Cys Val Arg Arg Ala Gly Leu Arg Pro Ala Ser Cys Gly Pro
295 300 305

1269

cac aag gaa cta gcc gac aag tca tgt cag tgt aag aac aag
His Lys Glu Gln Asp Ser Arg Leu Asn Ser Arg Asp Arg His Aen Lys
310 315 320

1317

going cgc acc tga gcc cag cac gaa ttt gat gaa aac aca
Leu Phe Pro Asp Gin Cys Glu Arg Asn Asp Gin Aep Gin Pro Leu Aen
325 330 335

1365

tgc cag tgt cta tgt aag aac tgc gcc cgg aag tca cag cgg
Cys Gin Cys Val Arg Thr Cys Gin Thr Arg Gin Thr Gin Pro Leu Aen
340 345 350

1413

cct gga aag tgt gcc tgt gaa tgt cag gaa aag gat ccc cag aag tgt tgg
Pro Gly Lye Ala Cys Glu Thr Gin Thr Pro Gin Lye Aen Thr Gin Thr
355 360 365

1461

tta aag gga aag cag ccc cac cag caa ccc tgc aag tgt tac aag
Leu Lye Gin Lye Gin Thr Phe Gin Ser Gin Arg Cys Gin Arg Cys
375 380 385

1509

cca tgt aag cac cag cag aag cag gct tgt gag cca gta tta tac taa aat
Pro Cys Thr Aen Arg Gin Lye Ala Cys Gin Thr Pro Gin Lye Thr Ser
390 395 400

1557

gaa gaa tgt ggt tgt gtc ctt tca taa tgt aag cga cca aca atg
Glu Gin Val Cys Arg Phe Pro Val Pro Ser Thr Aen Thr Gin Met
405 410 415

1605

ago taagttgtga gcttttttcga gttctatgtc ttctttttttt gaaaaaagtgt
Ser

tggtgcacag tagaactcgc tgtgaacaga gagaccccttg tgtggtcatg ctaacaaaga
1718

cassagtct ctttctgga acactgtgta taacttttaca gaaatgactc ggaacgcatac
1778

tgcacacagc ccctttgca gactttttttctgccatgc tgaacacacgc aagatttttcc
1838

tctttgtatt tcttttaaga aagactata taatattgtaa caactaaaattat tattggttct
1898

gcttttcatttcttaaacac acacattgtg aaaaactcact gtgtcataa attttatatac
tttttatc
1958

2668

<210> SEQ ID NO 2
<211> LENGTH: 419
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<400> SEQUENCE: 2

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Ser Leu Leu Ala Ala
1 5 10 15

1997
Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Ala Phe 20 25 30
Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala 35 40 45
Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Leu Arg Ser Val Ser 50 55 60
Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met 65 70 75 80
Tyr Lys Cys Glu Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln 85 90 95
Ala Asn Leu Asn Ser Arg Thr Glu Thr Ile Lys Phe Ala Ala Ala 100 105 110
His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys 115 120 125
Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe 130 135 140
Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr 145 150 155 160
Arg Cys Gly Gly Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr 165 170 175
Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu 180 185 190
Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser 195 200 205
Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile 210 215 220
Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Glu Ala Ala Asn 225 230 235 240
Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys 245 250 255
Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser 260 265 270
Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu 275 280 285
Glu Thr Cys Glu Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys 290 295 300
Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Glu Cys Val Cys Lys 305 310 315 320
Asn Lys Leu Phe Pro Ser Glu Cys Gly Ala Asn Arg Glu Phe Asp Glu 325 330 335
Asn Thr Cys Glu Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro 340 345 350
Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys 355 360 365
Cys Leu Leu Lys Gly Lys Phe His His Gln Thr Cys Ser Cys Tyr 370 375 380
Arg Arg Pro Cys Thr Asn Arg Glu Ala Cys Glu Pro Gly Phe Ser 385 390 395 400
Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro 405 410 415
Gln Met Ser
\begin{verbatim}
<210> SEQ ID NO 3
<211> LENGTH: 2029
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<221> NAME/KEY: CD6
<222> LOCATION: (411)..(1475)

<400> SEQUENCE: 3

gtggttgccc agcttgcttg agctgtaagc attggtggccc acaccacctc cttaacacgc 60
aactagaacc tgggccatcc atgggagaga tttttttaat ttttggagaca tgaagtaaat 120
ttgaggtgct ttttaatttc agytgagaaga catgtccaccc ttctgtattat ttttggagaa 180
cctttttgct ttttttctct ctctctcccc acccctagtag ttgtgaaaaa aaagctgacc 240
ttgcttaattt gaaatatttt cattggtatt tgcattgacag tcattatggtt tttttctgtg 300
gtaatttggtt aagttttaaaa ctttctctct ggaattgcgtc ttttttgaaca atttttctta 360
gttgccctag gctaatgcttg tagtactcag tggatattga aatattcaca aatagctcg 416

Met Tyr

aga gag tgg tga tgg tga aat gtt ttc atg ttc atg tgg tac gtc cag ctc 464
Arg Glu Thr Val Val Val Val Phe Met Met Leu Tyr Val Glu Leu
5     10     15

gtt cag ggc tcc agt aat gaa gta cca gca tga tct cag 512
Val Glu Gly Ser Ser Arg Ala Arg Val His Gly Pro Val Lys Arg Ser Ser Glu
20    25    30

tcc aca tgg gaa cga tcc gca cag cag atc agg gct gtt tct agt tgg 560
Ser Thr Leu Glu Gly Ser Arg Glu Gln Ile Arg Ala Ala Ser Ser Leu
35    40    45    50

gag gaa cta ctt cga att act cac tct gag gac tgg aag ctt gta gga 608
Glu Glu Leu Arg Ile Thr His Ser Glu Aep Thr Lys Tyr Leu Trp Arg
55    60    65

tgc agg ctc agg ctc aac aat ttt acc aat ttc gac tca gct gca 656
Cys Arg Leu Arg Leu Ser Phe Ser Ser Met Arg Ser Arg Ser Ala
70    75    80

tcc cat cgg tcc act agg ttt ggc gaa act ttc tat gac att gaa aca 704
Ser His Arg Ser Thr Arg Phe Ser Thr Ser Met Arg Ser Ser Ala
85    90    95

cga aag tgc gtt gaa gaa gaa tgg caa aag act cag bga aag oct aga 752
Leu Lys Val Ile Aep Glu Glu Tpc Glc Arg Thr Gln Ser Ser Pro Arg
100   105   110

gaa gac tgg gtc gat gat gaa gaa aag aag aag aag acc aac aca 800
Glu Thr Cys Val Glu Val Ala Ser Leu Gly Lys Ser Thr Aen Thr
115   120   125   130

ttc tcc aag ccc cct tgg ctc gaa gca tct gtt ggt gtt cgc tgc 848
Phe Phe Lys Pro Pro Cys Val Val Val Phe Arg Cys Gly Gly Cys Cys
135   140   145

aag gaa gac gac ctt tct gat gtg aac acc acc aag ctc aag ctc tct 896
Aen Glu Glu Ser Leu Ile Cys Met Aen Thr Ser Thr Ser Tyr Ile Ser
150   155   160

aaa cag ctc ttt gag tca tca tca tca tca tca gta cct tgg tca 944
Lys Glu Leu Phe Glu Ile Ser Val Pro Leu Thr Pro Glu Leu
165   170   175

gtt gct gtt gaa gtt gcc aat ctc gca ggt tga aag tgc tgg cct aca 992
Val Pro Val Lys Val Ala Aen His Thr Gly Cys Lys Cys Leu Pro Thr
180   185   190

gcc ccc ctc cca tca tca atc aag aag aag ctc aag aag ctc gca 1040
Ala Pro Arg His Pro Tyr Ser Ile Ile Arg Arg Ser Ile Glu Ile Pro
195   200   205   210

gaa gaa gac gtc tgt ctc ctc aag aac ctc tgt ctc att gac atg 1088

\end{verbatim}
Glu Glu Asp Arg Cys Ser His Ser Lys Lys Leu Cys Pro Ile Asp Met 215 220 225

Glu Trp Asp Ser Asn Lys Cys Val Leu Gln Glu Glu Asn Pro 230 235 240

Leu Ala Gly Thr Glu Asp His Ser His Leu Gln Pro Ala Leu Cys 245 250 255

Gly Pro His Met Met Phe Asp Glu Pro Asp Arg Cys Glu Cys Val Lys 260 265 270

Thr Pro Cys Pro Lys Asp Leu Ile Gln His Pro Lys Asn Ser Cys 275 280 285 290

Phe Glu Cys Leu Ser Leu Thr Cys Glu Cys Lys His Cys Leu 295 300 305

Thr Pro His Pro Thr Cys Ser Cys Glu Asp Arg Cys Pro Phe His Thr 310 315 320

Arg Pro Cys Pro Ser Gly Lys Thr Ala Cys Ala Cys Ala Arg Phe 325 330 335

Pro Lys Glu Lys Arg Ala Ala Gln Gly Pro His Ser Arg Lys Asn Pro 340 345 350

tga ttcagcgttc caagttccoc atocctgtaa ttttttacaag catgagctgtt 1525

tgccaagttg cttctagtctt tttttttttta ggtgttaaaaa aaaaaatccaa aaaaaaccag 1585

cacacaatgc aacottcatt tcacacaccgc taaagagtcccttgtttagctt 1645

gatgtggttc tttagcagtct gagctagccc gaattcagag gaagggggacc 1705

tagatattgg aatttttggttt aaaaataaggggttgagtct 1765

catgtggttc gaattcaggtt aagctagcgtt aacggagaa aagctagttcg 1825

tggttttttt taaagagtcccttgaaatcagggag 1885

tttctgtcgt gaattcaggtt ttttctttttttt aaaaaataagggag 1945

tatttttttc aaaagggag 2005

tattttttttt cagtcagttc aaaaaataagggag 2069

cagcagaaaaa aaaaaaaa aaaa
<table>
<thead>
<tr>
<th>85</th>
<th>90</th>
<th>95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu</td>
<td>Thr</td>
<td>Leu</td>
</tr>
<tr>
<td>Lys</td>
<td>Val</td>
<td>Ile</td>
</tr>
<tr>
<td>Asp</td>
<td>Glu</td>
<td>Glu</td>
</tr>
<tr>
<td>Trp</td>
<td>Gln</td>
<td>Arg</td>
</tr>
<tr>
<td>Thr</td>
<td>Gln</td>
<td>Cys</td>
</tr>
<tr>
<td>Ser</td>
<td>100</td>
<td>105</td>
</tr>
<tr>
<td>Pro</td>
<td>Arg</td>
<td>Glu</td>
</tr>
<tr>
<td>Thr</td>
<td>Cys</td>
<td>Val</td>
</tr>
<tr>
<td>Glu</td>
<td>Val</td>
<td>Ala</td>
</tr>
<tr>
<td>Ser</td>
<td>Ser</td>
<td>Glu</td>
</tr>
<tr>
<td>Leu</td>
<td>Gly</td>
<td>Lys</td>
</tr>
<tr>
<td>Ser</td>
<td>Thr</td>
<td>Thr</td>
</tr>
<tr>
<td>115</td>
<td>120</td>
<td>125</td>
</tr>
<tr>
<td>Asn</td>
<td>Thr</td>
<td>Phe</td>
</tr>
<tr>
<td>Phe</td>
<td>Phe</td>
<td>Lys</td>
</tr>
<tr>
<td>Pro</td>
<td>Pro</td>
<td>Cys</td>
</tr>
<tr>
<td>Val</td>
<td>Asn</td>
<td>Val</td>
</tr>
<tr>
<td>Pro</td>
<td>Arg</td>
<td>Cys</td>
</tr>
<tr>
<td>Gly</td>
<td>Gly</td>
<td>140</td>
</tr>
<tr>
<td>Cys</td>
<td>Cys</td>
<td>Asn</td>
</tr>
<tr>
<td>Glu</td>
<td>Glu</td>
<td>Ser</td>
</tr>
<tr>
<td>Leu</td>
<td>Ile</td>
<td>Cys</td>
</tr>
<tr>
<td>Met</td>
<td>Asn</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>Thr</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>Ser</td>
<td>Tyr</td>
</tr>
<tr>
<td>145</td>
<td>150</td>
<td>155</td>
</tr>
<tr>
<td>Ile</td>
<td>Ser</td>
<td>Lys</td>
</tr>
<tr>
<td>Leu</td>
<td>Phe</td>
<td>Glu</td>
</tr>
<tr>
<td>Ile</td>
<td>Ser</td>
<td>Val</td>
</tr>
<tr>
<td>Pro</td>
<td>Leu</td>
<td>Thr</td>
</tr>
<tr>
<td>Ser</td>
<td>Val</td>
<td>Pro</td>
</tr>
<tr>
<td>165</td>
<td>170</td>
<td>175</td>
</tr>
<tr>
<td>Glu</td>
<td>Leu</td>
<td>Val</td>
</tr>
<tr>
<td>Val</td>
<td>Lys</td>
<td>Val</td>
</tr>
<tr>
<td>Ala</td>
<td>Asn</td>
<td>His</td>
</tr>
<tr>
<td>Thr</td>
<td>Tyr</td>
<td>Cys</td>
</tr>
<tr>
<td>Lys</td>
<td>Cys</td>
<td>Leu</td>
</tr>
<tr>
<td>180</td>
<td>185</td>
<td>190</td>
</tr>
<tr>
<td>Pro</td>
<td>Thr</td>
<td>Ala</td>
</tr>
<tr>
<td>Pro</td>
<td>Arg</td>
<td>His</td>
</tr>
<tr>
<td>Pro</td>
<td>Tyr</td>
<td>Ser</td>
</tr>
<tr>
<td>Ile</td>
<td>Ile</td>
<td>Arg</td>
</tr>
<tr>
<td>Ser</td>
<td>Arg</td>
<td>Ser</td>
</tr>
<tr>
<td>Ile</td>
<td>Gln</td>
<td>195</td>
</tr>
<tr>
<td>Ile</td>
<td>Pro</td>
<td>Glu</td>
</tr>
<tr>
<td>Glu</td>
<td>Asp</td>
<td>Arg</td>
</tr>
<tr>
<td>Cys</td>
<td>Ser</td>
<td>His</td>
</tr>
<tr>
<td>Ser</td>
<td>Ser</td>
<td>Lys</td>
</tr>
<tr>
<td>Leu</td>
<td>Cys</td>
<td>Pro</td>
</tr>
<tr>
<td>210</td>
<td>215</td>
<td>220</td>
</tr>
<tr>
<td>Asp</td>
<td>Met</td>
<td>Leu</td>
</tr>
<tr>
<td>Trp</td>
<td>Asp</td>
<td>Ser</td>
</tr>
<tr>
<td>Ser</td>
<td>Asn</td>
<td>Cys</td>
</tr>
<tr>
<td>Lys</td>
<td>Cys</td>
<td>Val</td>
</tr>
<tr>
<td>Leu</td>
<td>Gln</td>
<td>Glu</td>
</tr>
<tr>
<td>225</td>
<td>230</td>
<td>235</td>
</tr>
<tr>
<td>Asn</td>
<td>Pro</td>
<td>Leu</td>
</tr>
<tr>
<td>Ala</td>
<td>Gly</td>
<td>Thr</td>
</tr>
<tr>
<td>Asp</td>
<td>His</td>
<td>Ser</td>
</tr>
<tr>
<td>His</td>
<td>Ser</td>
<td>Leu</td>
</tr>
<tr>
<td>Gln</td>
<td>Glu</td>
<td>Pro</td>
</tr>
<tr>
<td>Ala</td>
<td>245</td>
<td>250</td>
</tr>
<tr>
<td>Leu</td>
<td>Cys</td>
<td>Gly</td>
</tr>
<tr>
<td>Pro</td>
<td>His</td>
<td>Met</td>
</tr>
<tr>
<td>Met</td>
<td>Phe</td>
<td>Asp</td>
</tr>
<tr>
<td>Glu</td>
<td>Asp</td>
<td>Arg</td>
</tr>
<tr>
<td>Cys</td>
<td>Glu</td>
<td>Cys</td>
</tr>
<tr>
<td>Val</td>
<td>260</td>
<td>265</td>
</tr>
<tr>
<td>Cys</td>
<td>Lys</td>
<td>Thr</td>
</tr>
<tr>
<td>Pro</td>
<td>Cys</td>
<td>Pro</td>
</tr>
<tr>
<td>Cys</td>
<td>Pro</td>
<td>Lys</td>
</tr>
<tr>
<td>Asp</td>
<td>Leu</td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td>Gln</td>
<td></td>
</tr>
<tr>
<td>His</td>
<td>Pro</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>Asn</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>275</td>
<td>280</td>
</tr>
<tr>
<td>Ser</td>
<td>Cys</td>
<td>Phe</td>
</tr>
<tr>
<td>Glu</td>
<td>Cys</td>
<td>Lys</td>
</tr>
<tr>
<td>Glu</td>
<td>Ser</td>
<td>Leu</td>
</tr>
<tr>
<td>Glu</td>
<td>Thr</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>Cys</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>Lys</td>
<td></td>
</tr>
<tr>
<td>290</td>
<td>295</td>
<td>300</td>
</tr>
<tr>
<td>Lys</td>
<td>Leu</td>
<td>Phe</td>
</tr>
<tr>
<td>His</td>
<td>Pro</td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>Thr</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>Ser</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>Glu</td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>Arg</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>Pro</td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>305</td>
<td>310</td>
</tr>
<tr>
<td>His</td>
<td>Thr</td>
<td>Arg</td>
</tr>
<tr>
<td>Pro</td>
<td>Cys</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Ser</td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Thr</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Cys</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Lys</td>
<td></td>
</tr>
<tr>
<td>His</td>
<td>Cys</td>
<td></td>
</tr>
<tr>
<td>325</td>
<td>330</td>
<td>335</td>
</tr>
<tr>
<td>Arg</td>
<td>Phe</td>
<td>Pro</td>
</tr>
<tr>
<td>Lys</td>
<td>Gly</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>Ala</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>Gln</td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>Pro</td>
<td></td>
</tr>
<tr>
<td>His</td>
<td>Ser</td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>Lys</td>
<td></td>
</tr>
<tr>
<td>340</td>
<td>345</td>
<td>350</td>
</tr>
<tr>
<td>Asn</td>
<td>Pro</td>
<td></td>
</tr>
</tbody>
</table>
| <210> SEQ ID NO 5 <211> LENGTH: 20 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: primer <400> SEQUENCE: 5
ttgagcgct aggcttttgc 20

<210> SEQ ID NO 6 <211> LENGTH: 20 <212> TYPE: DNA <213> ORGANISM: Artificial Sequence <220> FEATURE: <223> OTHER INFORMATION: Description of Artificial Sequence: primer <400> SEQUENCE: 6
tactgtgtc cgttccctcctca 20

<210> SEQ ID NO 7 <211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer

<400> SEQUENCE: 7

gttgaagacctccaaagactt 22

<210> SEQ ID NO: 8
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer

<400> SEQUENCE: 8

cgcattcgccattccag 17

<210> SEQ ID NO: 9
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer

<400> SEQUENCE: 9

cgtgcttactggtttatcgc 18

<210> SEQ ID NO: 10
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer

<400> SEQUENCE: 10

cctgtttctctgttatggtgc 20

<210> SEQ ID NO: 11
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer

<400> SEQUENCE: 11

tttccaaaaaggtttacaccg 19

<210> SEQ ID NO: 12
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer

<400> SEQUENCE: 12

cagttgctggtgagagg 18

<210> SEQ ID NO: 13
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer

<400> SEQUENCE: 13

casggtgtgtggtgagagg
tcgatcatg aacctctctgc 20
<210> SEQ ID NO 14
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer
<400> SEQUENCE: 14
tccgtttacg tcagctgtgc 20
<210> SEQ ID NO 15
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer
<400> SEQUENCE: 15
gacotggtgc ttacgtgtg 19
<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: primer
<400> SEQUENCE: 16
gaacatatta caagctgctg 20
<210> SEQ ID NO 17
<211> LENGTH: 2679
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: chimeric sequence in which CNV promoter sequence is ligated to Homo sapien VEGF-C sequence
<400> SEQUENCE: 17
cgtgtaacatt gattatggac tagtatattaa tagtaataca ttaagggggtc attagttcat 60
agccaccata tcgagcctcg cgttacatca cttacgttas atgggccccgc tggctgaccc 120
cccacaagcc ccgcgccgctt gacgctata atgaacagtg ttcctaatag aaagcataa 180
gggcccttcc attagacga cttgctggag tatttatgt aaacgcccc ccctgggaatgta 240
cataaggtct actatagcg accatggccc cctattgacg tcgaacggc taaagcccc 300
gctggcagg atcgcctta ctagcctta tggaaccttc ctacagcgca gtcatctaca 360
gattaagct gagttcagatcg gggtttttgc gggttatcatc acggcgctg ccccgggggg 420
tagcctatgg gttcaggg atcttccggg gctgctcagc ctcacgccca cttgctggag 480
tttggcacc aaattacgga ggcgtcttta aagttcgata acacattcgg ccccgtttgc 540
taaaggggg gcggggtgct cagcgtggag gttccgtctag gcacacggac cttgcccc 600
gagacccca ctctggcact gctttcggg attacaccga ctcactctag ggacacccca 660
gctggctagc gttacatcag aacggcccc gcttcctcagc aagcctcagc cagcgggac 720
gcggcgccgg cctcctctcg cctcctctcg acctcggcgc ggcggagcc ggacgctgc 780
gatgcgctagt ctccctctcg gctctccgtgc cttgcgatgc gcccgggg 840
ggaggcggc cctgcaagct ggagacggig gacccgctg ccacctgcgg 900
ctgcocccagg gggtgtgcc ggagagagcc cgggggagag ggaccagagag ggcaccgcg g 960
cctgcagggg ggcgccgcc cccacccct cccacccca cgggcacgca gggacggctgc cccacccccc 1020
eggatcttcc aaccatcaagtt tgtcggcggatt ctctcttgtgc gctgttttctc tgtctgcccgc 1080
tgctgtcgct cgggtgtcct gcggagagcc gcgcgccgcc gcgccttctgcc agtcgcgact 1140
cgacctcttc gacgcgcggc cgccacggcgg ccggccacgc cttatgcaag gcacaagctt 1200
gggagagcgct ttacgctttgg ttcctcagytt gatgaccata atgtcatctactattcaccaga 1260
atatcctaca attgcgagaa tgtcgcacgag gaaaggggcc aagcacaata aoagagaaca 1320
ggcacacctc aacatcagga cagaagaagc tataatattc gttgcgccac gattatatac 1380
agatccttgg aacaatgtatg ataattgagg ctatagacac cggagggagtt cgcgggagtt 1440
gtgataagtct ttgctgtaag gtgtgagagc atcgcacaaa acctttcttta aacatcagcg 1500
tgtgtactgc tagcaggttgt ggggtggtgct caatagtgac gggctgtcaag gatgaccacag 1560
cgcacagca tagctcagcc agacggttag tgaattacta gtcctctctct ctcaggggcc 1620
caaaacagta aacatcagtt ttggcaccaca ctcctcctgc tgtgctagtgt ctaaactgga 1680
tggttaagc ggatttcttctt gttttctc tgattttggtc gattgaccaaa cagatggatt 1740
tacggagcgg cagcaagccct gcccaacacc tttatcgtgg gaaatctaca actgtgcag 1800
ccttgcttcg gaaattttat lgattttcttc ggtgtgctga gatgaccataa cagatggttt 1860
ocactacatc tcggtggccac ccgccagctg ggtgagagct gcgtgcagctc tgtgtgctgc 1920
aggtgggctt cggctggcag ggctgttgccc accaagacaa cgggggacgcgcaactctggg 1980
gggtgtctgt tggcggcgcg acgaatttgg gccacacgag tattgtgtga 2040
aaaacactgc caggtgtatct gttaacagac gcccccacagc atcacaacccc ataatcctcg 2100
aaaagtgcgc ggattgtatg cagaagctcc cccagaaactg tttattaggg gaaagaagt 2160
ocaccaacag acagctgaact ttatacagcgc gcggatgtac gcaggcagag aggottgttga 2220
gccggacttc tataattatatg gaagatgtgg tcttggtgttc ctcctttatt gaaagacgcg 2280
ocaccaagrc taaaagtgttg ctttttttccctgtatcatt cttttctctatt gaaagaactgt 2340
gttgccccag ttttaactgtct gcggagcaga ggcacccttg tcggctcatg ctaaccaaga 2400
caaagttgct tttttctctga ccacaggtgaa cttcccattaa gagatggact ggggctcaca 2460
tggcaaggg ctttctttgt ggatgttcttt cttgcacact ccacacgacac cggatattctc 2520
tcggtgtatat cttataatagc cttatatattt cccaatattatt tgtttttttt 2580
gcacactatt tttcatcgaa acaoatttg gtaacactat cttgacaattt ctttttatct 2640
atcacaata gttttatat aataatgaaata ttgtattt 2679

<210> SEQ ID NO: 18
<211> LENGTH: 2240
<212> TYPE: DNA
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 18

<214> 18

tggttagagc agacaccytc aagcttaggt aagaactgaggg gacgcttcata ctcacacccct 60
tacggaggttt ggcgcgctag ctcagcagct ggcctactaat cttttcttgag tcgttaggtga 120
continued

gtttgtaact accatgcgg cctggccggt ttctgccccct attggcaccct tggotctagt 180
tccctgcaca ccctctctgct tgtgttgtgt tgtgtgtag gagaagatca ggaagacca 240
ggctcttcgt ctcggccaga ctggctcttc otctcagggcct ctggccagact acacagtcac 300
tacyggggttt tcacagagtc ggctcctccgc cgcagcagtc ctcacgccca aaccocccccc 360
cgctctcggt tcgccccacc taccttctcga aggagcagcct ttctgccaccc 420
tccccaggagga tccatcctca cccagggccc atcagotctga tgcagcagat gccaggaag 480
gttcagataa gcatctctcc ccccccgcgt aacccctct caacatata caacatccgt 540
atcctcctga gtcaccacaac gcacacttcttt ggtgtgtgtgt gatgtgcaca aaccagcaaat 600
tatgggagg ccaacactcgg gcagcatctc gagaactcttt tctcacccccct tctcaacagcc 660
cgctccattt gagacagagt tgggggtgtgc ataatgtgtgc cactagggggt cgctcccggca 720
tccgctgggag atcgaagctct gggcagcgcag agtcgctgtg aagggagacgcgttatgtc 780
tcgagttcggt tcggctgtgtgt tgtgtgtgtgt tgtgtgtgtgt gggagagacgcgt 840
caggggtcct gggctgttccc ccacctcccc acactcaacctc ctggtctcttc cgggctccac 900
coctcccttc tctactctcgtc tgggaaggct atcgctctgct caggtggtcctgccatataa 960
tacactctct gttcctcttc atctccataa atctcctaaca tctctcggat ccaactctttgctgccttaa 1020
attggaggtg gtcacagact aatattgagg cctgctccccc tgtgaaggct ttagctcctcc 1080
acattcctgg tagttgctca ggggctcacc cggagcgggt cctttgctcct gaccaagctccg 1140
cccccgggac ggtggctgggc ggggtgggaggtgtgtactctt atttcttctttttctcctcc 1200
cgccgccctgg cggcagcggc gggcagcgcg agtcgctgtg aagggagacgcgt 1260
acctgctcgt cgcagccggc ggactcctggc gcacgcagcgc gcacagagcgcg 1320
actactaggc gaccagcccg cccgggggtcct tgggcagcct gatgggtgat tcaaaaaactctcttctcct 1380
taaaatatt ttccttactt cctttttgtt acgtcttactt ttttttttct tctctcttctcctcc 1440
octtctaat gctggccagcct gtttgagggt gttgggtctttt cctcctcctc aaccactctct 1500
gattggtgaa atcagccgac gaaagaggca tcaagtgaac gcagcgagaagc tcaaggaaga 1560
gacagagagag cccgctcacc gaggccgctc gcggacgccg aacagagagagg gacagagagag 1620
aacagtgact gacctggtct tgggggtgca cccggcgcagc gggcagcgcg aaccaccctcctcctcctc 1680
aatctttgtcg ccagcagctc ggcctgacgc accacagagc agacaccggcc cccagccccca 1740
ggcctccct cccgctcggc ggctgctcgc gggctggacgg gggccagcgc gcagacaccggc 1800
aacacgcggc ccagcagcgc gggctcagcc ctgggggtcg cggagattcga gcagaaactcttctcctcctc 1860
tctgcccaacct tctggctttcc tctgctccgt cttagtgcgct gcggctgacgc gcagggagaca 1920
aacaccgtcc gactggctgggg gggcctgctgc gggcctgctgc gcggcggagagc gcagggagaca 1980
gaggagagagag cggagcagcagc gggggggcct gggcctgccgc tggggggcgt gcggcggagagc 2040
acagccctgc gctgccgagt ggctgctcgtc gcacgcagcgc gcagccggctcg ccggccggctcg 2100
ggccggccgg ggcctgcctgc tcaaggaagg gaccaangcc gcacagcagcgc gcagggagaca 2160
gggctgctgc gcagcagcagc gggagcagcgc gcacgcagcgc gcagccggctcg ccggccggctcg 2220
cctcggaaacc ctaagactttt 2240
What is claimed is:

1. A method of treating a mammalian subject to inhibit restenosis of a blood vessel, comprising the step of: administering to a mammalian subject in need of treatment to inhibit restenosis of a blood vessel a composition comprising a replication-deficient adenovirus comprising a polynucleotide, wherein said composition is administered locally at the site in need of treatment to inhibit restenosis,

2. A method according to claim 1 wherein said mammalian subject is human.

3. A method according to claim 1 wherein the polynucleotide further comprises a polyadenylation sequence operably connected to the sequence that encodes the VEGF-C polypeptide.

4. A method according to claim 2 wherein the composition further comprises a pharmaceutically acceptable carrier.

5. A method according to claim 2 wherein said composition comprises at least one intravascular injection of said composition.

6. A method according to claim 2 wherein said composition comprises a catheter-mediated transfer of said composition into a blood vessel of the mammalian subject.

7. A method according to claim 6 wherein said catheter-mediated gene transfer comprises introducing a catheter into a coronary artery of the mammalian subject, and releasing the composition into the coronary artery.

8. A method according to claim 2 wherein said composition comprises a catheter-mediated transfer of said composition into a blood vessel of a human, comprising delivering a replication-deficient adenovirus vector to the vessel, said vector comprising a polynucleotide encoding a VEGF-C polypeptide, and further comprising a promoter sequence to promote expression of the VEGF-C polypeptide in cells of the blood vessel, wherein expression of said VEGF-C polypeptide in said blood vessel cells inhibits restenosis of the blood vessel, and

9. A treatment to inhibit restenosis of a blood vessel in a human, comprising delivering a replication-deficient adenovirus vector to the vessel, said vector comprising a polynucleotide encoding a VEGF-C polypeptide, and further comprising a promoter sequence to promote expression of the VEGF-C polypeptide in cells of the blood vessel, wherein the VEGF-C polypeptide comprises

10. An improvement in a medical device designed to contact a surface of a blood vessel in the course of surgery to treat stenosis of the blood vessel, said improvement comprising integrating into the device a composition effective to prevent restenosis, said composition comprising a replication-deficient adenovirus comprising a VEGF-C polynucleotide operatively linked to a promoter that promotes expression of a VEGF-C polypeptide encoded by the polynucleotide in cells of blood vessels,

11. The improvement of claim 10, wherein the device is selected from the group consisting of intravascular stents, intravascular catheters, and combinations thereof.

12. The improvement of claim 10, wherein the device comprises an extravascular collar.

13. The improvement of claim 10, wherein the device comprises an elastic membrane adapted to cover a surface of an intravascular stent or catheter.

14. A medical device comprising an endovascular stent having an outer surface for contacting a surface of a blood vessel, and a composition on said surface, said composition comprising a replication-deficient adenovirus comprising a VEGF-C polynucleotide operatively linked to a promoter that promotes expression of VEGF-C polypeptide encoded by the polynucleotide in cells of blood vessels, wherein the VEGF-C polypeptide comprises

15. A medical device comprising a catheter having an outer surface for contacting a surface of a blood vessel, and a composition on said surface, said composition comprising a replication-deficient adenovirus comprising a VEGF-C polynucleotide operatively linked to a promoter that promotes expression of VEGF-C polypeptide encoded by the polynucleotide in cells of blood vessels, wherein the VEGF-C polypeptide comprises

16. A medical device comprising a balloon catheter having a void for holding a therapeutic agent for delivery to the interior of a blood vessel, and a composition contained in the void, the composition comprising a replication-deficient adenovirus comprising a VEGF-C polynucleotide operatively linked to a promoter that promotes expression of VEGF-C polypeptide encoded by the polynucleotide in cells of blood vessels, wherein the VEGF-C polypeptide comprises

17. A method of treating a mammalian subject to inhibit restenosis of a blood vessel, comprising identifying a mammalian subject that has been or will be treated for a stenosed blood vessel; and administering to the mammalian subject at the site of the stenosed blood vessel a composition comprising a replication-deficient adenovirus comprising a polynucleotide, said polynucleotide comprising a nucleotide sequence that encodes a vascular endothelial growth factor C (VEGF-C) polypeptide, wherein the VEGF-C polypeptide comprises

18. A method according to claim 17 wherein said mammalian subject is human.

19. A method according to claim 17 wherein the blood vessel is a coronary artery, and the administering is performed concurrently with percutaneous transluminal coronary angioplasty to treat the stenosed blood vessel.

20. A method according to claim 17 wherein the composition further comprises a pharmaceutically acceptable carrier.

21. A method according to claim 17 wherein said composition comprises at least one intravascular injection of said composition at the site of the stenosed blood vessel.

22. A method according to claim 17 wherein said composition comprises a catheter-mediated transfer of said composition to the site of the stenosed blood vessel.

23. A method according to claim 17 wherein said catheter-mediated gene transfer comprises introducing a catheter into a coronary artery of the mammalian subject, and releasing the composition into the coronary artery.

24. A method according to claim 17 wherein said administering comprises implanting an intravascular stent in said mammalian subject at the site of the stenosed blood vessel, and wherein the stent is coated or impregnated with the composition.
25. An extravascular collar designed to contact a surface of a blood vessel in the course of surgery to treat stenosis of the blood vessel, the collar comprising an outer wall shaped to surround the outer surface of a blood vessel, wherein the wall encloses a space containing a composition comprising a replication-deficient adenovirus comprising a polynucleotide that comprises a nucleotide sequence encoding a VEGF-C polypeptide; wherein the VEGF-C polypeptide comprises the amino acid sequence of SEQ ID NO: 2;

wherein the polynucleotide further comprises a promoter to promote expression of the polypeptide in mammalian cells.

26. A method according to any one of claims 2, 8, and 18, further comprising administering to said subject an inhibitor of smooth muscle cell growth or migration.

27. A device according to any one of claims claim 10, 14, 15 and 16, wherein the composition further comprises an inhibitor of smooth muscle cell growth or migration.
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

**On the Title Page, Item (75) Col. 1 delete “Marc G. Achen” as an inventor.**

Signed and Sealed this Twenty-first Day of November, 2006

JON W. DUDAS
Director of the United States Patent and Trademark Office
It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:
At field (54), “VEGF-C TO” should be -- VEGF-C GENE TO --.

At field (73), 2nd Assignee, “Yla-Herttuala” should be -- Ylä-Herttuala --.

At field (73), 3rd Assignee, “Institute of” should be -- Institute for --.

Signed and Sealed this
Eighth Day of April, 2008

Jon W. Dudas

Director of the United States Patent and Trademark Office
It is certified that an error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On the Title Page:

At field (54) and Column 1, line 1, "VEGF-C TO" should be -- VEGF-C GENE TO --.

At field (73), 2nd Assignee, "Yla-Hertuala" should be -- Ylä-Hertuala --.

At field (73), 3rd Assignee, "Institute of" should be -- Institute for --.

This certificate supersedes the Certificate of Correction issued April 8, 2008.

Signed and Sealed this

Sixth Day of May, 2008

[Signature]

JON W. DUDAS
Director of the United States Patent and Trademark Office