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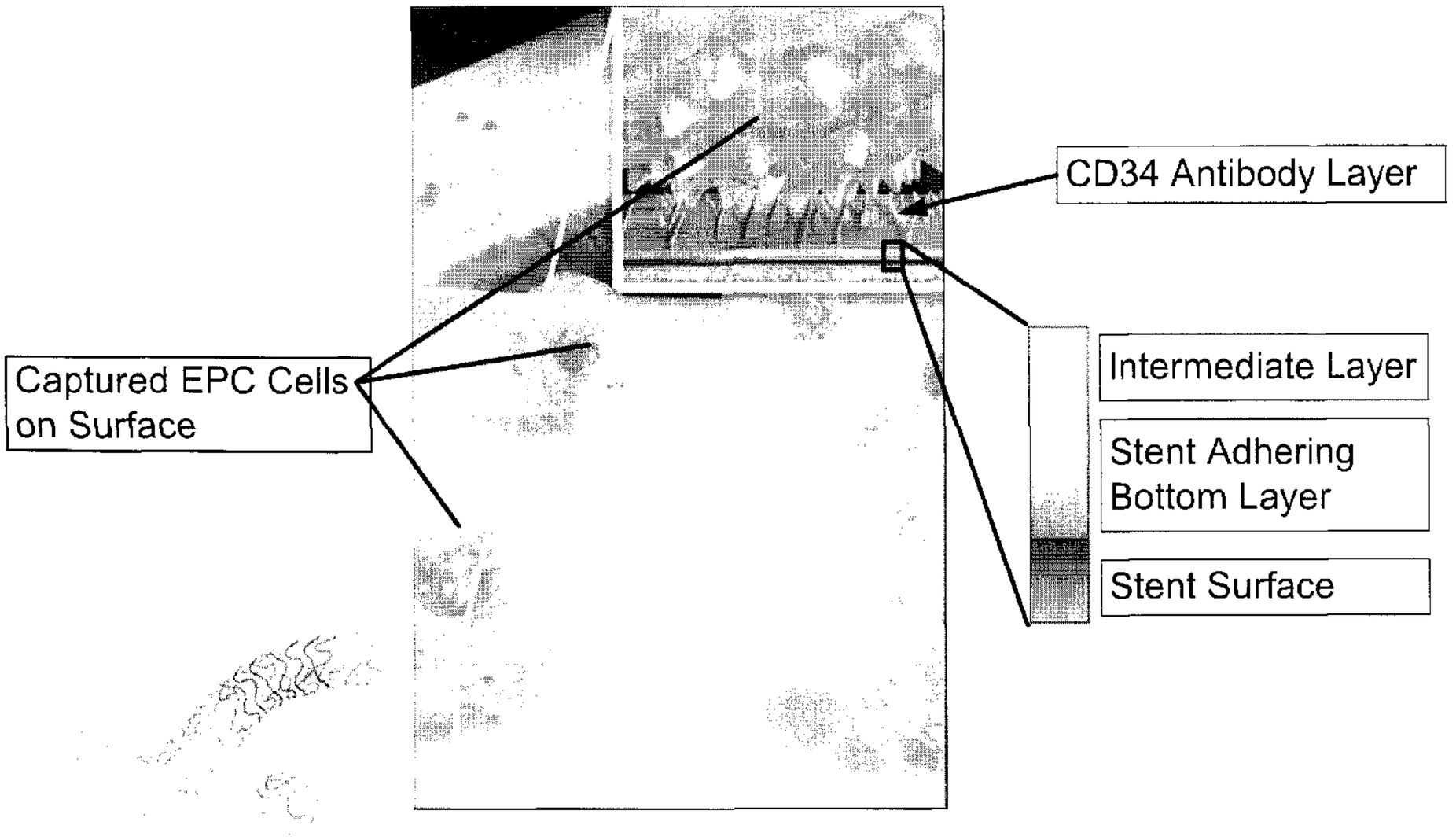
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(54) **Titre : DISPOSITIF MEDICAL DOTE D'UN REVETEMENT SERVANT A CAPTER DES CELLULES MODIFIEES GENETIQUEMENT ET METHODES D'UTILISATION ASSOCIEES**

(54) **Title: MEDICAL DEVICE WITH COATING FOR CAPTURING GENETICALLY-ALTERED CELLS AND METHODS FOR USING SAME**



(57) **Abrégé/Abstract:**
Therapeutic and drug delivery systems are provided in the form of medical devices with coatings for capturing and immobilizing target cells such as circulating progenitor or genetically-altered mammalian cells in vivo. The genetically-altered cells are transfected

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(57) **Abrégé(suite)/Abstract(continued):**
with genetic material for expressing a marker gene and at least one therapeutic gene in a constitutively or controlled manner. The marker gene is a cell membrane antigen not found in circulating cells in the blood stream and therapeutic gene encodes a peptide for the treatment of disease, such as, vascular disease and cancer. The coating on the medical device may be a biocompatible matrix comprising at least one type of ligand, such as antibodies, antibody fragments, other peptides and small molecules, which recognize and bind the target cells. The therapeutic and/or drug delivery systems may be provided with a signal source such as activator molecules for stimulating the modified cells to express and secrete the desired marker and therapeutic gene products.

ABSTRACT

Therapeutic and drug delivery systems are provided in the form of medical devices with coatings for capturing and immobilizing target cells such as circulating progenitor or genetically-altered mammalian cells in vivo. The genetically-altered cells are transfected with genetic material for expressing a marker gene and at least one therapeutic gene in a constitutively or controlled manner. The marker gene is a cell membrane antigen not found in circulating cells in the blood stream and therapeutic gene encodes a peptide for the treatment of disease, such as, vascular disease and cancer. The coating on the medical device may be a biocompatible matrix comprising at least one type of ligand, such as antibodies, antibody fragments, other peptides and small molecules, which recognize and bind the target cells. The therapeutic and/or drug delivery systems may be provided with a signal source such as activator molecules for stimulating the modified cells to express and secrete the desired marker and therapeutic gene products.

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MEDICAL DEVICE WITH COATING FOR CAPTURING GENETICALLY-ALTERED CELLS AND METHODS FOR USING SAME

This application claims benefit of U.S. Provisional Application No. 60/566,829, filed on April 30, 2004 and U.S. Application Serial No. 10/835,767, filed on April 30, 2004.

FIELD OF INVENTION

[0001] The invention relates to medical devices for implantation into vessels or hollow organs of patients such as coated stents, stent grafts, synthetic vascular grafts, heart valves, catheters and vascular prosthetic filters for treating various diseases. In particular, the invention relates to medical devices comprising a coating on the surface that contacts blood, which coating is engineered to capture cells on the surface of the device. The captured cells form a monolayer on the surface of the device and are useful in many therapeutic applications, such as a drug delivery system and/or in the treatment of vascular disease. For example, the cells binding to the implanted medical device may be native, progenitor endothelial cells from the circulating blood and/or cells genetically modified *in vitro* to express and secrete molecules or substances *in vivo* having a local or generalized therapeutic effect in the patient.

BACKGROUND

[0002] Diseases such as atherosclerosis and cancer are two of the leading causes of death and disability in the world. Atherosclerosis involves the development of fatty plaques on the luminal surface of arteries. These fatty plaques cause narrowing of the cross-sectional area of the artery. Ultimately, blood flow distal to the lesion is reduced causing ischemic damage to the tissues supplied by the artery.

[0003] Coronary arteries supply the heart with blood. Coronary artherosclerosis or coronary artery disease (CAD) is the most common, serious, chronic, life-threatening illness in the United States, affecting more than 11 million persons. The social and economic costs of coronary atherosclerosis vastly exceed those of most other diseases. Narrowing of the coronary artery lumen affects heart muscle resulting first in angina, followed by myocardial infarction and finally death, and more than three hundred thousand of those patients die before reaching the hospital. (Harrison's Principles of Internal Medicine,14th Edition, 1998).

[0004] CAD can be treated using percutaneous transluminal coronary angioplasty (PTCA). More than 400,000 PTCA procedures are performed each year in the United States. In PTCA, a balloon catheter is inserted into a peripheral artery and threaded through the arterial system into the blocked coronary artery. The balloon is then inflated, the artery stretched, and the obstructing fatty plaque flattened, thereby increasing the cross-sectional flow of blood through the affected artery. The therapy, however, does not usually result in a permanent opening of the affected coronary artery. As many as 50% of the patients who are treated by PTCA require a repeat procedure within six months to correct a re-narrowing of the coronary artery. Medically, this re-narrowing of the artery after treatment by PTCA is called restenosis. Acutely, restenosis involves recoil and shrinkage of the vessel. Subsequently, recoil and shrinkage of the vessel are followed by proliferation of medial smooth muscle cells in response to injury of the artery from PTCA. In part, proliferation of smooth muscle cells is mediated by release of various inflammatory factors from the injured area including thromboxane A_2, platelet derived growth factor (PDGF) and fibroblast growth factor (FGF). A number of different techniques have been used to overcome the problem of restenosis, including treatment of patients with various pharmacological agents or mechanically holding the artery open with a stent. (Harrison's Principles of Internal Medicine,14th Edition, 1998).

[0005] Of the various procedures used to overcome restenosis, stents have proven to be the most effective. Stents are metal scaffolds that are positioned

in the diseased vessel segment to create a normal vessel lumen. Placement of the stent in the affected arterial segment prevents recoil and subsequent closing of the artery. Stents can also prevent local dissection of the artery along the medial layer of the artery. By maintaining a larger lumen than that created using PTCA alone, stents reduce restenosis by as much as 30%. Despite their success, stents have not eliminated restenosis entirely. (Suryapranata et al. 1998. Randomized comparison of coronary stenting with balloon angioplasty in selected patients with acute myocardial infarction. Circulation 97:2502-2502).

[0006] Narrowing of the arteries can occur in vessels other than the coronary arteries, including the aortoiliac, infrainguinal, distal profunda femoris, distal popliteal, tibial, subclavian and mesenteric arteries. The prevalence of peripheral artery atherosclerosis disease (PAD) depends on the particular anatomic site affected as well as the criteria used for diagnosis of the occlusion. Traditionally, physicians have used the test of intermittent claudication to determine whether PAD is present. However, this measure may vastly underestimate the actual incidence of the disease in the population. Rates of PAD appear to vary with age, with an increasing incidence of PAD in older individuals. Data from the National Hospital Discharge Survey estimate that every year, 55,000 men and 44,000 women had a first-listed diagnosis of chronic PAD and 60,000 men and 50,000 women had a first-listed diagnosis of acute PAD. Ninety-one percent of the acute PAD cases involved the lower extremity. The prevalence of comorbid CAD in patients with PAD can exceed 50%. In addition, there is an increased prevalence of cerebrovascular disease among patients with PAD.

[0007] PAD can be treated using percutaneous transluminal balloon angioplasty (PTA). The use of stents in conjunction with PTA decreases the incidence of restenosis. However, the post-operative results obtained with medical devices such as stents do not match the results obtained using standard operative revascularization procedures, i.e., those using a venous or prosthetic bypass material. (Principles of Surgery, Schwartz et al. eds., Chapter 20, Arterial Disease, 7th Edition, McGraw-Hill Health Professions Division, New York 1999).

[0008] Preferably, PAD is treated using bypass procedures where the blocked section of the artery is bypassed using a graft. (Principles of Surgery, Schwartz et al. eds., Chapter 20, Arterial Disease, 7th Edition, McGraw-Hill Health Professions Division, New York 1999). The graft can consist of an autologous venous segment such as the saphenous vein or a synthetic graft such as one made of polyester, polytetrafluoroethylene (PTFE), or expanded polytetrafluoroethylene (ePTFE), or other polymeric materials. The post-operative patency rates depend on a number of different factors, including the luminal dimensions of the bypass graft, the type of synthetic material used for the graft and the site of outflow. Excessive intimal hyperplasia and thrombosis, however, remain significant problems even with the use of bypass grafts. For example, the patency of infrainguinal bypass procedures at 3 years using an ePTFE bypass graft is 54% for a femoral-popliteal bypass and only 12% for a femoral-tibial bypass.

[0009] Consequently, there is a significant need to improve the performance of stents, synthetic bypass grafts, and other chronic blood contacting surfaces and or devices, in order to further reduce the morbidity and mortality of CAD and PAD. For example, procedures that can cause radial enlargement of vessels (outward or positive remodeling) can compensate for progressive growth of atherosclerotic plaques, thus should postpone the development of flow-limiting stenosis.

[0010] With stents, the approach has been to coat the stents with various anti-thrombotic or anti-restenotic agents in order to reduce thrombosis and restenosis. For example, impregnating stents with radioactive material appears to inhibit restenosis by inhibiting migration and proliferation of myofibroblasts. (U.S. Patent Nos. 5,059,166, 5,199,939 and 5,302,168). Irradiation of the treated vessel can cause severe edge restenosis problems for the patient. In addition, irradiation does not permit uniform treatment of the affected vessel.

[0011] Alternatively, stents have also been coated with chemical agents such as heparin, phosphorylcholine, rapamycin, and taxol, all of which appear to decrease thrombosis and/or restenosis. Although heparin and phosphorylcholine appear to markedly reduce thrombosis in animal models in

the short term, treatment with these agents appears to have no long-term effect on preventing restenosis. Additionally, heparin can induce thrombocytopenia, leading to severe thromboembolic complications such as stroke. Therefore, it is not feasible to load stents with sufficient therapeutically effective quantities of either heparin or phosphorylcholine to make treatment of restenosis in this manner practical.

[0012] Synthetic grafts have been treated in a variety of ways to reduce postoperative restenosis and thrombosis. (Bos et al. 1998. Small-Diameter Vascular Graft Prostheses:Current Status Archives Physio. Biochem. 106:100-115). For example, composites of polyurethane such as meshed polycarbonate urethane have been reported to reduce restenosis as compared with ePTFE grafts. The surface of the graft has also been modified using radiofrequency glow discharge to fluorinate the polyterephthalate graft. Synthetic grafts have also been impregnated with biomolecules such as collagen. However, none of these approaches has significantly reduced the incidence of thrombosis or restenosis over an extended period of time.

[0013] The endothelial cell (EC) layer is a crucial component of the normal vascular wall, providing an interface between the bloodstream and the surrounding tissue of the blood vessel wall. Endothelial cells are also involved in physiological events including angiogenesis, inflammation and the prevention of thrombosis (Rodgers GM. FASEB J 1988;2:116-123.). In addition to the endothelial cells that compose the vasculature, recent studies have revealed that ECs and endothelial progenitor cells (EPCs) circulate postnatally in the peripheral blood (Asahara T, et al. Science 1997;275:964-7; Yin AH, et al. Blood 1997;90:5002-5012; Shi Q, et al. Blood 1998;92:362-367; Gehling UM, et al. Blood 2000;95:3106-3112; Lin Y, et al. J Clin Invest 2000;105:71-77). EPCs are believed to migrate to regions of the circulatory system with an injured endothelial lining, including sites of traumatic and ischemic injury (Takahashi T, et al. Nat Med 1999;5:434-438). In normal adults, the concentration of EPCs in peripheral blood is 3-10 cells/mm^3 (Takahashi T, et al. Nat Med 1999;5:434-438; Kalka C, et al. Ann Thorac Surg. 2000;70:829-834). It is now evident that each phase of the vascular response to injury is influenced (if not controlled) by the endothelium. It is

believed that the rapid re-establishment of a functional endothelial layer on damaged stented vascular segments may help to prevent these potentially serious complications by providing a barrier to circulating cytokines, preventing the adverse effects of a thrombus, and by their ability to produce substances that passivate the underlying smooth muscle cell layer. (Van Belle et al. 1997. Stent Endothelialization. Circulation 95:438-448; Bos et al. 1998. Small-Diameter Vascular Graft Prostheses:Current Status Archives Physio. Biochem. 106:100-115).

[0014] Endothelial cells have been encouraged to grow on the surface of stents by local delivery of vascular endothelial growth factor (VEGF), an endothelial cell mitogen, after implantation of the stent (Van Belle et al. 1997. Stent Endothelialization. Circulation 95:438-448.). While the application of a recombinant protein growth factor VEGF in saline solution at the site of injury induces desirable effects, the VEGF is delivered after stent implantation using a channel balloon catheter. This technique is not desirable since it has demonstrated that the efficiency of a single dose delivery is low and produces inconsistent results. Therefore, this procedure cannot be reproduced accurately every time.

[0015] Synthetic grafts have also been seeded with endothelial cells, but the clinical results with endothelial seeding have been generally poor, i.e., low post-operative patency rates (Lio et al. 1998. New concepts and Materials in Microvascular Grafting: Prosthetic Graft Endothelial Cell Seeding and Gene Therapy. Microsurgery 18:263-256) due most likely to the fact the cells did not adhere properly to the graft and/or lost their EC function due to *ex-vivo* manipulation.

[0016] Endothelial cell growth factors and environmental conditions *in situ* are therefore essential in modulating endothelial cell adherence, growth and differentiation at the site of blood vessel injury. Accordingly, with respect to restenosis and other blood vessel diseases, there is a need for the development of new methods and compositions for coating medical devices, including stents and synthetic grafts, which would promote and accelerate the formation of a functional endothelium on the surface of implanted devices so

that a confluent EC monolayer is formed on the target blood vessel segment or grafted lumen thereby inhibiting neo-intimal hyperplasia.

[0017] In regard to diseases such as cancer, most therapeutic agents used to date have generalized systemic effects on the patient, not only affecting the cancer cells, but any dividing cell in the body due to the use of drugs in conventional oral or intravenous formulations. Yet in many cases, systemic administration is not effective due to the nature of the disease that is in need of treatment and the properties of the drug such as solubility, *in vivo* stability, bioavailability, etc. Upon systemic administration, the drug is conveyed by blood circulation and distributed into body areas including normal tissues. At diseased sites, the drug concentration is first low and ineffective which frequently increases to toxic levels, while in non-diseased areas, the presence of the drug causes undesired side effect. In certain instances, drugs are readily susceptible to metabolic degradation after being administered. Therefore, drug dose is often increased to achieve pharmacological efficacy and prolong duration, which causes increased systemic burden to normal tissues as well as cost concern for the patient. In other instances, the therapeutic potential of some potent drugs cannot be fulfilled due to their toxic side effects.

[0018] Therefore, much effort has been made to improve efficacy and targeting of drug delivery systems. For example, the use of liposomes to deliver drugs has been advantageous in that, in general, they increase the drug circulation time in blood, reduce side effects by limiting the concentration of free drug in the bloodstream, decrease drug degradation, prolong the therapeutic effect after each administration, reduce the need for frequent administration, and reduce the amount of drug needed. However, liposome systems that are currently available show limited efficiency of delivering drugs to target sites *in vivo*. See Kaye et al., 1979, Poznansky et al. 1984, US 5,043,165, and US 4,920,016.

[0019] To yield highly efficient delivery of therapeutic compounds, viral vectors able to incorporate transgenic DNA have been developed, yet the number of successful clinical applications has been limited. Despite the

number of successes *in vitro* and in animal models, gene transfer technology is therefore proposed to marry with cell therapy. The *ex vivo* transfer of gene combinations into a variety of cell types will likely prove more therapeutically feasible than direct *in vivo* vector transfer. See Kohn et al., (1987) "Retroviral-Mediated Gene Transfer into Mammalian Cells," *Blood Cell*s 13(1-2): 285-298, Bilbao et al., (1997) "Adenoviral/retroviral vector chimeras: a novel strategy to achieve high-efficiency stable transduction in vivo," *FASEB J.* 11(8): 624-634, and Giannoukakis et al. (2003) "Gene therapy technology applied to disorders of glucose metabolism: promise, achievements, and prospects," *Biotechniques* 35(1): 122-145.

[0020] More recently local drug delivery vehicles such as drug eluting stents (DES) have been developed. See US 6,273,913, US 6,258,121, and US 6,231,600. However, drug eluting stents of the prior art are limited by many factors such as, the type of drug, the amount of drug to be released and the amount of time it takes to release the drug. Other factors which need to be considered in regards to drug eluting stents are the drug interactions with other stent coating components, such as polymer matrices, and individual drug properties including hydrophobicity, molecular weight, intactness and activity after sterilization, as well as efficacy and toxicity. With respect to polymer matrices of drug eluting stents, one must consider the polymer type, polymer ratio, drug loading capability, and biocompatibility of the polymer and the drug-polymer compatibility such as drug pharmacokinetics.

[0021] Additionally, the drug dose in a drug eluting stent is pre-loaded and an adjustment of drug dose upon individual conditions and need cannot be achieved. In regard to drug release time, drug eluting stents instantly start to release the drug upon implantation and an ideal real-time release cannot be achieved.

[0022] It is therefore a long-felt need to develop an efficient systemic and local drug delivery system to overcome limitations of current available techniques. The present invention provides a system for the delivery of therapeutic agents locally or systemically in a safe and controlled manner.

SUMMARY OF INVENTION

[0023] It is an object of the invention to provide a therapeutic, drug delivery system and method for treating diseases in a patient. The therapeutic or drug

delivery system comprises a medical device with a coating composed of a matrix comprising at least one type of ligand for recognizing and binding target

cells such as progenitor endothelial cells or genetically-altered mammalian cells and genetically-altered mammalian cells which have been at least singly or dually-transfected.

[0024] The medical device of the invention can be any device that is implantable into a patient. For example, in one embodiment the device is for insertion into the lumen of a blood vessels or a hollowed organ, such as stents, stent grafts, heart valves, catheters, vascular prosthetic filters, artificial heart, external and internal left ventricular assist devices (LVADs), and synthetic vascular grafts, for the treatment of diseases such as cancer, vascular diseases, including, restenosis, artherosclerosis, thrombosis, blood vessel obstruction, or any other applications additionally covered by these devices.

[0025] In one embodiment, the coating on the present medical device comprises a biocompatible matrix and at least one type of substance or ligand, which specifically recognize and bind target cells such as progenitor endothelial cells such as in the prevention or treatment of restenosis, or genetically-altered mammalian cells, onto the surface of the device, such as in the treatment of blood vessel remodeling and cancer.

[0026] Additionally, the coating of the medical device may optionally comprise at least an activating compound for regulating the expression and secretion of the engineered genes of the genetically-altered cells. Examples of activator stimulatory compounds, include but is not limited to chemical moieties, and peptides, such as growth factors. In embodiments when the coating comprises at least one compound, the stimulus, activator molecule or compound may function to stimulate the cells to express and/or secrete at least one therapeutic substance for the treatment of disease.

[0027] In one embodiment, the coating on the medical device comprises a biocompatible matrix which comprises an outer surface for attaching a therapeutically effective amount of at least one type of ligand such as an antibody, antibody fragment, or a combination of the antibody and the antibody fragment, or at least one type of molecule for binding the engineered marker on the surface of the genetically-modified cell. The present antibody

or antibody fragment recognizes and binds an antigen or the specific genetically-engineered cell surface marker on the cell membrane or surface of target cells so that the cells are immobilized on the surface of the device. In one embodiment, the coating may optionally comprise an effective amount of at least one compound for stimulating the immobilized progenitor endothelial cells to either accelerate the formation of a mature, functional endothelium if the target cells are circulating progenitor cells, or to stimulate the bound cells to express and secrete the desired gene products if the target are genetically-altered cells on the surface of the medical device.

[0028] The medical device of the invention can be any device used for implanting into an organ or body part comprising a lumen, and can be, but is not limited to, a stent, a stent graft, a synthetic vascular graft, a heart valve, a catheter, a vascular prosthetic filter, a pacemaker, a pacemaker lead, a defibrillator, a patent foramen ovale (PFO) septal closure device, a vascular clip, a vascular aneurysm occluder, a hemodialysis graft, a hemodialysis catheter, an atrioventricular shunt, an aortic aneurysm graft device or components, a venous valve, a suture, a vascular anastomosis clip, an indwelling venous or arterial catheter, a vascular sheath and a drug delivery port. The medical device can be made of numerous materials depending on the device. For example, a stent of the invention can be made of stainless steel, Nitinol™ (NiTi), or chromium alloy and biodegradable materials. Synthetic vascular grafts can be made of a cross-linked PVA hydrogel, polytetrafluoroethylene (PTFE), expanded polytetrafluoroethylene (ePTFE), porous high density polyethylene (HDPE), polyurethane, and polyethylene terephthalate, or biodegradable materials.

[0029] The biocompatible matrix forming the coating of the present medical device comprises without limitation a synthetic material such as polyurethanes, segmented polyurethane-urea/heparin, poly-L-lactic acid, cellulose ester, polyethylene glycol, polyvinyl acetate, dextran and gelatin, and/or naturally-occurring material such as basement membrane components such as collagen, elastin, tropoelastin, laminin, fibronectin, vitronectin, heparin, fibrin, cellulose, and amorphous carbon, or fullerenes.

[0030] In an embodiment of the invention, the medical device comprises a biocompatible matrix comprising fullerenes. In this embodiment, the fullerene can range from about C_{20} to about C_{150} in the number of carbon atoms, and more particularly, the fullerene is C_{60} or C_{70}. The fullerene of the invention can also be arranged as nanotubes on the surface of the medical device.

[0031] In one embodiment of the invention, the ligand is applied to the blood contacting surface of the medical device and the ligand specifically recognizes and binds a desired component or epitope on the surface of target cells in the circulating blood. In one embodiment, the ligand is specifically designed to recognize and bind only the genetically-altered mammalian cell by recognizing only the genetically-engineered marker molecule on the cell membrane of the genetically-altered cells. The binding of the target cells immobilizes the cells on the surface of the device.

[0032] In one embodiment, the ligand on the surface of the medical device for binding the genetically-altered cell is selected depending on the genetically engineered cell membrane marker molecule. That is, the ligand binds only to the cell membrane marker molecule or antigen which is expressed by the cell from extrachromosomal genetic material provided to the cell so that only the genetically-modified cells can be recognized by the ligand on the surface of the medical device. In this manner, only the genetically-modified cells can bind to the surface of the medical device. For example, if the mammalian cell is an endothelial cell, the ligand can be at least one type of antibody, antibody fragments or combinations thereof; the antibody is specifically raised against a specific target epitope or marker molecule on the surface of the target cell. In this aspect of the invention, the antibody can be a monoclonal antibody, a polyclonal antibody, a chimeric antibody, or a humanized antibody which recognizes and binds only to the genetically-altered endothelial cell by interacting with the surface marker molecule and, thereby modulating the adherence of the cells onto the surface of the medical device. The antibody or antibody fragment of the invention can be covalently or noncovalently attached to the surface of the matrix, or tethered covalently by a linker molecule to the outermost layer of the matrix coating the medical device. In this embodiment, for example, the monoclonal antibodies can further

comprises Fab or $F(ab')_2$ fragments. The antibody fragment of the invention comprises any fragment size, such as large and small molecules which retain the characteristic to recognize and bind the target antigen as the antibody.

[0033] In another embodiment, the antibody or antibody fragment of the invention recognize and bind antigens with specificity for the mammal being treated and their specificity is not dependent on cell lineage. In one embodiment, for example, in treating restenosis wherein the cells may not be genetically modified to contain specific cell membrane marker molecules, the antibody or fragment is specific for selecting and binding circulating progenitor endothelial cell surface antigen such as CD133, CD34, CDw90, CD117, HLA-DR, VEGFR-1, VEGFR-2, Muc-18 (CD146), CD130, stem cell antigen (Sca-1), stem cell factor 1 (SCF/c-Kit ligand), Tie-2, MHC such as $H\text{-}2K^k$ and HAD-DR.

[0034] In another embodiment, the coating of the medical device comprises at least one layer of a biocompatible matrix as described above, the matrix comprises an outer surface for attaching a therapeutically effective amount of at least one type of small molecule of natural or synthetic origin. The small molecule recognizes and interacts with, for example, progenitor endothelial cells in the treatment of restenosis, to immobilize the cells on the surface of the device to form an endothelial layer. The small molecules can be used in conjunction with the medical device for the treatment of various diseases, and can be derived from a variety of sources such as cellular components such as fatty acids, proteins, nucleic acids, saccharides and the like and can interact with an antigen on the surface of a progenitor endothelial cell with the same results or effects as an antibody. In this aspect of the invention, the coating on the medical device can further comprise a compound such as a growth factor as described herewith in conjunction with the coating comprising an antibody or antibody fragment.

[0035] In one embodiment, the compound of the coating of the invention, for example in treating restenosis, comprises any compound which stimulates or accelerates the growth and differentiation of the progenitor cell into mature, functional endothelial cells. In another embodiment, the compound is for stimulating the genetically modified cells to express and secrete the desired

gene product. For example, a compound for use in the invention may be a growth factor such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, platelet-induced growth factor, transforming growth factor beta 1, acidic fibroblast growth factor, osteonectin, angiopoietin 1 (Ang-1), angiopoietin 2 (Ang-2), insulin-like growth factor, granulocyte-macrophage colony-stimulating factor, platelet-derived growth factor AA, platelet-derived growth factor BB, platelet-derived growth factor AB and endothelial PAS protein 1.

[0036] In another embodiment, for example when using genetically-altered mammalian cells, the activating agents or compounds useful for stimulating the cells to express and secrete the genetically-engineered gene products include, but are not limited to estrogen, tetracycline and other antibiotics, tamoxiphen, etc., and can be provided to the patient via various routes of administration, such as through the skin via a patch and subcutaneously.

[0037] The invention also provides methods for treating a variety of diseases, such as vascular disease, cancer, blood vessel remodeling, severe coronary artery disease. artherosclerosis, restenosis, thrombosis, aneurysm and blood vessel obstruction. In one embodiment, there is provided a method for retaining or sealing the medical device insert to the vessel wall, such as a stent or synthetic vascular graft, heart valve, abdominal aortic aneurysm devices and components thereof, and for establishing vascular homeostasis, thereby preventing excessive intimal hyperplasia as in restenosis. In the present method of treating atherosclerosis, the artery may be either a coronary artery or a peripheral artery such as the femoral artery. Veins can also be treated using these techniques and medical device.

[0038] With respect to the treatment of restenosis, the invention also provides an engineered method for inducing a healing response. In one embodiment, a method is provided for rapidly inducing the formation of a confluent layer of endothelium in the luminal surface of an implanted device in a target lesion of an implanted vessel, in which the endothelial cells express nitric oxide synthase and other anti-inflammatory and inflammation-modulating factors. The invention also provides a medical device which has increased biocompatibility over prior art devices, and decreases or inhibits tissue-based

excessive intimal hyperplasia and restenosis by decreasing or inhibiting smooth muscle cell migration, smooth muscle cell differentiation, and collagen deposition along the inner luminal surface at the site of implantation of the medical device.

[0039] In an embodiment, a method for coating a medical device comprises the steps of: applying at least one layer of a biocompatible matrix to the surface of the medical device, wherein the biocompatible matrix comprises at least one component selected from the group consisting of a polyurethane, a segmented polyurethane-urea/heparin, a poly-L-lactic acid, a cellulose ester, a polyethylene glycol, a polyvinyl acetate, a dextran, gelatin, collagen, elastin, tropoelastin, laminin, fibronectin, vitronectin, heparin, fibrin, cellulose and carbon and fullerene, and applying to the biocompatible matrix, simultaneously or sequentially, a therapeutically effective amounts of at least one type of antibody, antibody fragment or a combination thereof, and at least one compound which stimulates endothelial cell growth and differentiation.

[0040] The invention further provides a method for treating vascular disease in a mammal comprising implanting a medical device into the lumen of a vessel or tubular organ of the mammal, wherein the medical device is coated with (a) a biocompatible matrix, (b) therapeutically effective amounts of at least one type of antibody, antibody fragment or a combination thereof, and (c) at least one compound; wherein the antibody or antibody fragment recognizes and binds an antigen on a progenitor endothelial cell surface so that the progenitor endothelial cell is immobilized on the surface of the matrix, and the compound is for stimulating the immobilized progenitor endothelial cells to form an endothelium on the surface of the medical device.

[0041] In one embodiment, a therapeutic/drug delivery system for treating a disease in a patient is also provided. The therapeutic or drug delivery system comprises genetically-altered mammalian cells, comprising exogenous nucleic acid encoding a genetically-engineered cell membrane marker and at least one therapeutic gene product, and a medical device for implantation into a patient. In one embodiment, the genetic engineered cells are transfected *in vitro* with an appropriate transfection vector comprising the exogenous genetic material for providing the desired genes to the cells. In this embodiment, the

cells can be any mammalian cell, either autologous, allogenic or xenogenic, such as endothelial cells, fibroblasts, myoblasts and the like. In this embodiment, the medical device is coated with a biocompatible matrix comprising a ligand which binds only to the genetically-altered mammalian cells by way of binding the genetically-engineeered cell membrane marker molecule or antigen on the surface of the cells.

[0042] In the therapeutic and/or drug delivery system of this embodiment, the genetically-altered cells are provided with exogenous genetic material to introduce at least one desired gene which encodes a cell surface marker molecule or antigen and at least one gene which encodes a therapeutic gene product. The system optionally comprises a signal system, such as an activating compound or molecule for stimulating the genetically-altered mammalian cells to express and/or secrete the desired gene product and/or the marker gene.

[0043] Thus, in one embodiment, the exogenous genetic material for introducing into mammalian cells is engineered to encode a cell membrane marker which specifically binds to the ligand on the device. For example, if the device is for implantation in a blood vessel lumen, the exogenous genetic material encodes a cell membrane marker not found in any cell circulating in the blood stream, other than the genetically-engineered cells provided to the patient.

[0044] There is also provided a coated medical devices and methods for the treatment of a variety of diseases such as vascular disease including but not limited to atherosclerosis, cancer, and rheumatoid arthritis. The medical device of the invention comprises a coating for the specific *in vivo* capturing and immobilization of genetically-altered mammalian cells which are introduced, simultaneously or sequentially, into the patient upon implantation of the coated medical device.

[0045] There is also provided immobilized genetically-altered cells which express and/or secrete at least one type of substance or therapeutic agent for the treatment of a specific disease. In this aspect of the invention, for example in the treatment of cancer, the cells, e.g., endothelial cells are

genetically-altered by introducing exogenous genetic material into the cells. In one embodiment, the genetic material is introduced into the nucleus of the cells and is DNA, such as extrachromosomal DNA. The extrachromosomal DNA may be a vector such as an adenoviral vector, a plasmid such as a naked plasmid, linear or short DNA, and the like. In one embodiment, the DNA comprises a regulatory/expression cassette for controlling the expression of the desired marker and/or therapeutic genes. In one embodiment, the regulatory cassette may comprise regulatory elements for constitutive expression of the therapeutic genes or may comprise elements that can be controlled or expressed as needed by the patient.

[0046] In one embodiment, the medical device for implantation into the patient comprises a coating; the coating comprises a matrix bearing at least one type of ligand, which recognizes and binds target cells. In the embodiment where the cells are genetically-altered, the ligand only recognizes and binds to a specific cell membrane marker molecule or antigen which is engineered into the cells. Thus in this embodiment, such ligand only recognizes the genetically-altered mammalian cells introduced into the patient, and the genetically-altered mammalian cells bind to said medical device and express and secrete the marker molecule or antigen as well as at least one therapeutic gene product.

[0047] In another embodiment, the therapeutic or drug delivery system may further comprise an activating molecule for stimulating said genetically-altered mammalian cells to express and/or secrete the desired therapeutic gene products. In this aspect of the invention, a compound such as a chemical stimulus or a peptide can be provided to the patient by several methods, including, oral route, a thermal patch, intravenously, intradermally and the like. In this embodiment, the genetically-altered mammalian cells may be autogenic or xenogenic, such as mature endothelial cells, fibroblasts, muscle cells, epithelial cells, etc. and comprise exogenous nucleic acid which can be extrachromosomal DNA. In one embodiment, the DNA is provided in the form of a vector, such as an adenovirus vector, naked plasmid DNA, linear DNA and the like. In one embodiment, the extrachromosomal DNA comprises a regulatory cassette, a gene which encodes a cell membrane antigen and at

least one gene which encodes a peptide for treating a disease. In one aspect of this embodiment, the cell membrane specific gene encodes, for example, an osteogenic or a prostatic cell membrane protein.

[0048] In one embodiment, the extrachromosomal genetic material comprises a gene which encodes the therapeutic/drug product, such as vascular endothelial growth factor and angiogenin for use in blood vessel remodeling, or anti-angiogenic factor in the treatment of cancer.

[0049] In another embodiment, a method for treating disease in a patient is provided. The method comprises:

providing genetically-altered mammalian cells to the patient; comprising an exogenous nucleic acid encoding a genetically-engineered cell membrane marker molecule and at least one therapeutic gene product;

implanting a medical device comprising a coating into the patient; the coating comprising a matrix bearing at least one ligand, wherein the ligand recognizes and binds the genetically-engineered cell membrane marker molecule on the genetically-altered mammalian cells, and wherein the genetically-altered mammalian cells bind to the medical device and express and secrete the therapeutic gene product. In an embodiment of the invention, the therapeutic gene and gene product comprises, for example, vascular endothelial growth factor, angiogenin, anti-angiogenic factor, and fibroblast growth factor.

[0050] The invention also provides a method for treating disease in a patient, the method comprises: providing genetically-altered mammalian cells to the patient; implanting a medical device into the patient; wherein the medical device comprises a coating which comprises a matrix bearing at least one ligand, wherein the ligand specifically recognizes and binds at least one marker molecule such as a receptor on the genetically-altered mammalian cells, and wherein the genetically-altered mammalian cells bind to the medical device and comprise exogenous nucleic acid for expressing and secreting a therapeutic gene product.

[0051] In another embodiment, a method for recruiting cells to a blood contacting surface *in vivo* is provided. The method comprises providing a

blood contacting surface positioned in the blood stream of a subject, said blood contacting surface configured to recruit target cells circulating in the blood stream of the subject to the blood contacting surface; and recruiting the target cells to the blood contacting surface. In this embodiment, the blood contacting surface comprises the luminal surface of a medical device implanted into the subject. In this embodiment of the invention, the recruited target cells on the blood contacting surface, for example, a stent or graft, can self-endothelialize the surface of the device in restoring normal endothelium at a site of blood vessel injury. The blood contacting surface can be a biodegradable scaffolding or can be coated with a biodegradable, biocompatible material. In this aspect of the invention, the biodegradable scaffolding when implanted into a blood vessel undergoes *in situ* degradation and the neo-endothelium formed on the luminal surface of the device restores the blood vessel continuity through the injured site so as to form a functional neo-vessel.

[0052] In another embodiment, the invention comprises a prosthesis, comprising: (a) a support member having an exterior surface and a blood contacting surface; (b) a first layer of a cross-linked polymeric compound coated onto said blood contacting surface of said support member; and, (c) a second layer coated on said first layer, said second layer comprising at least one ligand having an affinity for a target cell *in vivo*.

[0053] In another embodiment, a method for generating a self-endothelializing graft *in vivo*, the method comprising: (a) providing a scaffolding configured to function as a vascular graft, said scaffolding having a lumen surface and exterior surface, said lumen surface comprising ligands specific for binding to endothelial progenitor cells; (b) implanting said scaffolding into a blood vessel of a subject; and (c) recruiting circulating endothelial progenitor cells to said lumen surface of said scaffolding to form a neo-endothelium.

[0054] In yet another embodiment, there is provided a method for generating a self-endothelializing graft *in situ*, the method comprising: (a) providing a prosthetic structure having a surface exposed to circulating blood; (b) implanting the prosthetic structure into a subject; and (c) recruiting circulating

cells such as endothelial progenitor cells and genetically-altered mammalian cells from the blood to bind onto the surface of the prosthetic structure to form a neo-endothelium thereon.

[0055] In another embodiment, a method for generating a self-endothelializing graft *in situ*, the method comprising: (a) providing a biodegradable scaffolding configured to function as a temporary vascular graft, the scaffolding having a lumen surface and an exterior surface; (b) implanting the biodegradable scaffolding into a blood vessel; (c) recruiting circulating cells such as progenitor endothelial cells and genetically- altered mammalian cells to bind to the luminal surface of a prosthesis such as a graft, stent or a biodegradable scaffolding to form a neo-endothelium; (d) encapsulating the exterior surface of the scaffolding by vascular tissue to form an exterior hemostatic vascular structure; and (e) degrading the biodegradable scaffolding under *in vivo* conditions within a time frame which allows the neo-endothelium and the exterior vascular structure to form a functional neo-vessel.

[0056] In an embodiment, there is provided a biodegradable scaffolding for forming an endothelialized vascular graft *in situ*, the scaffolding comprising: (a) a porous biodegradable support member having a lumen and an exterior surface; (b) the lumen surface comprising a first layer of at least one species of a polymeric compound coated to the support member, and wherein the compound is cross-linked to itself with a cross-linking agent that forms covalent bonds that are subject to enzymatic cleavage or non-enzymatic hydrolysis under *in vivo* conditions, and (c) a ligand with specific affinity for binding genetically-altered mammalian cells *in vivo*.

[0057] In another embodiment, a method for generating a self-endothelializing graft *in situ*, the method comprising: (a) providing a prosthetic structure, having a surface exposed to circulating blood to a patient; (b) implanting the prosthetic structure into a subject or patient; (c) administering genetically-altered mammalian cells to the patient and (d) recruiting cells such as circulating genetically-altered mammalian cells from the blood to bind to the surface of the prosthetic structure to form a layer of genetically-altered cells on the surface of the prosthetic structure.

[0058] In yet another embodiment, a method is provided to promote vascular remodeling such as to increase the circumference of an artery by outward or positive remodeling to partially or totally compensate for the encroachment of the lumen caused by the formation of atherosclerotic plaques or by intimal hyperplasia after arterial injury so as to prevent or inhibit inward or negative remodeling of the injured vessel. In this embodiment, for example, a stent which is coated with a matrix and a ligand as described above in conjunction with genetically engineered cells, is provided for capturing genetically modified autologous cells such as endothelial progenitor cells, which are capable of secreting at least one potent anticoagulant and vasodilator such as prostacyclin, for example, prostaglandin I2, PGI2; calcitonin gene-related peptide such as α-CGRP and the like. Other products which can be engineered to be produced by the cells include, nitric oxide (nitric oxide synthase gene), matrix metalloproteinases, acetylcholine, adenosine, 5-hydroxytryptamine, substance P, adrenomedulin, and the like. Any gene which product acts as or has vasodilator and/or anticoagulant properties can be used, for example, a vasodilator can cause the vascular smooth muscle relaxation. The gene encoding the vasodilator, for example, prostacyclin synthase gene can be provided to progenitor endothelial cells or endothelial cells by gene transfer technologies such as viral gene transfer using, for example, a cistronic gene construct, in the case of prostacyclin, for example, a cistronic cyclooxygenase-1/prostacyclin synthase gene construct can provide continuous delivery of prostacyclin locally. In this embodiment, the local delivery system for prostacyclin can be used to treat, for example, cerebral infarct and coronary blood vessel disease. Positive remodeling of blood vessels can also be used as therapy for regulating arteriogenesis, i.e., formation of mature blood vessels such as arterioles and arteries in adults, to form collateral blood vessels.

[0059] In another embodiment, suitable cells such as fibroblasts, endothelial cells, or progenitor endothelial cells can be transfected with a bicistronic vector encoding both a vasodilatory compound and a unique cell surface marker such as a truncated MHC-I, which can be recognized by a ligand such as an antibody immobilized on an intravascular prosthesis. For example,

ligand such as an antibody, coated stent can be implanted into the coronary arteries of a patient, followed by transplantation of genetically modified cells such as genetically modified endothelial cells into the patient in need of treatment for vascular disease. In this embodiment and other embodiment using genetically modified cells, exogenous genes can be delivered into cells prior to transplantation of the cells using standard genetic engineering techniques using for example, a plasmid vector such as the bicistronic pMACS^{TM} K^{K}.II plasmid vector (Miltenyi Biotec, Germany), which contains multiple cloning sites and wherein the gene of interest can be inserted, for example, prostacyclin synthase as well as a marker gene, such as the truncated MHC class I molecule, H-2K^{K} as the selection marker for the mammalian cell lineage used.

[0060] In yet another embodiment, the exogenous gene delivery system for transfecting mammalian cells for use in therapy can comprise, for example, a lentivirus vector which may contain a truncated MHC class I antigen and vasodilator transgenes, for example, prostacyclin synthase and/or α-CGRP gene for treating vascular disease. In this embodiment, the mammalian cells to be transfected can be autologous endothelial cells, or endothelial progenitor cells, and the prosthetic device can be coated with ligands specific to the truncated MHC class 1 antigen such as and anti-H-2K^{k} antibody.

BRIEF DESCRIPTION OF DRAWINGS

[0061] FIG. 1A is a schematic representation of an antibody tethered covalently to the matrix by a cross-linking molecule. FIG. 1B shows a diagram of the $C_{60}O$ molecule anchoring the matrix. FIG. 1C depicts a schematic representation of a stent coated with the matrix of the invention.

[0062] FIG. 2A is a phase contrast micrograph of progenitor endothelial cells adhered to a fibronectin-coated slide containing cells isolated by enriched medium. FIG. 2B is a phase contrast micrograph of progenitor endothelial cells adhered to a fibronectin-coated slide containing cells isolated by anti-CD34 antibody coated magnetic beads. FIGs. 2D and 2F are micrographs of the progenitor endothelial cells which had been incubated for 7 days and stained with PI nuclear stain. As seen in these figures, the cells express

mature endothelial cell markers as shown by the antibody fluorescence for Tie-2 (FIGs. 2E and 2G) and VEGFR-2 (FIG. 2C) antibody reactivity.

[0063] FIGs. 3A and 3B are photographs of a 2% agarose gel stained with ethidium bromide of a semiquantitative RT-PCR for endothelial nitric oxide synthatase, eNOS and glyceraldehyde phosphate dehydrogenase, GAPDH. After 3 days (FIG. 3B) and 7 days (FIG. 3A) in culture on fibronectin-coated slides, the progenitor endothelial cells begin to express eNOS mRNA.

[0064] FIGs. 4A-4E are photomicrographs of HUVECs attached to the CMDx and anti-CD34 antibody (4A); gelatin and anti-CD34 antibody (4B); bare stainless steel disc (4C); CMDx coated (4D) and gelatin coated (4E) stainless steel disc which were incubated with HUVEC cell and stained with propidium iodide.

[0065] FIGs. 5A-5C are photomicrographs of a control, coated with CMDx without antibody which were incubated with the white cell fraction of human blood. The cells were stained with propidium iodide and FITC labeled anti-KDR antibody. FIGs. 5D-5F are photomicrographs of control stainless steel discs coated with gelatin without antibody bound to its surface which were incubated with the white cell fraction of human blood. The cells were stained with propidium iodide and FITC labeled anti-KDR antibody.

[0066] FIGs. 6A-6C are photomicrographs of stainless steel discs coated with CMDx matrix with anti-CD34 antibody bound to its surface which were incubated with the HUVECs. The cells were stained with propidium iodide and FITC labeled anti-KDR antibody. FIGs. 6D-6F are photomicrographs of stainless steel discs coated with gelatin matrix with antibody bound to its surface, which were incubated with HUVECS. The cells were stained with propidium iodide and FITC labeled anti-KDR antibody.

[0067] FIG. 7 is a photomicrograph of stainless steel discs coated with CMDx matrix with antibody bound to its surface, which was incubated with progenitor cells for 24 hours. The cells were stained with propidium iodide and FITC labeled anti-KDR antibody.

[0068] FIGs. 8A and 8B are photomicrographs of a stainless steel disc coated with CMDx matrix containing anti-CD34 antibody bound to its surface

incubated with progenitor cells for 7 days. The cells were stained with propidium iodide and FITC labeled anti-KDR antibody.

[0069] FIGs. 9A and 9B photomicrograph of a stainless steel disc coated with CMDx matrix containing anti-CD34 antibody bound to its surface incubated with progenitor cells for 7days. The cells were stained with propidium iodide and FITC labeled anti-Tie-2 antibody.

[0070] FIGs. 10A-10C are phase contrast photomicrographs of stainless steel CMDx coated discs incubated with progenitor cells for 3 weeks in endothelial growth medium which show mature endothelial cells.

[0071] FIG. 11 is schematic diagram of a functional fullerene coated stent surface of the invention binding a progenitor cell.

[0072] FIGs. 12A –12D are photomicrographs of fullerene-coated samples without or with anti-CD34 antibody. The samples were incubated with a human white blood cell fraction and stained with Propidium iodide and FITC labeled anti-VEGFR-2 antibody.

[0073] 13A-13D are photomicrographs of histological cross-sections of coronary artery explants which had been implanted for 4 weeks with a bare stainless steel stent (FIGs. 13A and 13C) and a fullerene-coated sample (FIGs.13B and 13D) taken at low and high magnification. The sections were stained with hematoxylin-eosin stain.

[0074] FIGs.14A –14G are scanning electron micrographs of stent explants 1 and 48 hours after implantation in male Yorkshire swine. Explants of dextran-coated (FIG. 14A) and dextran/anti-CD34 antibody-coated (14B) stents at 1 hour after implantation. FIGs. 14C and 14D show explants of control samples and FIGs. 14E-G are dextran/anti-CD34 antibody-coated stents at 48 hours after implantation. FIGs. 14H-14M are histological photomicrographs of cross-sections through coronary arteries of explants from male Yorkshire swine which were implanted for 4 weeks: uncoated (Bare stainless steel) (14H and 14I), dextran-coated control (14J and 14K), and dextran/anti-CD34 antibody-coated (14L and 14M).

[0075] FIGs. 15A, 15B and 15C are, respectively, fluorescent photomicrographs of 48 hours explants of a dextran-plasma-coated stent

without antibody on its surface, and a dextran-plasma-coated/anti-CD34 antibody-coated stent of 18 mm in length.

[0076] FIGs. 16A and 16B are photomicrographs of a Propidium iodide and anti-lectin/FITC-conjugated sample.

DETAILED DESCRIPTION

[0077] The present invention provides a coated, implantable medical device such as a stent or graft, methods and compositions for coating the medical device, and methods of treating vascular disease with the coated medical device. There is also provided a method for treating diseases such as restenosis and cancer, which method comprises implanting a medical device with a coating to a patient in need of treatment, and providing the patient with genetically engineered mammalian cells which bind *in vivo* to the surface of the medical device and can produce an engineered and desired therapeutic agent such as a gene product. FIGs. 1A-1C illustrates a schematic representation of the surface coat of a medical device of the invention. The coating on the medical device comprises a biocompatible matrix for promoting the formation of a confluent layer of cells such as genetically-altered mammalian cells such as endothelial cells or fibroblasts on the surface of the device for regulating or producing a desired therapeutic event in the patient such as producing an anti-angiogenic factor or an anti-thrombotic agent, or producing a product which inhibits excessive intimal hyperplasia in preventing restenosis and/or thrombosis. In one embodiment, the coating on the prosthetic device comprises a matrix comprising a synthetic or naturally-occurring material in which a therapeutically effective amount of at least one type of antibody that promotes adherence of circulating cells such as genetically-altered mammalian cells such as endothelial, progenitor or stem cells to the medical device, and at least one compound such as a growth factor, which stimulates endothelial cell growth and differentiation. Upon implantation of the device, the cells that adhere to the surface of the device transform into a mature, confluent, functional layer of cells such as an endothelium on the luminal surface of the medical device. The presence of a confluent layer of endothelial cells on the medical device, for example, can

reduce the occurrence of restenosis and thrombosis at the site of implantation.

[0078] As used herein, "medical device" refers to a device that is introduced temporarily or permanently into a mammal for the prophylaxis or therapy of a medical condition. These devices include any that are introduced subcutaneously, percutaneously or surgically to rest within an organ, tissue or lumen of an organ, such as an artery, vein, ventricle, or atrium of the heart. Medical devices may include stents, stent grafts, covered stents such as those covered with polytetrafluoroethylene (PTFE), expanded polytetrafluoroethylene (ePTFE), or other natural or synthetic coverings, or synthetic vascular grafts, artificial heart valves, artificial hearts and fixtures to connect the prosthetic organ to the vascular circulation, venous valves, abdominal aortic aneurysm (AAA) grafts, inferior venal caval filters, permanent drug infusion catheters, embolic coils, embolic materials used in vascular embolization (e.g., cross-linked PVA hydrogel), vascular sutures, vascular anastomosis fixtures, transmyocardial revascularization stents and/or other conduits.

[0079] Coating of the medical device with the present compositions and methods stimulates the development of a confluent mammalian cell layer *in vivo* on the surface of the device. For example, an endothelial cell layer on the surface of the medical device is formed when the ligand provided binds endothelial cells forming a functional endothelial layer on the blood contacting surface of the device, thereby preventing restenosis as well as modulating the local chronic inflammatory response and thromboembolic complications that result from implantation of the medical device.

[0080] The matrix coating the medical device can be composed of synthetic material, such as polymeric gel foams, such as hydrogels made from polyvinyl alcohol (PVA), polyurethane, poly-L-lactic acid, cellulose ester or polyethylene glycol. In one embodiment, very hydrophilic compounds such as dextran compounds can comprise the synthetic material for making the matrix. In another embodiment, the matrix can be composed of naturally occurring materials, such as collagen, fibrin, elastin, tropoelastin, and/or amorphous carbon. The matrix may also comprise several layers with, for example, a first

layer being composed of synthetic or naturally occurring materials and a second layer composed of, for example, a ligand such as antibodies. The layers may be ordered sequentially, with the first layer directly in contact with the medical device such as a stent or synthetic graft surface and the second layer having one surface in contact with the first layer and the opposite surface in contact with the vessel lumen.

[0081] The matrix may further comprise at least a growth factor, cytokine, vasodilator, anticoagulants, or the like. Growth factors which can stimulate endothelial cell proliferation and differentiation are, for example, vascular endothelial cell growth factor (VEGF) and isoforms, basic fibroblast growth factor (bFGF), platelet-induced growth factor (PIGF), transforming growth factor beta 1 (TGF.*b*1), acidic fibroblast growth factor (aFGF), osteonectin, angiopoietin 1, angiopoietin 2, insulin-like growth factor (ILGF), platelet-derived growth factor AA (PDGF-AA), platelet-derived growth factor BB (PDGF-BB), platelet-derived growth factor AB (PDGF-AB), granulocyte-macrophage colony-stimulating factor (GM-CSF), and the like, or functional fragments thereof can be used in the invention. Vasodilators include prostacyclin, α-CGRP, and the like.

[0082] In another embodiment, the matrix may comprise fullerenes, where the fullerenes range from about C_{20} to about C_{150} in carbon number. The fullerenes can also be arranged as nanotubes, that incorporate molecules or proteins. The fullerene matrix can also be applied to the surface of stainless steel, PTFE, or ePTFE medical devices, which layer is then functionalized and coated with antibodies and growth factor on its surface. Alternatively, the PTFE or ePTFE can be layered first on, for example, a stainless steel medical device followed by a second layer of fullerenes and then the antibodies and the growth factor are added.

[0083] The matrix may be noncovalently or covalently attached to the medical device. Antibodies and growth factors can be covalently attached to the matrix using hetero- or homobifunctional cross-linking reagents. The growth factor can be added to the matrix using standard techniques with the antibodies or after antibody binding.

[0084] As used herein, the term "antibody" refers to one type of monoclonal, polyclonal, humanized, or chimeric antibody or a combination thereof, wherein the monoclonal, polyclonal, humanized or chimeric antibody binds to one antigen or a functional equivalent of that antigen. The term antibody fragment encompasses any fragment of an antibody such as Fab, $F(ab')_2$, and can be of any size, i.e., large or small molecules, which have the same results or effects as the antibody. (An antibody encompasses a plurality of individual antibody molecules equal to 6.022×10^{23} molecules per mole of antibody).

[0085] In an embodiment, for example, a stent or synthetic graft can be coated with a biocompatible matrix comprising antibodies, antibody fragments or combinations thereof that modulate adherence of circulating cells such as genetically-altered mammalian therapeutic cells and progenitor endothelial cells to the medical device. For example, the antibodies of the invention recognize and bind specific cell membrane marker molecules such as progenitor endothelial cells surface antigens and/or cell membrane molecules which are produced by genetically modified mammalian cells in the circulating blood so that the cells are immobilized on the surface of the device to form a layer of functional cells on the device such as a functional endothelium. In one embodiment, the antibodies comprise monoclonal antibodies reactive (recognize and bind) with genetically-altered mammalian cell surface molecule, progenitor endothelial cell surface antigens, or a progenitor or stem cell surface antigen, such as vascular endothelial growth factor receptor-1, -2 and -3 (VEGFR-1, VEGFR-2 and VEGFR-3 and VEGFR receptor family isoforms), Tie-1, Tie2, CD34, Thy-1, Thy-2, Muc-18 (CD146), CD30, stem cell antigen-1 (Sca-1), stem cell factor (SCF or c-Kit ligand), CD133 antigen, VE-cadherin, P1H12, TEK, CD31, Ang-1, Ang-2, or an antigen expressed on the surface of the cells. In one embodiment, a single type of antibody that reacts with one antigen can be used. Alternatively, a plurality of different antibodies directed against different progenitor endothelial cell surface antigens can be mixed together and added to the matrix. In another embodiment, a cocktail of monoclonal antibodies is used to increase the rate of endothelium formation by targeting specific cell surface antigens. In this embodiment, for example, anti-CD34 and anti-CD133 can be used in combination or combinations of

these with any or several of the above listed antigens can be used attached to the surface of the matrix on the medical device, for example, a stent or graft. Antibodies, fragments of the antibodies and/or combinations thereof can be used for coating the medical device.

[0086] As used herein, a "therapeutically effective amount of the antibody" means the amount of an antibody that promotes adherence of cells such as native or genetically-altered mammalian cells, including, endothelial, progenitor, or stem cells to the medical device. The amount of an antibody needed to practice the invention varies with the nature of the antibody used. For example, the amount of an antibody used depends on the binding constant and/or affinity between the antibody and the antigen against which it reacts. It is well known to those of ordinary skill in the art how to determine therapeutically effective amounts of an antibody to use with a particular antigen.

[0087] As used herein, the term "compound" refers to any substance which stimulates genetically-altered mammalian cells to express and/or secrete the therapeutic gene product.

[0088] As used herein, the term "growth factor" refers to a peptide, protein, glycoprotein, lipoprotein, or a fragment or modification thereof, or a synthetic molecule, which stimulates cells such as endothelial, stem or progenitor cells which may or may not have been genetically-altered to grow and differentiate into mature, functional endothelial cells. Mature endothelial cells express nitric oxide synthetase, thereby releasing nitric oxide into the tissues. Table 1 below lists some of the growth factors that can be used for coating the medical device.

Table 1

Growth Factor	Endothelial cell specific
Acidic fibroblast growth factor (aFGF)	No
Basic fibroblast growth factor (bFGF)	No
Fibroblast growth factor 3 (FGF-3)	No
Fibroblast growth factor 4 (FGF-4)	No
Fibroblast growth factor 5 (FGF-5)	No
Fibroblast growth factor 6 (FGF-6)	No
Fibroblast growth factor 7 (FGF-7)	No
Fibroblast growth factor 8 (FGF-8)	No
Fibroblast growth factor 9 (FGF-9)	No

Angiogenin 1	Yes
Angiogenin 2	Yes
Hepatocyte growth factor / scatter factor (HGF/SF)	No
Platelet-derived growth factor (PDE-CGF)	Yes
Transforming growth factor-α (TGF-α)	No
Transforming growth factor-β (TGF-β)	No
Tumor necrosis factor-α (TNF-α)	No
Vascular endothelial growth factor 121 (VEGF 121)	Yes
Vascular endothelial growth factor 145 (VEGF 145)	Yes
Vascular endothelial growth factor 165 (VEGF 165)	Yes
Vascular endothelial growth factor 189 (VEGF 189)	Yes
Vascular endothelial growth factor 206 (VEGF 206)	Yes
Vascular endothelial growth factor B (VEGF-B)	Yes
Vascular endothelial growth factor C (VEGF-C)	Yes
Vascular endothelial growth factor D (VEGF-D)	Yes
Vascular endothelial growth factor E (VEGF-E)	Yes
Vascular endothelial growth factor F (VEGF-F)	Yes
Placental growth factor	Yes
Angiopoietin-1	No
Angiopoietin-2	No
Thrombospondin (TSP)	No
Proliferin	Yes
Ephrin-A1 (B61)	Yes
E-selectin	Yes
Chicken chemotactic and angiogenic factor (cCAF)	No
Leptin	Yes
Heparin affinity regulatory peptide (HARP)	No
Heparin	No
Granulocyte colony stimulating factor	No
Insulin-like growth factor	No
Interleukin 8	No
Thyroxine	No
Sphingosine 1-phosphate	No

[0089] As used herein, the term "VEGF" means any of the isoforms of the vascular endothelium growth factor listed in Table 1 above unless the isoform is specifically identified with its numerical or alphabetical abbreviation.

[0090] As used herein, the term "therapeutically effective amounts of growth factor" means the amount of a growth factor that stimulates or induces a specific cell population, for example, a native or modified endothelial, progenitor or stem cell to grow and differentiate, thereby forming a confluent layer of mature and functional cell layer such as endothelial cells forming functional endothelium on the luminal surface of the medical device. The amount of a growth factor needed to practice the invention varies with the nature of the growth factor used and binding kinetics between the growth factor and its receptor on the target cell. For example, 100 μg of VEGF has

been shown to stimulate the adherence of endothelial cells on a medical device and form a confluent layer of epithelium. It is well known to those of ordinary skill in the art how to determine therapeutically effective amounts of a growth factor for use in stimulating cell growth and differentiation of cells, for example, endothelial cells.

[0091] As used herein, "intimal hyperplasia" is the undesirable increased in smooth muscle cell proliferation and/or matrix deposition in the vessel wall. As used herein "restenosis" refers to the recurrent narrowing of the blood vessel lumen. Vessels may become obstructed because of restenosis. After PTCA or PTA, smooth muscle cells from the media and adventitia, which are not normally present in the intima, proliferate and migrate to the intima and secrete proteins, forming an accumulation of smooth muscle cells and matrix protein within the intima. This accumulation causes a narrowing of the lumen of the artery, reducing blood flow distal to the narrowing. As used herein, "inhibition of restenosis" refers to the inhibition of migration and proliferation of smooth muscle cells accompanied by prevention of protein secretion so as to prevent restenosis and the complications arising therefrom.

[0092] The subjects that can be treated using the medical device, methods and compositions of this invention are mammals, including humans, dogs, cats, pigs, horses, rodents and monkeys.

[0093] The present methods of treatment may be practiced *in vivo* or *in vitro*.

[0094] The term "progenitor endothelial cell" refers to endothelial cells at any developmental stage, from progenitor or stem cells to mature, functional endothelial cells from bone marrow, blood or local tissue origin and which are non-malignant.

[0095] The coated medical device can be fully provided with genetically modified mammalian cells such as genetically-altered differentiated endothelial cells which can be isolated from an explanted artery or vein such as a human umbilical vein, which have been genetically-altered with a desired nucleic acid construct *in vitro*, while progenitor endothelial cells can be isolated from peripheral blood or bone marrow. In one embodiment, the endothelial cells can be bound to the medical devices by incubation of the

endothelial cells with a medical device coated with the matrix that incorporates an antibody, and optionally at least one growth factor, or other ligands that adhere to endothelial cells. In another embodiment, the endothelial cells can be transformed endothelial cells. The transfected endothelial cells can contain vectors which express growth factors or other peptides or proteins which directly or indirectly inhibit thrombogenesis, restenosis, or any other therapeutic end.

[0096] In another embodiment, endothelial or any other type of stable mammalian cells such as fibroblasts can be transfected with any mammalian expression vector that contains any cloned genes encoding proteins or peptides suitable for specific applications. For example, the vector can be constructed consisting an expression cassette comprising a gene encoding platelet derived growth factor (PDGF), fibroblast growth factor (FGF), or nitric oxide synthase (NOS) and the expression cassette can be constructed using conventional methods, and supplies from commercially available sources. (See, for example, mammalian expression vectors and transfection kits commercially available from Stratagene, San Diego, CA). For example, purified porcine progenitor endothelial cells are transfected with vascular endothelial growth factor (VEGF) using an adenoviral expression vector expressing the VEGF cDNA according to the methods of Rosengart et al. (Six-month assessment of a phase I trial of angiogenic gene therapy for the treatment of coronary artery disease using direct intramyocardial administration of an adenovirus vector expressing the VEGF121 cDNA. Ann. Surg. 230(4):466-470, 1999). In this embodiment, the mammalian cells can be autologous, allogenic or xenogenic in origin. Once the cells are genetically-altered by transfection of exogenous DNA or RNA expression cassettes comprising the desired genes, the cells can be grown using standard tissue culture techniques. Samples of cells which express and secrete desired genes can be stored frozen in liquid nitrogen using standard techniques. Frozen cells can be regrown using standard tissue culture techniques prior to use. Genetically-altered mammalian cells can be administered to the patient at the time of implantation of the device either locally at the implant site, or intravenously, or intra-

arterially into the patient, preferably after the coated medical device is implanted. Transformed cells can further comprise a marker or reporter gene for the accurate detection and identification of the cells prior to cell administration to the patient.

[0097] The methods of treatment of vascular disease of the invention can be practiced on any artery or vein. Included within the scope of this invention is atherosclerosis of any artery including coronary, infrainguinal, aortoiliac, subclavian, mesenteric and renal arteries. Other types of vessel obstructions, such as those resulting from a dissecting aneurysm are also encompassed by the invention.

[0098] The method of treating a mammal with vascular disease comprises implanting a coated medical device into the patient's organ or vessel, for example, in the case of a coated stent during angioplasty. Once *in situ*, progenitor endothelial cells are captured on the surface of the coated stent by the recognition and binding of cellular antigens, for example, genetically-modified mammalian cells or on the progenitor cell surface by the antibody alone or in combination with other ligands which are present on the coating of the device. Once the progenitor cell is adhered to the matrix, the growth factor on the coating promotes the newly-bound progenitor endothelial cells to grow and differentiate and form a confluent, mature and functional endothelium on the luminal surface of the stent. Alternatively, the medical device can be coated with native or genetically-modified mammalian cells such as endothelial cells *in vitro* before implantation of the medical device which cells can be progenitor, stem cells, or mature endothelial cells isolated from the patient's blood, bone marrow, or blood vessel. In either case, the presence of functional cells on the luminal surface of the medical device can produced the desired or engineered function such as inhibiting or preventing excessive intimal hyperplasia and thrombosis.

Endothelial Cells

[0099] In certain embodiments, human umbilical vein endothelial cells (HUVEC) can be obtained from umbilical cords according to the methods of Jaffe, et al., J. Clin. Invest., 52:2745-2757, 1973,

and were used in experiments. Briefly, cells are stripped from the blood vessel walls by treatment with collagenase and cultured in gelatin-coated tissue culture flasks in M199 medium containing 10% low endotoxin fetal calf serum, 90 ug/ml preservative-free porcine heparin, 20 ug/ml endothelial cell growth supplement (ECGS) and glutamine.

[00100] Progenitor endothelial cells (EPC) can be isolated from human peripheral blood according to the methods of Asahara et al. (Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964-967, 1997). Magnetic beads coated with antibody to CD34 are incubated with fractionated human peripheral blood. After incubation, bound cells are eluted and can be cultured in EBM-2 culture medium. (Clonetics, San Diego, CA). Alternatively enriched medium isolation can be used to isolate these cells. Briefly, peripheral venous blood is taken from volunteers and the mononuclear cell fraction is isolated by density gradient centrifugation, and the cells are plated on fibronectin coated culture slides (Becton Dickinson) in EC basal medium-2 (EBM-2) (Clonetics) supplemented with 5% fetal bovine serum, human VEGF-A, human fibroblast growth factor-2, human epidermal growth factor, insulin-like growth factor-1, and ascorbic acid. EPCs are grown for 7-days, with culture media changes every 48 hours. Cells are characterized by fluorescent antibodies to CD45, CD34, CD31, VEGFR-2, Tie-2, and E-selectin.

[00101] In another embodiment, mammalian cells can be transfected with any expression cassette that may contain any cloned gene that encodes a specific marker molecule not normally found in circulating cells such as prostatic specific antigen or a bone cell antigen, and can also express peptides and/or proteins such as platelet derived growth factor (PDGF), fibroblast growth factor (FGF), or nitric oxide synthase (NOS) using conventional methods. (See, for example, mammalian expression vectors and transfection kits commercially available from Stratagene, San Diego, CA). For example, purified porcine progenitor endothelial cells are transfected with vascular endothelial growth factor (VEGF) using a mammalian expression cassette expressing the VEGF cDNA according to the methods of Rosengart et al. (Six-month assessment of a phase I trial of angiogenic gene therapy for

the treatment of coronary artery disease using direct intramyocardial administration of an adenovirus vector expressing the VEGF121 cDNA. Ann. Surg. 230(4):466-470 (1999)).

Antibodies

[00102] Monoclonal antibodies useful in the method of the invention may be produced according to the standard techniques of Kohler and Milstein (Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 265:495-497, 1975), or can be obtained from commercial sources. Endothelial cells can be used as the immunogen to produce monoclonal antibodies directed against endothelial cell surface antigens.

[00103] Monoclonal antibodies directed against endothelial cells are prepared by injecting HUVEC or purified progenitor endothelial cells into a mouse or rat. After a sufficient time, the mouse is sacrificed and spleen cells are obtained. The spleen cells are immortalized by fusing them with myeloma cells or with lymphoma cells, generally in the presence of a non-ionic detergent, for example, polyethylene glycol. The resulting cells, which include the fused hybridomas, are allowed to grow in a selective medium, such as HAT-medium, and the surviving cells are grown in such medium using limiting dilution conditions. The cells are grown in a suitable container, e.g., microtiter wells, and the supernatant is screened for monoclonal antibodies having the desired specificity, i.e., reactivity with endothelial cell antigens.

[00104] Various techniques exist for enhancing yields of monoclonal antibodies such as injection of the hybridoma cells into the peritoneal cavity of a mammalian host which accepts the cells and then harvesting the ascitic fluid. Where an insufficient amount of monoclonal antibody collects in the ascitic fluid, the antibody is harvested from the blood of the host. Various conventional ways exist for isolation and purification of monoclonal antibodies so as to free the monoclonal antibodies from other proteins and other contaminants.

[00105] Also included within the scope of the invention are useful binding fragments of antibodies such as anti-endothelial cell monoclonal antibodies

such as the Fab, $F(ab')_2$ of these monoclonal antibodies. The antibody fragments can be obtained by conventional techniques. For example, useful binding fragments may be prepared by peptidase digestion of the antibody using papain or pepsin abd cab be used alone or in combination with its antibody of origin or with other types of antibodies and fragments thereof.

[00106] The antibodies can be directed to an antibody of the IgG class from a murine source; however, this is not meant to be a limitation. Specific antibodies such as the above antibody and those antibodies having functional equivalency with the above antibody, whether from a murine source, mammalian source including human, or other sources, or combinations thereof are included within the scope of this invention, as well as other classes such as IgM, IgA, IgE, and the like, including isotypes within such classes. Such antibodies specifically recognize and bind with high affinity to the target antigen on the membrane of target cells, whether on a native molecule or a genetically-engineered antigen. In the case of antibodies, the term "functional equivalency" means that two different antibodies each bind to the same antigenic site on an antigen, in other words, the antibodies compete for binding to the same antigen. The antigen may be on the same or different molecule.

[00107] In one embodiment, monoclonal antibodies and/or fragments thereof reacting with the endothelial cell surface antigen, for example, CD34 can be used. Anti-CD34 monoclonal antibodies attached to a solid support have been shown to capture progenitor endothelial cells from human peripheral blood. After capture, these progenitor cells are capable of differentiating into endothelial cells. (Asahara et al. 1997. Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964-967.) Hybridomas producing monoclonal antibodies directed against CD34 can be obtained from the American Type Tissue Collection. (Rockville, MD). In another embodiment, monoclonal antibodies reactive with endothelial cell surface antigens such as VEGFR-1 and VEGFR-2, CD133, or Tie-2 are used. In the embodiment using genetically-altered cell, antibodies are produced against the genetically engineered gene product using standard techniques in

the same manner as described above, and then applied to the blood contacting surface of the medical device following matrix application.

[00108] Polyclonal antibodies reactive against endothelial cells isolated from the same species as the one receiving the medical device implant may also be used.

Stent

[00109] The term "stent" herein means any medical device which when inserted or implanted into the lumen of a vessel expands the cross-sectional lumen of a vessel. The term "stent" includes, stents commercially available manufactured from stainless steel or other alloys which have been coated by the methods of the invention; covered stents such as those covered with PTFE or ePTFE. In one embodiment, this includes stents delivered percutaneously to treat coronary artery occlusions or to seal dissections or aneurysms of the splenic, carotid, iliac and popliteal vessels. In another embodiment, the stent is delivered into a venous vessel. The stent can be composed of polymeric or metallic structural elements onto which the matrix comprising the antibodies and the compound, such as growth factors, is applied or the stent can be a composite of the matrix intermixed with a polymer. For example, a deformable metal wire stent can be used, such as that disclosed in U.S. Patent No. 4,886,062 to Wiktor. A self-expanding stent of resilient polymeric material such as that disclosed in published international patent application WO91/12779 and U.S. Patent No. 5,871,535 entitled "Intraluminal Drug Eluting Prosthesis" can also be used. Other stents that can be used are disclosed in U.S. Patent Nos 6,432,132 and 6,821,292. Stents may also be manufactured using stainless steel, polymers, nickel-titanium, tantalum, gold, platinum-iridium, cobalt-based alloys or Elgiloy and MP35N and other ferrous materials. Stents are delivered through the body lumen on a catheter to the treatment site where the stent is released from the catheter, allowing the stent to expand into direct contact with the luminal wall of the vessel. In another embodiment, the stent comprises a biodegradable stent

(H. Tamai, pp 297 in Handbook_of_Coronary_Stents_3rd_Edition, Eds. PW Serruys and MJB Kutryk, Martin Dunitz (2000). It will be apparent to those skilled in the art that other self-expanding stent designs (such as resilient metal stent designs) could be used with the antibodies, growth factors and matrices of this invention.

Synthetic Graft

[00110] The term "synthetic graft" means any artificial prosthesis having biocompatible characteristics. In one embodiment, the synthetic grafts can be made of polyethylene terephthalate (Dacron®, PET) or polytetrafluoroehtylene (Teflon®, ePTFE). In another embodiment, synthetic grafts are composed of polyurethane, cross-linked PVA hydrogel, and/or biocompatible foams of hydrogels. In yet another embodiment, a synthetic graft is composed of an inner layer of meshed polycarbonate urethane and an outer layer of meshed polyethylene terephthalate. It will be apparent to those skilled in the art that any biocompatible synthetic graft can be used with the present coating components such as antibodies, growth factors, and matrices. (Bos et al. 1998. Small-Diameter Vascular Prostheses: Current Status. Archives Physio Biochem. 106:100-115). Synthetic grafts can be used for, for example, end-to-end, end to side, side to end, side to side or intraluminal and in anastomosis of vessels or for bypass of a diseased vessel segments, for example, as abdominal aortic aneurysm devices.

Matrix

[00111] (A) Synthetic Materials - The matrix that is used to coat the stent or synthetic graft may be selected from synthetic materials such as polyurethane, segmented polyurethane-urea/heparin, poly-L-lactic acid, cellulose ester, polyethylene glycol, cross-linked PVA hydrogel, biocompatible foams of hydrogels, or hydrophilic dextrans, such as carboxymethyl dextran.

[00112] (B) Naturally Occurring Material - The matrix may be selected from naturally occurring substances such as collagen, fibronectin, vitronectin, elastin, laminin, heparin, fibrin, cellulose or carbon. A primary requirement for the matrix is that it be sufficiently elastic and flexible to remain unruptured on the exposed surfaces of the stent or synthetic graft.

[00113] (C) Fullerenes - The matrix may also comprise a fullerene (the term "fullerene" encompasses a plurality of fullerene molecules). Fullerenes are carbon-cage molecules. The number of carbon (C) molecules in a fullerene species varies from about C_{20} to about C_{150}. Fullerenes are produced by high temperature reactions of elemental carbon or of carbon-containing species by processes well known to those skilled in the art; for example, by laser vaporization of carbon, heating carbon in an electric arc or burning of hydrocarbons in sooting flames. (U.S. Patent No. 5,292,813, to Patel et al., and U.S. Patent No. 5,558,903 to Bhushan et al.). In each case, a carbonaceous deposit or soot is produced. From this soot, various fullerenes are obtained by extraction with appropriate solvents, such as toluene. The fullerenes are separated by known methods, in particular by high performance liquid chromatography (HPLC). Fullerenes may be synthesized or obtained commercially from Dynamic Enterprises, Ltd., Berkshire, England or Southern Chemical Group, LLC, Tucker, Georgia, or Bucky USA, Houston Texas.

[00114] Fullerenes may be deposited on surfaces in a variety of different ways, including, sublimation, laser vaporization, sputtering, ion beam, spray coating, dip coating, roll-on or brush coating as disclosed in U.S. Patent No. 5,558,903, or by derivatization of the surface of the stent.

[00115] An important feature of fullerenes is their ability to form "activated carbon." The fullerene electronic structure is a system of overlapping pi-orbitals, such that a multitude of bonding electrons are cooperatively presented around the surface of the molecule. (Chemical and Engineering News, Apr. 8, 1991, page 59). As forms of activated carbon, fullerenes exhibit substantial van der Waals forces for weak interactions. The adsorptive nature of the fullerene surface may lend itself to additional modifications for the purpose of directing specific cell membrane interactions. For example, specific molecules that possess chemical properties that selectively bind to cell membranes of particular cell types or to particular components of cell membranes, e.g., lectins or antibodies, can be adsorbed to the fullerene surface. Attachment of different molecules to the

fullerene surface may be manipulated to create surfaces that selectively bind various cell types, e.g., progenitor endothelial cells, epithelial cells, fibroblasts, primary explants, or T-cell subpopulations. U.S. Patent No. 5,310,669 to Richmond et al.; Stephen R. Wilson, Biological Aspects of Fullerenes, Fullerenes:Chemistry, Physics and Technology, Kadish et al. eds., John Wiley & Sons, NY 2000.

[00116] Fullerenes may also form nanotubes that incorporate other atoms or molecules. (Liu et al. Science 280:1253-1256 (1998)). The synthesis and preparation of carbon nanotubes is well known in the art. (U.S. Patent No. 5,753,088 to Olk et al., and U.S. Patent No. 5,641,466 to Ebbsen et al.). Molecules such as proteins can also be incorporated inside carbon nanotubes. For example, nanotubes may be filled with the enzymes, e.g., Zn_2Cd_2-metallothionein, cytochromes C and C3, and beta-lactamase after cutting the ends of the nanotube. (Davis et al. Inorganica Chim. Acta 272:261 (1998); Cook et al. Full Sci. Tech. 5(4):695 (1997)).

[00117] Three dimensional fullerene structures can also be used. U.S. Patent No. 5,338,571 to Mirkin et al. discloses three-dimensional, multilayer fullerene structures that are formed on a substrate surface by (i) chemically modifying fullerenes to provide a bond-forming species; (ii) chemically treating a surface of the substrate to provide a bond-forming species effective to covalently bond with the bond-forming species of the fullerenes in solution; and, (iii) contacting a solution of modified fullerenes with the treated substrate surface to form a fullerene layer covalently bonded to the treated substrate surface.

(D) Application of the Matrix to the Medical Device

[00118] The matrix should adhere tightly to the surface of the medical device including stent or synthetic graft. In one embodiment, this is accomplished by applying the matrix in successive thin layers. Alternatively, antibodies and growth factors are applied only to the surface of the outer layer

in direct contact with the vessel lumen. Different types of matrices may be applied successively in succeeding layers. The antibodies may be covalently or noncovalently coated on the matrix after application of the matrix to the stent.

[00119] In order to coat a medical device such as a stent, the stent is dipped or sprayed with a liquid solution of the matrix of moderate viscosity. After each layer is applied, the stent is dried before application of the next layer. In one embodiment, a thin, paint-like matrix coating does not exceed an overall thickness of 100 microns.

[00120] In one embodiment, the medical device's surface is, for example, a stent surface which is first functionalized, followed by the addition of a matrix layer. Thereafter, the antibodies, as well as other components of the coating such as a growth factor, are coupled to the surface of the matrix. In this aspect, the techniques used to apply the matrix on, for example, the stent surface creates chemical groups which are functional. For example, the chemical groups can be amines, which can be reactive with functional groups of the polymer to immobilize an intermediate layer of matrix, which serves as support for the ligands such as antibodies, peptides, and/or growth factors to identify and capture the target cells.

[00121] In another embodiment, a suitable matrix coating solution is prepared by dissolving 480 milligrams (mg) of a drug carrier, such as poly-D, L-lactid (available as R203 of Boehringer Inc., Ingelheim, Germany) in 3 milliliters (ml) of chloroform under aseptic conditions. In principle, however, any biodegradable (or non-biodegradable) matrix that is blood-and tissue-compatible (biocompatible) and can be dissolved, dispersed or emulsified may be used as the matrix if, after application, it undergoes relatively rapid drying to a self-adhesive lacquer- or paint-like coating on the medical device.

[00122] For example, coating a stent with fibrin is well known to one of ordinary skill in the art. In U.S. Patent No. 4,548,736 issued to Muller et al., fibrin is clotted by contacting fibrinogen with thrombin. Preferably, the fibrin in the fibrin-containing stent of the present invention has Factor XIII and calcium

present during clotting, as described in U.S. Pat. No. 3,523,807 issued to Gerendas, or as described in published European Patent Application 0366564, in order to improve the mechanical properties and biostability of the implanted device. In this embodiment, the fibrinogen and thrombin used to make fibrin in the present invention are from the same animal or human species as that in which the stent will be implanted in order to avoid any inter-species immune reactions, e.g., human anti-cow. The fibrin product can be in the form of a fine, fibrin film produced by casting the combined fibrinogen and thrombin in a film and then removing moisture from the film osmotically through a semipermeable membrane. In the European Patent Application 0366564, a substrate (preferably having high porosity or high affinity for either thrombin or fibrinogen) is contacted with a fibrinogen solution and with a thrombin solution. The result is a fibrin layer formed by polymerization of fibrinogen on the surface of the medical device. Multiple layers of fibrin applied by this method could provide a fibrin layer of any desired thickness. Alternatively, the fibrin can first be clotted and then ground into a powder which is mixed with water and stamped into a desired shape in a heated mold (U.S. Patent No. 3,523,807). Increased stability can also be achieved in the shaped fibrin by contacting the fibrin with a fixing agent such as glutaraldehyde or formaldehyde. These and other methods known by those skilled in the art for making and forming fibrin may be used in the present invention.

[00123] If a synthetic graft is coated with collagen, the methods for preparing collagen and forming it on synthetic graft devices are well known as set forth in U.S. Patent No. 5,851,230 to Weadock et al. This patent describes methods for coating a synthetic graft with collagen. Methods for adhering collagen to a porous graft substrate typically include applying a collagen dispersion to the substrate, allowing it to dry and repeating the process. Collagen dispersions are typically made by blending insoluble collagen (approximately 1-2% by weight) in a dispersion at acidic pH (a pH in a range

of 2 to 4). The dispersion is typically injected via syringe into the lumen of a graft and massaged manually to cover the entire inner surface area with the collagen slurry. Excess collagen slurry is removed through one of the open ends of the graft. Coating and drying steps are repeated several times to provide sufficient treatment.

[00124] In yet another embodiment, the stent or synthetic graft is coated with amorphous carbon. In U.S. Patent No. 5,198,263, a method for producing a high-rate, low-temperature deposition of amorphous carbon films in the presence of a fluorinated or other halide gas is described. Deposition according to the methods of this invention can be performed at less than 100°C, including ambient room temperature, with a radio-frequency, plasma-assisted, chemical-vapor deposition process. The amorphous carbon film produced using the methods of this invention adheres well to many types of substrates, including for example glasses, metals, semiconductors, and plastics.

[00125] Attachment of a fullerene moiety to reactive amino group sites of an amine-containing polymer to form the fullerene-graft, amine-containing polymers may be performed as described in U.S. Patent No. 5,292,813. Chemical modification in this manner allows for direct incorporation of the fullerenes into the stent. In another embodiment, the fullerenes may be deposited on the surface of the stent or synthetic grafts as described above. (see, WO 99/32184 to Leone et al.). Fullerenes, for example, C_{60} may also be attached through an epoxide bond to the surface of stainless steel (Yamago et al., Chemical Derivatization of Organofullerenes through Oxidation, Reduction and C-O and C-C Bond Forming Reactions. J. Org. Chem., 58 4796-4798 (1998)). The attachment is through a covalent linkage to the oxygen. This compound and the protocols for coupling are commercially available from BuckyUSA. (BuckyUSA, Houston, Texas).

[00126] (E) Addition of ligands such as antibodies, peptides and/or growth factor to the Matrix - Antibodies that promote adherence of progenitor

endothelial cells, and growth factors for promoting cell growth and differentiation are incorporated into the matrix, either covalently or noncovalently. The ligands of the coating such as antibodies, antibody fragments, hormones, peptides, growth factor and/or the like can be incorporated into the matrix layer by mixing the ligand with the matrix coating solution and then applied the solution to the surface of the device. In certain embodiments, antibodies, fragments or combinations thereof, and/or growth factors are attached to the surface of the outermost layer of matrix that is applied on the luminal surface of the device, so that the ligand such as antibodies are projecting on the surface that is in contact with the circulating blood and maintain their binding affinity for the target cells. In these embodiments, the ligand such as antibodies are applied to the surface of the matrix using standard techniques.

[00127] In one embodiment, the antibodies are added to a solution containing the matrix. For example, Fab fragments on anti-CD34 monoclonal antibody are incubated with a solution containing human fibrinogen at a concentration of between 500 and 800 mg/dl. It will be appreciated that the concentration of anti-CD34 Fab fragment will vary and that one of ordinary skill in the art could determine the optimal concentration without undue experimentation. The stent is added to the Fab/fibrin mixture and the fibrin activated by addition of concentrated thrombin (at a concentration of at least 1000U/ml). The resulting polymerized fibrin mixture containing the Fab fragments incorporated directly into the matrix is pressed into a thin film (less than 100 μm) on the surface of the stent or synthetic graft. Virtually any type of antibody or antibody fragment can be incorporated in this manner into a matrix solution prior to coating of a stent or synthetic graft.

[00128] For example, in another embodiment, whole antibodies with or without antibody fragments and growth factors are covalently coupled to the matrix. In one embodiment, the antibodies and growth factor(s) are tethered covalently the matrix through the use of hetero- or homobifunctional linker molecules. As used herein the term "tethered" refers to a covalent coupling of the antibody to the matrix by a linker molecule. The use of linker molecules in connection with the present invention typically involves covalently coupling the

linker molecules to the matrix after it is adhered to the stent. After covalent coupling to the matrix, the linker molecules provide the matrix with a number of functionally active groups that can be used to covalently couple one or more types of antibody. In an example of this embodiment, FIG. 1A provides an illustration of coupling via a cross-linking molecule. An endothelial cell, 1.01, binds to an antibody, 1.03, by a cell surface antigen, 1.02. The antibody is tethered to the matrix, 1.05-1.06, by a cross-linking molecule, 1.04. The matrix, 1.05-1.06, adheres to the stent, 1.07. The linker molecules may be coupled to the matrix directly (i.e., through the carboxyl groups), or through well-known coupling chemistries, such as, esterification, amidation, and acylation. The linker molecule may be a di- or tri-amine functional compound that is coupled to the matrix through the direct formation of amide bonds, and provides amine-functional groups that are available for reaction with the antibodies. For example, the linker molecule could be a polyamine functional polymer such as polyethyleneimine (PEI), polyallylamine (PALLA) or polyethyleneglycol (PEG). A variety of PEG derivatives, e.g., mPEG-succinimidyl propionate or mPEG-N-hydroxysuccinimide, together with protocols for covalent coupling, are commercially available from Shearwater Corporation, Birmingham, Alabama. (See also, Weiner et al., Influence of a poly-ethyleneglycol spacer on antigen capture by immobilized antibodies. J. Biochem. Biophys. Methods 45:211-219 (2000)). It will be appreciated that the selection of the particular coupling agent may depend on the type of antibody used and that such selection may be made without undue experimentation. Mixtures of these polymers can also be used. These molecules contain a plurality of pendant amine-functional groups that can be used to surface-immobilize one or more antibodies, peptides, proteins, hormones and other coating components.

[00129] In one embodiment, antibodies may be attached to C_{60} fullerene layers that have been deposited directly on the surface of the stent. Cross linking agents may be covalently attached to the fullerenes. The antibodies are then attached to the cross-linking agent, which in turn is attached to the stent. FIG. 1B provides an illustration of coupling by fullerene C_{60}. The endothelial cell, 2.01, is bound via a cell surface antigen, 2.02, to an antibody,

2.03, which in turn is bound, covalently or non-covalently to the matrix, 2.04. The matrix, 2.04, is coupled covalently via C_{60}, 2.05, to the stent, 2.06.

[00130] Small molecules of the invention can comprise synthetic or naturally occurring molecules or peptides which can be used in place of antibodies, antibody fragments, growth factors and the like. For example, lectin is a sugar-binding peptide of non-immune origin which occurs naturally. The endothelial cell specific Lectin antigen (Ulex Europaeus Uea 1) (Schatz et al. 2000 Human Endometrial Endothelial Cells: Isolation, Characterization, and Inflammatory-Mediated Expression of Tissue Factor and Type 1 Plasminogen Activator Inhibitor. Biol Reprod 62: 691-697), for example, can selectively bind the cell surface of progenitor endothelial cells.

[00131] Synthetic "small molecules" have been created to target various cell surface , proteins, glucoproteins, polysaccharides and receptors. These molecules selectively bind a specific surface moieties and can target specific cell types such as progenitor endothelial cells. Small molecules can be synthesized to recognize endothelial cell surface markers such as VEGF. SU11248 (Sugen Inc.) (Mendel et al. 2003 In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. Clin Cancer Res. Jan;9(1):327-37), PTK787/ZK222584 (Drevs J. et al. 2003 Receptor tyrosine kinases: the main targets for new anticancer therapy. Curr Drug Targets. Feb;4(2):113-21) and SU6668 (Laird, AD et al. 2002 SU6668 inhibits Flk-1/KDR and PDGFRbeta in vivo, resulting in rapid apoptosis of tumor vasculature and tumor regression in mice. FASEB J. May;16(7):681-90) are small molecules which bind to VEGFR-2.

[00132] Another subset of synthetic small molecules which target the endothelial cell surface are the alpha(v)beta(3) integrin inhibitors. SM256 and SD983 (Kerr JS. et al. 1999 Novel small molecule alpha v integrin antagonists: comparative anti-cancer efficacy with known angiogenesis inhibitors. Anticancer Res Mar-Apr;19(2A):959-68) are both synthetic molecules which target and bind to alpha(v)beta(3) present on the surface of endothelial cells.

[00133] The present invention provides a drug delivery system comprising: coated medical devices such as stents, stent grafts, heart valves, catheters, vascular prosthetic filters, artificial heart, external and internal left ventricular assist devices (LVADs), and synthetic vascular grafts, for the treatment of diseases, including tumor and vascular diseases, such as restenosis, artherosclerosis, thrombosis, blood vessel obstruction, and the like. In one embodiment, the coating on the present medical device comprises a biocompatible matrix, at least one antibody, antibody fragments or combinations thereof, and/or at least one compound such as a ligand or a therapeutic agent such as estradiol, angiogenin, FGF and the like.

[00134] In one embodiment, transgenic cells incorporating at least one transgene that is introduced into the cells by viral or non-viral based genetic procedures. The transgene may code for at least one therapeutic drug and can be expressed continuously or upon induction by a stimulus. In one embodiment, the therapeutic drug can be a hormone, a peptide, a protein, and the like. The transgenic cells also present at least one antigen on its cell surface that can be recognized and bound by the antibody that is coated on the surface of the medical device.

[00135] As used herein "antibody" refers to antibody or antibody fragment, or a combination of antibody and fragments, which can be a monoclonal antibody, a polyclonal antibody, a chimeric antibody, or a humanized antibody. The antibody fragment of the invention comprises any fragment size, such as large and small molecules of, for example, the antibody which retain the characteristic to recognize and bind the target antigen as the antibody (FIGs. 1A, 1B, and 11).

[00136] As used herein "ligand" refers to a molecule that binds another molecule such as a receptor on the mammalian cell. For example, a ligand can be an antibody, antibody fragment (FIGs. 1A, 1B, 11, and 17), cell adhesion molecule, or basement membrane component which recognizes and binds a specific epitope or structure on the membrane of the target cell. In the embodiment which uses genetically altered mammalian cells, the ligand to be used on the coating of the medical device can be specifically selected to

recognize and bind to a gene product produced by the exogenous DNA introduced into the transgenic cells.

[00137] As used herein "protein" refers to a polymer of amino acids of any length. The polymer may be linear or branched, may comprise modified amino acids, and may be interrupted by non-amino acids. The polymer may be naturally occurring peptides, proteins, or modified and synthetic forms thereof including biologically active fragments, derivatives, analogues, mimetics, and non-functional or dominant negative mutants.

[00138] The medical device can be any device used for implanting into an organ or body part comprising a lumen, and can be, but is not limited to, a stent, a stent graft, a synthetic vascular graft, a heart valve, a catheter, a vascular prosthetic filter, a pacemaker, a pacemaker lead, a defibrilator, a patent foramen ovale (PFO) septal closure device, a vascular clip, a vascular aneurysm occluder, a hemodialysis graft, a hemodialysis catheter, an atrioventricular shunt, an aortic aneurysm graft device or components, a venous valve, a suture, a vascular anastomosis clip, an indwelling venous or arterial catheter, a vascular sheath and a drug delivery port. The medical device can be made of numerous materials depending on the device. For example, a stent of the invention can be made of stainless steel, Nitinol (NiTi), or chromium alloy. Synthetic vascular grafts can be made of a cross-linked PVA hydrogel, polytetrafluoroethylene (PTFE), expanded polytetrafluoroethylene (ePTFE), porous high density polyethylene (HDPE), polyurethane, and polyethylene terephthalate.

[00139] The biocompatible matrix forming the coating of the present device comprises a synthetic material such as polyurethanes, segmented polyurethane-urea/heparin, poly- L-lactic acid, cellulose ester, polyethylene glycol, polyvinyl acetate, dextran and gelatin, a naturally-occurring material such as basement membrane components such as collagen, elastin, tropoelastin, laminin, fibronectin, vitronectin; heparin, fibrin, cellulose, and amorphous carbon, or fullerenes and the like.

[00140] In one embodiment, the medical device comprises a biocompatible matrix comprising fullerenes. In this embodiment, the fullerene can range from

about C_{20} to about C_{150} in the number of carbon atoms, and more particularly, the fullerene is C_{60} or C_{70}. The fullerene of the invention can also be arranged as nanotubes on the surface of the medical device.

[00141] The antibody for providing to the coating of the medical device comprises at least one antibody that recognizes and binds a transgenic cell surface antigen which can be expressed by an endogenous gene or by a transgene and modulates the adherence of the cells onto the surface of the medical device. The antibody can be covalently or noncovalently attached to the surface of the matrix, or tethered covalently by a linker molecule to the outermost layer of the matrix coating the medical device. In this aspect of the invention, for example, the monoclonal antibodies can further comprises Fab or F (ab') 2 fragments.

[00142] The antibody can recognize and bind antigens with specificity for the mammal being treated and their specificity is not dependent on cell lineage. In one embodiment, the antibody is specific for a human progenitor endothelial cell surface antigen such as CD133, CD14, CD34, CDw90, CD117, HLA-DR, VEGFR-1, VEGFR-2, Muc-18 (CD146), CD130, stem cell antigen (Sca-1), stem cell factor 1(SCF/c-Kit ligand), Tie-2, HAD-DR and others, such as anti-H-$2K^k$ antibody.

[00143] In another embodiment, the coating of the medical device comprises at least one layer of a biocompatible matrix as described above, the matrix comprising an outer surface for attaching a therapeutically effective amount of at least one type of small molecule of natural or synthetic origin. The small molecule recognizes and interacts with an antigen on a transgenic cell surface to immobilize the transgenic cell on the surface of the device and to induce transgene expression. The small molecules can be derived from a variety of sources such as cellular components such as fatty acids, proteins, nucleic acids, saccharides and the like and can interact with a receptor on the surface of a transgenic cell. In this embodiment of the invention, the coating on the medical device can further comprise a compound such as a ligand in conjunction with the coating comprising an antibody.

[00144] Both viral and non-viral based genetic procedures can be used to introduce transgenes for generating transgenic cells. Transgenic cells of the invention express and secrete therapeutic drugs coded by transgenes that are either transiently or stably incorporated. Additional transgenes can be incorporated to confer survival, selection and/or growth advantage. Various cells such as endothelial cells or leukocytes including neutrophil, eosinophil, basophil, monocyte and lymphocytes or somatic cells, or a combination of these cells can be modified to produce transgenic cells, which may be either non-repopulating or repopulating. Transgenic cells can be cultured in vitro, collected, and stored. Transgenic cells producing a variety of therapeutic drugs can be generated by incorporating different transgenes to serve different therapeutic purposes. Transgenic cells can be administered as a single or mixed populations via systemic or local routes. Various amounts of transgenic cells can be administered to release different amount of therapeutic drugs upon individual conditions. In one embodiment, transgenic cells can repopulate progenitor endothelial cells. In a further embodiment, transgenic progenitor endothelial cells can be administered locally with catheter based delivery or dual balloon inflation method.

[00145] In one embodiment, transgenic cells further comprise an additional transgene that expresses an exogenous cell surface antigen, which can be specifically recognized and bound by the antibody that is coated in the matrix of the medical device. Transgene expression and product secretion can be continuous or contingent upon the activation of an inducible promoter via exogenous excitation.

[00146] The therapeutic compounds coded by the transgenes of the invention can be any molecule with a desired physiological effect, and can be, but is not limited to, proteins as defined including growth factors, chemokines and cytokines, ligands and receptors, and other functional proteins and non-protein excretable compounds. In one embodiment, a therapeutic compound is a protein selected from the group consisting of endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor (VEGF), an anti-inflammatory factor, and an inflammation-modulating factor.

[00147] A drug, for example, a compound that can stimulate transgene expression and target product secretion in the embodiment using genetically altered mammalian cells can be a ligand or another component of the coating of the medical device that binds a transgenic cell surface antigen and triggers downstream signaling pathway activation of the extrachromosomal nucleic acid, for example, the DNA construct introduced into the target cells. In another embodiment, transgene expression of the genetically-altered mammalian cells can be stimulated by, for example, a ligand or drug that can be taken up by the transgenic cell and stimulate gene expression through an inducible promoter. In one embodiment, the ligand or drug is administered systemically. In another embodiment, the ligand or drug is coated in the matrix of the implanted device and administered locally.

[00148] The invention provides methods for treating a variety of diseases, which can be, but not limited to, tumors, vascular diseases, and healing response. The methods provide improvement over prior art in terms of target site delivery of a variety of drugs of desired amount upon demand.

[00149] The invention provides a method for treating tumors and their metastases. In this embodiment, the transgene can code for (1) an antiangiogenic factor, such as interferons (IFNs), thrombospondin (TSP), angiostatin, endostatin, oncostatin M (OSM), and Rho, which inhibits neovascularization that is a prerequisite for tumor progressive growth; or (2) an tumor suppressive protein, such as p53, Rb, E1, BRCA1, antibody or dominant negative mutant of a cell growth activator such as a growth factor, a cyclin dependent kinase (CDK) or a cyclin, E2F, NFκB; or a combination of these genes. In one embodiment, the transgene may comprise a gene encoding, for example, prostacyclin and/or a cyclooxygenase, α-CGRP, a matrix metalloprotein, and/or endothelial nitric oxide synthase.

[00150] As used herein the phrase "anti-angiogenic factor" refers to a molecule that is capable of inhibiting angiogenesis, or blood vessel growth.

[00151] The invention also provides methods for treating vascular disease. In one embodiment, there is provided a method to treat ischemic conditions, in which the transgene codes for an angiogenic factor such as pleiotrophin,

angiogenin, angiopoietin, an integrin stimulating factor, and/or an antibody or dominant negative mutant of an anti-angiogenic factor.

[00152] As used herein the phrase "angiogenic factor" refers to a molecule that is capable of stimulating angiogenesis, or blood vessel growth.

[00153] In another embodiment, the invention is used to treat atherosclerosis, restenosis, thrombosis, aneurysm or blood vessel obstruction. In this embodiment of the invention, transgene can code for (a) eNOS or VEGF that promotes re-endothelialization; or (b) an anti-inflammatory or inflammation-modulating factor such as IFN-β, IFN-α, TGF-β, or interleukin-10 (IL-10); or (c) an inhibitor of smooth muscle cell growth, migration, or differentiation that inhibits intimal hyperplasia; or a combination of these genes.

[00154] The invention also provides an engineered method for inducing a healing response. In one embodiment, a method is provided for rapidly inducing the formation of a confluent layer of endothelium in the luminal surface of an implanted device in a target lesion of an implanted vessel, in which transgenic cells are progenitor endothelial cells that express eNOS, VEGF, or an anti-inflammatory or inflammation-modulating factor. In this embodiment, a medical device is provided of increased biocompatibility over prior art devices, and decreases or inhibits tissue-based excessive intimal hyperplasia and restenosis by decreasing or inhibiting smooth muscle cell migration, smooth muscle cell differentiation, and collagen deposition along the inner luminal surface at the site of implantation of the medical device.

[00155] In one embodiment, a method for coating a medical device comprises the steps of: applying at least one layer of a biocompatible matrix to the surface of the medical device, wherein the biocompatible matrix can comprise at least one component selected from the group consisting of a polyurethane, a segmented polyurethane-urea/heparin, a poly-L-lactic acid, a cellulose ester, a polyethylene glycol, a polyvinyl acetate, a polysaccharide such as dextran, gelatin, collagen, elastin, tropoelastin, laminin, fibronectin, vitronectin, heparin, fibrin, cellulose and carbon and fullerene, and applying to

the biocompatible matrix, simultaneously or sequentially, at least one antibody, and optionally one compound which induces transgene expression.

[00156] The invention further provides a method for treating diseases such as tumor, vascular disease, and wound healing in a mammal. The method comprises implanting a medical device into a vessel or tubular organ of the mammal, wherein the medical device is coated with (a) a biocompatible matrix; (b) at least one antibody; and optionally (c) one compound, introducing transgenic cells into the mammal that is need of the treatment, and optionally administering a compound, wherein the antibody coated in the matrix of the medical device recognizes and binds an antigen expressed on the transgenic cell surface, so that the transgenic cells are immobilized on the surface of the matrix, and at least one therapeutic drug coded by a transgene is expressed by the immobilized cells upon excitation of the cells by a compound such as a drug and the therapeutic gene product is secreted at a designated site.

[00157] The invention further provides a method for treating vascular disease in a mammal comprises implanting a medical device into a vessel or tubular organ of the mammal, wherein the medical device is coated with (a) a biocompatible matrix, (b) at least one antibody, and optionally (c) one compound, and introducing transgenic cells into the mammal that is in need of the treatment, and optionally administering a compound, wherein the antibody coated in the matrix of the medical device recognizes and binds an antigen expressed only on the transgenic cell membrane surface so that the transgenic cells are immobilized on the surface of the matrix coating the medical device. The transgenic (genetically-altered) cells can also contain genetic material which encodes at least one therapeutic gene product which can be expressed constitutively or upon activation by a signal such as a compound including hormones and peptides.

[00158] The present transgenic cells can contain at least one expressible transgene that can code for, but not limited to (1) growth factors including family members such as platelet derived growth factor (PDGF), transforming growth factor (TGF), epidermal growth factor (EGF), fibroblast growth factor (FGF), insulin like growth factors (IGF), vascular endothelial growth factor (VEGF), heparin binding growth factors, hepatoma-derived growth factor

(HDGF), hepatocyte growth factor/scatter factor (HGF), placental growth factor (PlGF), platelet derived endothelial cell growth factor (PD-ECGF), stem cell factor (SCF), and their other protein forms; (2) Chemokines such as CXC families, CC families, C families, and their other protein forms; (3) cytokines such as a disintegrin and metalloprotease (ADAM), annexin V, B7 & CD28/CTLA-4 receptor families, bone morphogenetic protein (BMP), caspase, CD44, CD44H, endothelin-1 (ET-1), eph, erythropoietin (Epo), intercellular adhesion molecule-3/CD50 (ICAM-3), macrophage stimulating protein (MSP), matrix metalloproteinase (MMP), neurotrophic factors, endothelial nitric oxide synthase (eNOS), NKG2D, platelet endothelial cell adhesion molecule-1 (PECAM-1/CD31), pleiotrophin/midkine (PTN/MK), transferrin receptor (sTfR), hedgehog peptide, STAT, stem cell marker, Th1/Th2, thrombopoietin (Tpo), tumor necrosis factor family, VCAM-1/CD16, monoclonal non-specific suppressor factor beta (MNSFbeta), 6Ckine (SLC), B-lymphocyte chemoattractant (BCA-1/BLC), leukemia inhibitory factor, monocyte-derived neutrophil-activating peptide (GRO), and their other protein forms; (4) other functional proteins invovled in the regulation of signal transduction, cell cycle regulation, cell division, and/or cell differentiation, such as ligands, receptors, phosphorylases, kinases, transcriptional factors, and their other protein forms.

[00159] In one embodiment, antiangiogenic factors for use in the invention are, for example, interferons (IFNs), thrombospondin (TSP), angiostatin, and endostatin, oncostatin M (OSM), blockers of integrin engagement, metalloproteinases inhibitors, inhibitors of endothelial cell phosphorylation, dominant negative receptors for angiogenesis inducers, antibodies of angiogenesis inducers, other proteins acting by other means, and their other protein forms. Other angiogenic factors include angiogenin, angiopoietins, integrin stimulating factors such as Del-1, and their other protein forms.

[00160] Additional growth factors for use in the invention are, for example, pleiotrophin, midkines, VEGF family including VEGF-2, VEGF-C, and VEGF-D, FGF family, including FGF-1, FGF-2, FGF-5, and FGF-18, hepatoma-derived growth factor (HDGF), hepatocyte growth factor/scatter factor (HGF), members of the epidermal growth factor (EGF) family, including transforming

growth factor alpha, EGF, and TGF-alpha-HIII, and platelet derived growth factor (PDGF), including AA, AB, and BB isoforms.

EXPERIMENTAL EXAMPLES

[00161] This invention is illustrated in the experimental details section which follows. These sections set forth below the understanding of the invention, but are not intended to, and should not be construed to limit in any way the invention as set forth in the claims which follow thereafter.

EXAMPLE 1

Endothelial Progenitor Cell Phenotyping

[00162] Endothelial Progenitor Cells (EPC) were isolated either by CD34+ Magnetic Bead Isolation (Dynal Biotech) or enriched medium isolation as described recently (Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964-7). Briefly, peripheral venous blood was taken from healthy male volunteers and the mononuclear cell fraction was isolated by density gradient centrifugation, and the cells were plated on human fibronectin coated culture slides (Becton Dickinson) in EC basal medium-2 (EBM-2) (Clonetics) supplemented with 5% fetal bovine serum, human VEGF-A, human fibroblast growth factor-2, human epidermal growth factor, insulin-like growth factor-1, and ascorbic acid. EPCs were grown up to seven days with culture media changes every 48 hours. The results of these experiments are shown in FIGs. 2A and 2B. FIGs. 2A and 2B show that the anti-CD34 isolated cells appear more spindle-like, which indicates that the cells are differentiating into endothelial cells.

[00163] EC phenotype was determined by immunohistochemistry. Briefly, EPC were fixed in 2% Paraformaldehyde (PFA) (Sigma) in Phosphate buffered saline (PBS) (Sigma) for 10 minutes, washed 3X with PBS and stained with various EC specific markers; rabbit anti-human VEGFR-2 (Alpha Diagnostics Intl. Inc.), mouse anti-human Tie-2 (Clone Ab33, Upstate Biotechnology), mouse anti-human CD34 (Becton Dickinson), EC-Lectin (Ulex Europaeus Uea 1) (Sigma) and mouse anti-human Factor 8 (Sigma). The presence of antibody was confirmed by exposure of the cells to a fluorescein

isothiocyanate-conjugated (FITC) secondary antibody. Propidium Iodine (PI) was used as a nuclear marker. The results of these experiments are shown in FIGs. 2C-2G. FIG. 2C shows that VEGFR-2 is expressed after 24 hours in culture, confirming that the cells are endothelial cells. FIGs. 2D and 2F show the nuclear staining of the bound cells after 7 days of incubation and FIGs. 2E and 2G the same field of cells stained with an FITC conjugated anti-Tie-2 antibody.

[00164] EPCs ability to express endothelial nitric oxide synthase (eNOS), a hallmark of EC function, was determined by Reverse Transcriptase-Polymerase Chain Reaction (rt-PCR) for eNOS mRNA. EPCs were grown up to seven days in EBM-2 medium after which total RNA was isolated using the GenElute™ Mammalian total RNA kit (Sigma) and quantified by absorbance at 260 nm. Total RNA was reverse-transcribed in 20 µL volumes using Omniscript™ RT kit (Qiagen) with 1 µg of random primers. For each RT product, aliquots (2-10 µL) of the final reaction volume were amplified in two parallel PCR reactions using eNOS (299 bp product, sense 5'-TTCCGGGGATTCTGGCAGGAG-3', SEQ ID NO: 1, antisense 5'-GCCATGGTAACATCGCCGCAG-3', SEQ ID NO: 2) or GAPDH (343 bp product, sense 5'-CTCTAAGGCTGTGGGCAAGGTCAT-3', SEQ ID NO: 3, antisense 5'-GAGATCCACCACCCTGTTGCTGTA-3', SEQ ID NO: 4) specific primers and Taq polymerase (Pharmacia Biotech Amersham). PCR cycles were as follows: 94°C for 5 minutes, 65°C for 45 seconds, 72°C for 30 seconds (35 cycles for eNOS and 25 cycles for GAPDH). rt-PCR products were analyzed by 2% agarose gel electrophoresis, visualized using ethidium bromide and quantified by densitometry. The results of this experiment are shown in FIGs. 3A and 3B. As seen in FIGs. 3A and 3B, nitric oxide synthetase (eNOS) is expressed after the cells have been incubated in medium for 3 days in culture in the presence or absence of oxygen. eNOS mRNA expression continues to be present after 7-days in culture. The presence of eNOS mRNA indicates that the cells have differentiated into mature endothelial cells by day 3 and have begun to function like fully differentiated endothelial cells.

EXAMPLE 2

[00165] Endothelial Cell Capture by anti-CD34 coated Stainless Steel Disks: Human Umbilical Vein Endothelial Cells (HUVEC) (American Type Culture Collection) are grown in endothelial cell growth medium for the duration of the experiments. Cells are incubated with CMDX and gelatin coated samples with or without bound antibody on their surface or bare stainless steel (SST) samples. After incubation, the growth medium is removed and the samples are washed twice in PBS. Cells are fixed in 2% paraformaldehyde (PFA) for 10 minutes and washed three times, 10 minutes each wash, in PBS, to ensure all the fixing agent is removed. Each sample is incubated with blocking solution for 30 minutes at room temperature, to block all non-specific binding. The samples are washed once with PBS and the exposed to 1:100 dilution of VEGFR-2 antibody and incubated overnight. The samples are subsequently washed three times with PBS to ensure all primary antibody has been removed. FITC-conjugated secondary antibody in blocking solution is added to each respective sample at a dilution of 1:100 and incubated for 45 minutes at room temperature on a Belly Dancer™ apparatus. After incubation, the samples are washed three times in PBS, once with PBS containing 0.1% Tween™ 20, and then again in PBS. The samples are mounted with Propidium Iodine (PI) and visualized under confocal microscopy.

[00166] FIGs. 4A-4E are photomicrographs of SST samples coated as described above with CMDX and anti-CD34 antibody (FIG. 4A), gelatin and anti-CD34 antibody coated (FIG. 4B), bare SST (FIG. 4C), CMDX coated and no antibody (FIG, 4D) and gelatin-coated and no antibody (FIG. 4E). The figures show that only the antibody coated samples contain numerous cells attached to the surface of the sample as shown by PI staining. The bare SST control disk shows few cells attached to its surface.

[00167] FIGs. 5A-5C are photomicrographs of control samples CMDX-coated without antibody bound to its surface. FIG. 5A shows very few cells as seen by PI staining adhered to the surface of the sample. FIG. 5B shows that the adherent cells are VEGFR-2 positive indicating that they are endothelial cells and FIG. 5C shows a combination of the stained nuclei and the VEGFR-

2 positive fluorescence. FIGs. 5D-F are photomicrographs of control samples coated with gelatin without antibody on its surface. FIG. 5D shows no cells are present since PI staining is not present in the sample and there is no fluorescence emitted by the samples (see FIGs. 5E and 5F).

[00168] FIGs. 6A-6C are photomicrographs of CMDX coated SST samples having anti-CD34 antibody bound on its surface. The figures show that the samples contain numerous adherent cells which have established a near confluent monolayer (FIG. 6A) and which are VEGFR-2 positive (FIGs. 6B and 6C) as shown by the fluorescence. Similarly, FIGs. 6D-6F are photomicrographs of a gelatin-coated sample with anti-CD34 antibody bound to its surface. These figures also show that HUVECs attached to the surface of the sample as shown by the numerous stained nuclei and fluorescence from the VEGFR-2/FITC antibody (FIGs. 6E and 6F).

EXAMPLE 3

[00169] VEGFR-2 and Tie-2 Staining of Progenitor Endothelial Cells: Progenitor cell are isolated from human blood as described in the in Example 1 and incubated in growth medium for 24 hours, 7 days, and 3 weeks *in vitro*. After incubation, the growth medium is removed and the samples are washed twice in PBS. Cells are fixed in 2% paraformaldehyde (PFA) for 10 minutes and washed three times, 10 minutes each wash, in PBS, to ensure all the fixing agent is removed. Each sample is incubated with 440 µl of Goat (for VEGFR-2) or Horse (for Tie-2) blocking solution for 30 minutes at room temperature, to block all non-specific binding. The samples are washed once with PBS and the VEGFR-2 or Tie-2 antibody was added at a dilution of 1:100 in blocking solution and the samples are incubated overnight. The samples are then washed three times with PBS to ensure all primary antibody has been washed away. FITC-conjugated secondary antibody (200 µl) in horse or goat blocking solution is added to each respective sample at a dilution of 1:100 and incubated for 45 minutes at room temperature on a Belly Dancer apparatus. After incubation, the samples are washed three times in PBS, once with PBS containing 0.1% Tween 20, and then again in PBS. The

samples are mounted with Propidium Iodine (PI) and visualized under confocal microscopy.

[00170] FIG.7 is a photomicrograph of a CMDX-coated sample containing CD34 antibody on its surface which was incubated with the cells for 24 hours, and shows that progenitor cells were captured on the surface of the sample and as demonstrated by the stained nuclei present on the surface of the sample. The figure also shows that about 75% of the cells are VEGFR-2 positive (indicated by white arrows) with a round morphology.

[00171] FIGs. 8A and 8B are from a sample which was incubated with the cells for 7 days. As seen in FIG. 8A, there are cells present on the sample as shown by the stained nuclei, which are VEGFR-2 positive (FIG. 8B, 100%) and are more endothelial in structure as shown by the spindle shape of the cells. FIGs. 9A and 9B are photomicrographs of CMDX-coated sample containing CD34 antibody on its surface, which was incubated for 7 days with the cells and after incubation, the sample was exposed to Tie-2 antibody. As seen in FIGs. 9A, there are numerous cells attached to the surface of the samples as shown by the stained nuclei. The cells adhered to the sample are also Tie-2 positive (100%) as seen by the fluorescence emitted from the cells (FIG. 9B). In summary, after 7 days of incubation of the cells with the samples, the CD34 antibody-coated samples are able to capture endothelial cells on their surface as seen by the numerous cells attached to the surface of the samples and the presence of VEGFR-2 and Tie-2 receptors on the surface of the adhered cells. In addition, the presence of 100% endothelial cells on the surface of the samples at 7 days indicates that the non-endothelial cells may have detached or that all adherent cells have begun to express endothelial cell markers by day 7.

[00172] FIGs. 10A-10C are phase contrast photomicrographs of the progenitor endothelial cells grown for 3 weeks in endothelial cell growth medium. FIG. 10A demonstrates the cells have differentiated into matured endothelial cells as shown by the two-dimensional tube-like structures (arrow) reminiscent of a lumen of a blood vessel at the arrow. FIG. 10B shows that there is a three-dimensional build-up of cells in multiple layers; i.e.; one on top of the other, which confirms reports that endothelial cells grown for prolonged

periods of time begin to form layers one on top of the other. FIG. 10C shows progenitor cells growing in culture 3 weeks after plating which have the appearance of endothelial cells, and the figure confirms that the cells are endothelial cells as demonstrated by the fluorescence of the CD34/FITC antibodies present on their surface.

[00173] The above data demonstrate that white blood cells isolated from human blood have CD34 positive progenitor cells and that these cells can develop into mature endothelial cells and readily express endothelial cell surface antigens. (VEGFR-2 and Tie-2) The data also show that antibodies against progenitor or stem cell surface antigens can be used to capture these cells on the surface of a coated medical device of the invention.

EXAMPLE 4

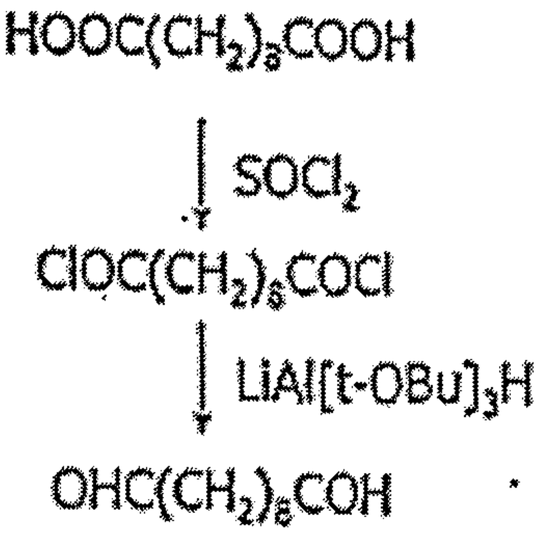
Fullerene Coated and Fullerene Coated with anti-CD34 Antibody and/or an Endothelial Cell Growth Factor (Ang-2, VEGF) Stainless Steel

[00174] Stainless steel stents and disks are derivatized with a functional fullerene layer for attaching antibodies and/or growth factors (i.e., VEGF or Ang-2) using the following procedure:

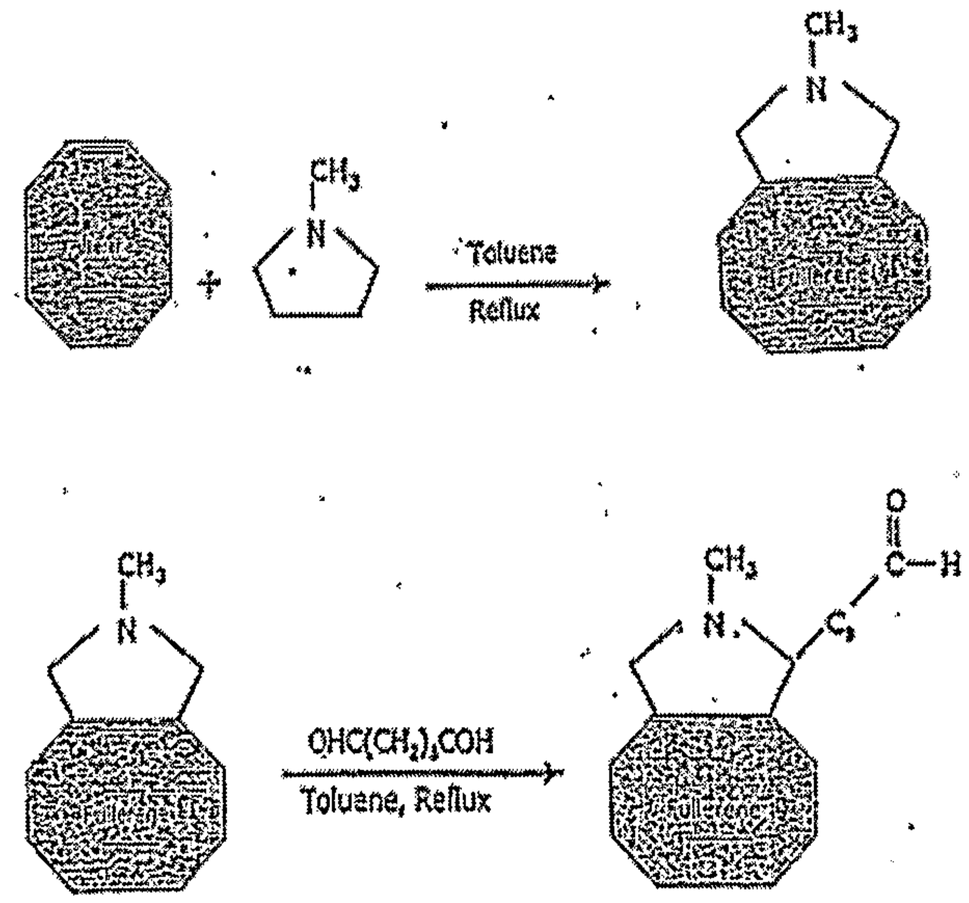
[00175] In the first step, the surface of the SST stent or disk is activated with 0.5M HCL which also cleans the surface of any passivating contaminants. The metal samples are removed from the activation bath, rinsed with distilled water, dried with methanol and oven-dried at 75 °C. The stents are then immersed in the toluene derivative solution with fullerene oxide (C_{60}-O), for a period of up to 24 hours. The fullerene oxide binds to the stent via Fe-O, Cr-O and Ni-O found on the stent. The stents are removed from the derivatizing bath, rinsed with toluene, and placed in a Soxhlet Extractor for 16 hours with fresh toluene to remove any physisorbed C_{60}. The stents are removed and oven-dried at 105 °C overnight. This reaction yields a fully derivatized stent or disk with a monolayer of fullerenes.

[00176] In step 2 a di-aldehyde molecule is formed in solution by reacting sebacic acid with thionyl chloride or sulfur oxychloride ($SOCl_2$) to form

Sebacoyl chloride. The resultant Sebacoyl chloride is reacted with LiAl[t-OButyl]$_3$ H and diglyme to yield 1,10-decanediol as shown below:



[00177] In step 3, an N-methyl pyrolidine derivate is formed on the surface of the stent or disk (from step 1). The fullerene molecule is further derivatized by reacting equimolar amounts of fullerene and N-methylglycine with the 1,10-decanediol product of the reaction of step 2, in refluxing toluene solution under nitrogen for 48 hours to yield N-methyl pyrolidine-derivatized fullerene-stainless steel stent or disk as depicted below.



[00178] The derivatized stainless steel stent or disk is washed to remove any chemical residue and used to bind the antibodies and/or (VEGF or Ang-2) using standard procedures. Progenitor cell are isolated from human blood as described in Example 1 and exposed to the anti-CD34 antibody coated fullerene disks. After incubation, the growth medium is removed and the

samples are washed twice in PBS. Cells are fixed in 2% paraformaldehyde (PFA) for 10 minutes and washed three times, 10 minutes each wash, in PBS, to ensure all the fixing agent is removed. Each sample is incubated with blocking solution for 30 minutes at room temperature, to block all non-specific binding. The samples are washed once with PBS and the exposed to 1:100 dilution of VEGFR-2 antibody and incubated overnight. The samples are subsequently washed three times with PBS to ensure all primary antibody has been removed. FITC-conjugated secondary antibody in blocking solution is added to each respective sample at a dilution of 1:100 and incubated for 45 minutes at room temperature on a Belly Dancer apparatus. After incubation, the samples are washed three times in PBS, once with PBS containing 0.1% Tween 20, and then again in PBS. The samples are mounted with Propidium Iodine (PI) and visualized under confocal microscopy. FIG. 11 shows a schematic representation of a functional fullerene coated stent surface of the invention binding a progenitor cell. FIGs. 12A-12B are, respectively, photomicrographs of fullerene-coated control sample without antibody stained with PI (12A) and anti-VEGFR-2/FITC-conjugated antibody stained. FIGs. 12C and 12D are photomicrographs of a sample coated with a fullerene/anti-CD34 antibody coating. As shown in the figures, the anti-CD34 antibody coated sample contains more cells attached to the surface which are VEGFR-2 positive.

[00179] Fullerene-coated samples with and without antibodies are implanted into Yorkshire pigs as described in Example 5. The stents are explanted for histology and the stented segments are flushed with 10% buffered Formalin for 30 seconds followed by fixation with 10% buffered Formalin until processed. Five sections are cut from each stent; 1mm proximal to the stent, 1mm from the proximal end of the stent, mid stent, 1mm from the distal edge of the stent and 1mm distal to the stent. Sections are stained with Hematoxylin & Eosin (HE) and Elastin Trichrome. FIGs. 13A – 13D are photomicrographs of cross-sections through coronary artery explants of stents which had been implanted for 4 weeks. The data show that the fullerene-coated (FIGs. 13B and 13D) stents inhibit excessive intimal hyperplasia at the stent site over the control (bare stent, FIGs. 13A and 13C).

EXAMPLE 5

[00180] PORCINE BALLOON INJURY STUDIES: Implantation of antibody-covered stents is performed in juvenile Yorkshire pigs weighing between 25 and 30 kg. Animal care complies with the "Guide for the Care and Use of Laboratory Animals" (NIH publication No. 80-23, revised 1985). After an overnight fast, animals are sedated with ketamine hydrochloride (20mg/kg). Following the induction of anesthesia with thiopental (12 mg/kg) the animals are intubated and connected to a ventilator that administers a mixture of oxygen and nitrous oxide (1:2 [vol/vol]). Anesthesia is maintained with 0.5-2.5 vol% isoflurane. Antibiotic prophylaxis is provided by an intramuscular injection of 1,000 mg of a mixture of procaine penicillin-G and benzathine penicillin-G (streptomycin).

[00181] Under sterile conditions, an arteriotomy of the left carotid artery is performed and a 8F-introducer sheath is placed in the left carotid artery. All animals are given 100 IU of heparin per kilogram of body weight. Additional 2,500 IU boluses of heparin are administered periodically throughout the procedure in order to maintain an activated clotting time above 300 seconds. A 6F guiding catheter is introduced through the carotid sheath and passed to the ostia of the coronary arteries. Angiography is performed after the administration of 200ug of intra coronary nitro glycerin and images analyzed using a quantitative coronary angiography system. A 3F-embolectomy catheter is inserted into the proximal portion of the coronary artery and passed distal to the segment selected for stent implantation and the endothelium is denuded. A coated R stent incorporating an anti-CD34 antibody is inserted through the guiding catheter and deployed in the denuded segment of the coronary artery. Bare stainless steel stents or stents coated with the matrix but without antibodies are used as controls. Stents are implanted into either the Left Anterior Descending (LAD) coronary artery or the Right Coronary Artery (RCA) or the Circumflex coronary artery (Cx) at a stent to artery ration of 1.1. The sizing and placement of the stents is evaluated angiographically and the introducer sheath was removed and the skin closed in two layers. Animals are placed on 300 mg of ASA for the duration of the experiment.

[00182] Animals are sacrificed at 1, 3, 7, 14, and 28 days after stent implantation. The animals are first sedated and anesthetized as described above. The stented coronary arteries are explanted with 1 cm of non-stented vessel proximal and distal to the stent. The stented arteries are processed in three ways, histology, immunohistochemistry or by Scanning Electron Microscopy.

[00183] For immunohistochemistry the dissected stents are gently flushed with 10% Formalin for 30seconds and the placed in a 10% Formalin/PBS solution until processing. Stents destined for immunohistochemistry are flushed with 2% Paraformaldehyde (PFA) in PBS for 30 seconds and then placed in a 2% PFA solution for 15min, washed and stored in PBS until immunohistochemistry with rabbit anti-human VEGFR-2 or mouse anti-human Tie-2 antibodies is performed.

[00184] Stents are prepared for SEM by flushing with 10% buffered Formalin for 30 seconds followed by fixation with 2% PFA with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer overnight. Samples are then washed 3X with cacodylate buffer and left to wash overnight. Post-fixation was completed with 1% osmium tetroxide (Sigma) in 0.1M cacodylate buffer which is followed by dehydration with ethanol (30% ethanol, 50%, 70%, 85%, 95%, 100%, 100%) and subsequent critical point drying with CO_2. After drying, samples are gold sputtered and visualized under SEM. (Reduction in thrombotic events with heparin-coated Palmaz-Schatz stents in normal porcine coronary arteries, Circulation 93:423-430).

[00185] For histology the stented segments are flushed with 10% buffered Formalin for 30seconds followed by fixation with 10% buffered Formalin until processed. Five sections are cut from each stent; 1mm proximal to the stent, 1mm from the proximal end of the stent, mid stent, 1mm from the distal edge of the stent and 1mm distal to the stent. Sections are stained with Hematoxylin & Eosin (HE) and Elastin Trichrome.

[00186] FIGs. 14A-14G show explants taken 1 (FIGs. 14A and 14B) and 48 hours (FIGs. 14C-14G) after implantation and observed under scanning

electron microscope. The photomicrographs clearly show that the dextran/anti-CD34 antibody-coated stents (14B, 14E-G) have capture progenitor endothelial cells as shown by the spindle-shaped appearance of the cells at higher magnification (400X) at 48 hours compared to the dextran-coated control (14A, 14C and 14D).

[00187] Cross-sections of the explants from the swine coronary arteries also showed that the dextran-anti-CD34 antibody-coated (14L, 14M) caused a pronounced inhibition of intimal hyperplasia (thickness of the arterial smooth muscle layer) compared to the controls (bare stainless steel 14H and 14I; dextran-coated 14J and 14K). Fullerene-coated stent implants also inhibit intimal hyperplasia better than bare, control stainless steel stents as shown in FIGs. 13B-13D.

[00188] FIGs. 15A and 15B show, respectively, confocal photomicrographs of 48 hours explants of a dextran-plasma coated stent without antibody on is surface, and a dextran-plasma coated anti-CD34 antibody-stent of 18 mm in length. The stents had been implanted into the coronary artery of juvenile male Yorkshire swine. The explants were immunohistochemically processed and stained for VEGFR-2, followed by FITC-conjugated secondary antibody treatment and studied under confocal microscopy. FIGs. 15B and 15C show that the antibody containing stent is covered with endothelial cells as demonstrated by the fluorescence of the section compared to the complete lack of endothelium on the stent without antibody (FIG. 15A).

EXAMPLE 6

[00189] Incorporation of an Endothelial Growth Factor into Immobilized Antibody Matrices Applied to Stents: The following describes the steps for immobilizing an antibody directed toward endothelial progenitor cell surface antigens to a biocompatible matrix applied to an intravascular stent to which an endothelial growth factor is then absorbed for the enhanced attachment of circulating endothelial progenitor cells and their maturation to functional endothelium when in contact with blood.

[00190] Matrix Deposition: Using methods known to those skilled in the art, stainless steel stents are treated with a plasma deposition technique to

introduce amine functionality on the stent surface. A layer of carboxy functional dextran (CMDX) is bound to the amine functional layer deposited on the stent through the activation of the CMDX carboxyl groups using standard procedures, known as water soluble carbodiimide coupling chemistry, under aqueous conditions to which the amine groups on the plasma deposited layer form an amide bond between the plasma layer and the functional CDMX.

[00191] Antibody Immobilization: Antibodies directed toward endothelial progenitor cell surface antigens, e.g., murine monoclonal anti-humanCD34, are covalently coupled to CDMX coated stents by incubation in aqueous water soluble carbodiimide chemistry in a buffered, acidic solution.

[00192] Absorption of Growth Factor: Subsequent to the immobilization of the monoclonal anti-humanCD34 to a CMDX matrix applied to a stent, the device is incubated in an aqueous solution of an endothelial growth factor, e.g. Angiopoietin-2, at an appropriate concentration such that the growth factor is absorbed into the CMDX matrix. The treated devices are rinsed in physiologic buffered saline solution and stored in a sodium azide preservative solution.

[00193] Using standard angiographic techniques, the above described devices when implanted in porcine coronary arteries and exposure to human blood produce an enhanced uptake and attachment of circulating endothelial progenitor cells on to the treated or coated stent surface and accelerate cellular maturation into functional endothelium. The rapid establishment of functional endothelium can decrease device thrombogenicity and modulate the extent of intimal hyperplasia.

EXAMPLE 7

[00194] Immobilization of an Endothelial Growth Factor and an Antibody on to Stents: The following describes the steps for immobilizing an antibody directed toward endothelial progenitor cells cell surface antigens and an endothelial growth factor to a biocompatible matrix applied to an intravascular stent for the enhanced attachment of circulating endothelial progenitor cells and their maturation to functional endothelium when in contact with blood.

[00195] Matrix Deposition: Matrix Deposition: Using methods known to those skilled in the art, stainless steel stents are treated with a plasma deposition technique to introduce amine functionality on the stent surface. A layer of carboxy functional dextran (CMDX) is bound to the amine functional layer deposited on the stent through the activation of the CMDX carboxyl groups using standard procedures, known as water soluble carbodiimide coupling chemistry, under aqueous conditions to which the amine groups on the plasma deposited layer form an amide bond between the plasma layer and the functional CDMX.

[00196] Antibody and Growth Factor Immobilization: Antibodies directed toward endothelial progenitor cell surface antigens, e.g. murine monoclonal anti-human CD34, and an endothelial growth factor, e.g. Angiopoietin-2, is covalently coupled with the CDMX coated stents by incubation at equimolar concentrations in a water soluble carbodiimide solution under acidic conditions. The treated devices are rinsed in physiologic buffered saline solution and stored in a sodium azide preservative solution.

[00197] Using standard angiographic techniques, the above described devices when implanted in porcine coronary arteries and exposed to human blood produce an enhanced uptake and attachment of circulating endothelial progenitor cells on to the treated or coated stent surface and accelerate their maturation into functional endothelium. The rapid establishment of functional endothelium can decrease device thrombogenicity and modulate the extent of intimal hyperplasia.

EXAMPLE 8

[00198] **Small Molecule Functionalization of a Stent:** Progenitor endothelial cells were isolated as described in Example 1. The cells were plated in fibronectin-coated slides and grown for 7 days in EBM-2 culture medium. Cells were fixed and stained with Propidium Iodine (PI) and a FITC-conjugated endothelial cell specific lectin. (Ulex Europaeus Uea 1) The results of these experiments are shown in FIGs. 16A and 16B. The figures show that progenitor endothelial cells are bound to the fibronectin-coated slides and that the cells express a ligand for the lectin on their surface.

EXAMPLE 9

[00199] Transfection of porcine Endothelial Progenitor Cells (EPCs) with a Bicistronic Vector Encoding Both a Vasodilatory Compound and a Unique Cell Surface Marker (truncated MHC-I). MHC-I can be recognized by a specific antibody immobilized on an intravascular prosthesis. Antibody coated stents are implanted into the coronary arteries of pigs, followed by transplantation of the genetically modified EPCs into the pigs. EPCs are captured by the coated stent due to the antibody-antigen interaction and an endothelial monolayer formed over the stent struts. The captured cells can secrete the over-expressed vasodilator, increasing distal flow, and trigger positive remodeling.

[00200] Plasmid selection: The MACSelect™ K System consisting of the pMASCSK^k plasmid vector has been developed by Miltenyi Biotec (Germany). The pMACSK .II plasmid is a bicistronic vector (5229 bp) containing a multiple cloning site (MCS) in which a cDNA encoding the prostacyclin synthase gene is cloned, as well as the gene encoding a truncated mouse MHC class I molecule, H-2K. This system was developed to select for transfected cells, with the truncated MHC molecule acting as the selection marker. Native H-2K expression is restricted to some rare murine strains (eg. AKRiJA or CBNJ), therefore, a monoclonal antibody to the H-2K^k surface protein (Miltenyi Biotec) should be substantially free of extraneous reactivity with other surface antigens.

[00201] Assessment of cross-reactivitv with whole blood: In order to ensure that the anti- H-2K^k antibody does not crossreact with cellular components of whole porcine blood, whole blood is reacted with FITC-conjugated anti-H-2K antibody and subjected to whole blood FACS analysis (Beckman Coulter Cytomics™ FC 500). As a positive control whole blood is "spiked" with the mouse spleen fibroblast cell line AKRIJASp (American Type Culture Collection (ATCC)), which expresses the H-2K^k surface antigen.

[00202] Fibroblast culture: AKR/JA.Sp fibroblast cells are cultured in non-coated T-75 plastic flasks (Sarstedt, Montreal) using Dulbeccos's Modified Eagle's Medium (DMEM) formulated with 4mM L-glutamine, 4500 mg/L

glucose, 1 mM sodium pyruvate, 1500 mg/L sodium bicarbonate, and 10% Fetal Bovine Serum at 37°C and 5% $C0_2$. Cells dissociation is performed using trypsin/EDTA (Invitrogen). H-2K^k expression is confirmed by immunohistochemical analysis using fluorescence labeled H-2K^k antibody. Briefly, cells are plated at 0.5 x 10^6 cells/cm^2 in 2-well non-coated chamber slides. Cultures are fixed at days 1, 2, 3, and 4 with 2% paraformaldehyde and stained with FITC-conjugated H-2K antibody (Miltenyi Biotec, Germany) and the nuclear marker propidium iodide (PI) (Vectashield ™ Mounting Medium, Vector Laboratories). Analysis and quantification are performed using confocal microscopy (Nikon Eclipse™ E800 - Biorad Radiance 2 100). Human fibroblasts are used as a negative control.

[00203] Analysis of non-adherent cells: AKRIJA.Sp cells in a non-adherent form are characterized for the retention of H-2K^k surface protein in order to confirm the feasibility of using this system in the presence of blood. Cells are cultured as described above in T-75, non-coated flasks. Adherent cells at day 4 are disassociated using Trypsin/EDTA and the number of cells expressing H-2K^k surface proteins is determined using FITC-conjugated H-2K^k antibody and FACS analysis (Beckman Coulter Cytomics FC500). FITC-labeled mouse IGg2a isotype is used as a negative control.

[00204] Plasmid construction: cDNA encoding prostacyclin synthase is cloned into the bicistronic plasmid vector pMACS K^k .II (Miltenyi Biotec, Germany) using BamHI and HindIII restriction sequences at the multiple cloning site. A cDNA of 1153 base pairs containing a prostacyclin synthase gene and pVAX-1 in a plasmid construct is used. Transformation of HG70 Ecoli is performed in the presence of ampicillin (50 ng/ml) as a selection agent.

[00205] Complete cDNA for human α-CGRP was obtained from Open Biosystems (Catalog # MHS 1768-9 1441 17; Huntsville AL) in the plasmid vector pPCR-Script Amp SK(+). The fragment is then ligated with BamHI/EcoRI into the bicistronic plasmid vector pMACS K .II. JM109 E coli is transformed to obtain large amounts of the plasmid.

[00206] EPC transfection: Porcine mononuclear cells are enriched from whole blood from pigs by Ficoll density centrifugation, and EPCs isolated by enriched culture as described above. After day 7 in culture the EPCs are transfected with the bicistronic plasmid vector containing the transgene containing the α-CGRP or prostacyclin synthase using nucleoporation (Amaxa Nucleofector, Germany). Electroporation transfection efficiencies of >70% of EPCs have been obtained using both a reporter gene and endothelial nitric oxide synthase (eNOS) in the pVAXt plasmid (data not shown). EPCs which have been successfully transfected and expressing H-2K^k surface proteins are purified and isolated using MACS™ Dead cell removal kit, MACSelect™ K^k MicroBeads and MS™ Separation Column (Miltenyi Biotec). MACSelect K^k MicroBeads are biodegradable, and are lost with cell culture within 24 hours.

Measurement of vasodilator expression:

[00207] Measurement of prostacyclin synthase activity: Transfected EPCs are maintained in culture after transfection for 2 days. The medium is changed, and prostacyclin synthase activity is assessed by measuring the level of the metabolite of prostacyclin synthase, 6-ketoprostaglandin Fla (6-keto-PGFlcu) in the medium by radioimmunoassay (Amersham Corp.) per the manufacturer's instructions.

[00208] Measurement of α-CGRP activity: α-CGRP expression is determined in transfected cells using the Immunohistochemistry Staining Kit (Bachem USA). Transfected EPCs in culture for 3 days are fixed in methanol at -IO°C for 5 minutes. The cells are washed and allowed to air dry. To quench endogenous peroxide activity the fixed cells are incubated in 0.5% solution of hydrogen peroxide in PBS for 7 minutes. To block nonspecific binding, the cells are incubated in serum block for 20 minutes. Cells are then treated with the primary antibody anti-α-CGRP (rabbit monoclonal, Bachem) at three dilutions, 1:100, 1:200 and 1:500 for 2h. The slides are then washed and exposed to biotinylated secondary antibody for 30 minutes. The cells are then rinsed and treated for 30 minutes with HRP-strepavidin complex. After a PBS wash, the cells are exposed to a substrate-chromogen mixture for 3 minutes. The reaction is stopped by the addition of deionized water. The slides are counterstained with Mayer's hematoxylin for 3 minutes. The slides

are then washed in tap water, placed in PBS until they turned blue, then rinsed with distilled water. The slides are then dehydrated using 95% and 100% ethanol and xylene. The slides are coverslipped and examined under light microscopy.

[00209] Antibodv coated stents: Stainless steel stents (9 mm long) are coated with dextran and anti- H-2K^{k} antibody as previously described.

[00210] In vivo cell capture: All experiments are performed in male Juvenile Yorkshire swine (>30 kg). Arterial access is obtained through an arteriotomy performed in the left carotid artery. After the administration of 200 pg of intracoronary nitroglycerin, coronary angiograms are obtained, and on-line quantitative coronary angiographic assessment performed. Stents are deployed 1.1 : 1 stent to vessel randomly to proximal segments of either the LAD, circumflex or right coronary arteries. Once implanted, 200 pg of intracoronary nitroglycerin is administered. Intravascular ultrasound (IVUS) is then performed to determine vessel caliber using a distal side-branch and the distal margin of the deployed stent as distal and proximal references. Administration of cells transfected with the bicistronic vector encoding either protacyclin synthase or a-CGRP cells are accomplished using a prototype tandem balloon catheter (Cordis Corporation). The catheter consisted of two highly compliant balloons located near the distal end of the device that are inflated through a single inflation port. Once inflated, a region of the vessel 1.0 cm in length is isolated between the balloons creating a localized infusion chamber. Distal blood flow is provided by a central lumen, and solutions are infused or aspirated throughout the chamber via two separated lumens. The infusion lumen terminates near the distal balloon, and the evacuation lumen terminates with one port near the proximal balloon. The tandem balloon catheter is advanced to the site of stent implantation and the balloons inflated to 25 psi (1.7 atm). Saline is delivered through the instillation port until the isolated segment is free of blood. Stented arterjal segments are randomized to receive either a saline infusion or cell delivery. A total of 3 x 10 EPCs are given in 2 mls of cell suspension an infusion rate of 200 pL/min over 10 minutes, followed by 10 minutes incubation time. The arteriotomy site is then closed, and the animals allowed to recover. Animals are housed for 28 days

after the cell treatment. A total of 34 animals are treated (10 saline control, 14 protacyclin synthase, 14 α-CGRP). Two animals fiom each group are sacrificed one hour after cell delivery. The stented segments are explanted and flushed stented arterial segments are prepared for SEM by fixation in 10% buffered formalin PBS for 30 seconds and further fixed in 2% PFA with 2.5% glutaraldehyde (BDH Inc.) in 0.1 M sodium cacodylate buffer (Sigma) overnight. Post-fixation is completed with 1% osmium tetroxide (Sigma) in 0.1M cacodylate buffer followed by serial dehydration with ethanol and subsequent critical point drylng with CO_2. After drying, samples are gold sputtered and visualized under scanning electron microscopy (SEM) for the presence of cells bound to the stent struts. Two animals from the prostacyclin synthase group and 2 animals fiom the α-CGRP group are sacrificed 5 days after stent implantation. The explanted stented arterial segments are placed in a 10% formalin/PBS solution until processing for standard histochemical analysis. Five sections are cut fiom each stent; 1 mm proximal to the stent, 1 mm from the proximal end of the stent, mid- stent, 1 mm from the distal edge of the stent and 1 mm distal to the stent. Sections are stained with hematoxylin & eosin (HE) and elastin trichrome. Inflammatory [Kornowski Score (0-3)] scores are determined to assess for evidence of rejection of the delivered cells. After the index procedure (about 28 days), the animals are anesthetized and coronary angiography is performed through an arteriotomy in the right carotid artery. Quantitative coronary angiography is performed and the vessels interrogated using IVUS, and changes in vessel caliber recorded using standard clinical algorithms.

EXAMPLE 10

[00211] Transfection of Mammalian Cells *in vitro* for Use in Blood Vessel Remodeling: Progenitor endothelial cells are transfected using electroporation of a bicistronic plasmid containing genes encoding a protein responsible for the production of adenosine and a prostate specific cell membrane protein. Both genes are under the control of their own promoter, so that the genes are expressed constitutively.

[00212] A vector is constructed similarly as described above comprising a gene encoding a prostatic specific membrane protein comprising its native

promoter and a gene encoding VEGF arranged in tandem within the same expression vector. The plasmid construct can be used to transfect cells mammalian cells for use in patients as describe in Example 9.

DEMANDE OU BREVET VOLUMINEUX

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CONTENANT LES PAGES	1	À	72

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

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CONTAINING PAGES	1	TO	72

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NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

What is claimed is:

1. A therapeutic system for providing at least one therapeutic gene product to a patient, the system comprising the following components for separate administration to said patient in need thereof:

(i) genetically-altered autologous human endothelial progenitor cells, comprising an exogenous nucleic acid encoding at least one unique genetically-engineered exogenous cell membrane marker molecule not normally found in circulating cells and said at least one therapeutic gene product; said autologous genetically altered human endothelial progenitor cells being capable of expressing said unique marker molecule and therapeutic gene product and said cells adapted for administration to the patient so as to be present in the circulating blood of the patient, wherein said unique cell membrane marker molecule is selected from the group consisting of a bone cell antigen, a prostatic specific antigen and an H-2K^k surface protein; and

(ii) a medical device for implantation into the patient comprising a luminal surface having a biocompatible coating; said coating comprising a flexible matrix bearing at least one ligand, wherein said at least one ligand specifically recognizes and binds said at least one unique genetically-engineered exogenous cell membrane marker molecule of said genetically-altered human endothelial progenitor cells *in vivo* and said at least one ligand fails to specifically recognize or bind genetically-unaltered human endothelial progenitor cells *in vivo*, and wherein the medical device surface is not coated with cells prior to implantation;

wherein, in use of the therapeutic system, said autologous genetically-altered circulating human endothelial progenitor cells are rapidly captured from the circulating blood and bound to said medical device *in situ*, proliferate to confluence, and express and secrete said at least one therapeutic gene product, wherein said at least one therapeutic gene product is selected from the group consisting of prostacyclin, calcitonin gene related peptide, vascular endothelial growth factor, angiogenin, anti-angiogenic factor, and fibroblast growth factor.

2. The therapeutic system of claim 1, further comprising a drug, a compound or an inducible promoter for stimulating said genetically-altered human endothelial progenitor cells to express and/or secrete said therapeutic gene product.

3. The therapeutic system of claim 1, wherein said exogenous nucleic acid is an extrachromosomal DNA.

4. The therapeutic system of claim 3, wherein said extrachromosomal DNA comprises a plasmid.

5. The therapeutic system of claim 3, wherein said extrachromosomal DNA comprises a regulatory cassette, at least one gene encoding at least one unique genetically-engineered exogenous cell membrane marker molecule and at least one gene encoding a peptide for treating a disease.

6. The therapeutic system of claim 1, wherein the ligand comprises a specific anti-unique genetically-engineered exogenous cell membrane marker molecule polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, antibody fragment, or combination of antibody and antibody fragments, or a synthetic peptide, or a small molecule, said marker molecule not normally expressed by an endothelial progenitor cell.

7. The therapeutic system of claim 1, wherein said exogenous nucleic acid present in said genetically-altered human endothelial progenitor cells comprises an RNA molecule comprising at least one gene encoding at least one therapeutic gene product.

8. The therapeutic system of claim 1 which is a drug delivery system.

9. The therapeutic system of claim 1, wherein the medical device comprises a metal, polymer, or biodegradable material.

10. The therapeutic system of claim 1, wherein the coating matrix comprises polyurethanes, segmented polyurethanes-urea/heparin, poly-L-lactic acid, cellulose ester, polyethylene glycol, polyvinyl acetate, dextran, heparin, amorphous carbon, fullerenes, gelatin, or basement membrane components comprising collagen, elastin, tropoelastin, laminin, fibronectin, or vitronectin.

11. A drug delivery system for providing at least one exogenous therapeutic gene product or drug to a subject in need thereof, the system comprising:

a medical device for implantation in said subject, the medical device comprising a biocompatible coating comprising one or more layers of a flexible matrix and a therapeutically effective amount of at least one single ligand comprising a polyclonal antibody, monoclonal antibody, chimeric antibody, humanized antibody, antibody fragment, or combination of antibody and antibody fragments, said ligand being compatible to binding selectively at least one specific exogenous genetically engineered cell membrane marker molecule not normally expressed by circulating cells, wherein said ligand fails to specifically recognize or bind genetically-unaltered human endothelial progenitor cells *in vivo*; wherein the medical device surface is not coated with cells prior to implantation, and

genetically altered autologous endothelial progenitor cells, said cells adapted for administration to said subject in peripheral circulating blood of the subject, and capture on a luminal surface of the device *in vivo*;

wherein said coating stimulates adherence and proliferation of said genetically altered autologous endothelial progenitor cells on the luminal surface of said medical device to form a confluent endothelium *in situ*; and

wherein, in use of the drug delivery system, said adherent genetically altered autologous endothelial progenitor cells are encoded to express said at least one specific exogenous genetically engineered endothelial progenitor cell membrane marker molecule and to express and secrete said at least one exogenous therapeutic gene product or drug, *in vivo*.

12. An implantable tube-shaped flexible medical device having an exterior, vascularly contacting surface and an interior, luminal blood contacting surface comprising a luminal surface coating comprising one or more layers of a flexible matrix containing a therapeutically effective amount of at least one single type of antibody or fragment thereof compatible to specifically recognizing and binding *in vivo* a genetically engineered unique exogenous cell membrane surface marker expressed by circulating genetically altered autologous human endothelial

progenitor cells bound on said luminal surface of said medical device to confluence, wherein said single type of antibody or fragment thereof fails to specifically recognize or bind genetically-unaltered human endothelial progenitor cells *in vivo*; and wherein the medical device surface is not coated with cells prior to implantation; and wherein in use of the medical device, said luminal coating adherent genetically altered autologous progenitor endothelial cells are encoded to express and secrete an exogenous therapeutic molecule *in situ*.

13. The implantable device of claim 12, further comprising one or more layers of a flexible matrix compatible to releasing pharmaceutical products into the adjacent vasculature.

14. A therapeutic system for providing at least one therapeutic gene product to a patient, the system comprising:

genetically altered autologous human endothelial progenitor cells, comprising exogenous nucleic acid encoding

a genetically engineered exogenous cell membrane marker molecule not normally found in circulating cells, selected from a bone cell antigen, a prostatic specific antigen, and an H-2K^k surface protein, and

said at least one therapeutic gene product, wherein said therapeutic gene product is selected from prostacyclin, calcitonin gene related peptide, vascular endothelial growth factor, angiogenin, anti-angiogenic factor, and fibroblast growth factor,

said genetically altered cells being capable of expressing said marker molecule and therapeutic gene product, and said cells being adapted for administration to the patient so as to be present in the circulating blood of the patient; and

a medical device for implantation into the patient comprising a coating; said coating comprising a flexible matrix bearing at least one ligand, wherein said ligand is an antibody capable of recognizing and binding said genetically engineered exogenous cell membrane marker molecule of said genetically altered human endothelial progenitor cells, and wherein said ligand fails to specifically recognize or bind genetically-unaltered human endothelial progenitor

cells *in vivo*; and wherein the medical device surface is not coated with cells prior to implantation,

wherein, in use of the therapeutic system, said genetically altered autologous human endothelial progenitor cells are selectively captured by said ligand from the circulating blood and bind to said medical device and express and secrete said at least one therapeutic gene product *in situ*.

15. A therapeutic drug delivery system for providing at least one exogenous therapeutic molecule to a patient, the system comprising:

a stent for implantation into the patient comprising a lumen and a luminal surface, and a covalently bound biocompatible luminal surface coating; said coating comprising

one or more layers of a flexible matrix and

a therapeutically effective amount of a single type of antibody or antibody fragment attached to said one or more layers of said matrix, said single type of antibody or antibody fragment being compatible to binding selectively an exogenous genetically engineered cell membrane marker molecule selected from an H-2K^k surface protein, expressed by genetically altered autologous progenitor human endothelial cells circulating in peripheral blood of said patient, wherein said single type of antibody or antibody fragment fails to specifically recognize or bind genetically-unaltered human endothelial progenitor cells *in vivo*;

wherein said luminal surface coating stimulates capture, adherence and proliferation of said genetically altered autologous progenitor human endothelial cells on the luminal surface of said stent to form rapidly a confluent endothelium *in situ* and wherein the stent surface is not coated with cells prior to implantation; and

wherein, in use of the therapeutic system, said circulating genetically altered autologous progenitor human endothelial cells rapidly bind to said stent luminal surface, proliferate to confluence, and express and secrete said at least one exogenous therapeutic molecule *in situ*.

16. Genetically altered mammalian endothelial progenitor cells, comprising an exogenous nucleic acid encoding

a genetically-engineered exogenous cell membrane marker molecule not normally found in circulating cells, wherein said cell membrane marker molecule is a foreign bone cell antigen, prostatic specific antigen, or H-2K^k surface protein and

at least one therapeutic gene product, wherein said therapeutic gene product is selected from prostacyclin, calcitonin gene related peptide, vascular endothelial growth factor, angiogenin, anti-angiogenic factor, and fibroblast growth factor;

said cells being capable of expressing said marker molecule and therapeutic gene product, and said cells being for use in providing said therapeutic gene product to a patient, wherein said cells are adapted for administration to the patient so as to be present in the circulating blood of the patient;

wherein said cells are adapted for capture by a medical device for implantation into the patient, the medical device comprising a biocompatible coating; said coating comprising a flexible matrix bearing at least one ligand, wherein said ligand is capable of specifically recognizing and binding said cell membrane marker molecule of said genetically altered endothelial progenitor cells, and said ligand fails to specifically recognize or bind genetically-unaltered human endothelial progenitor cells *in vivo* and

wherein said ligand comprises an antibody, antibody fragment, or combination of antibody and antibody fragments; and

wherein the medical device surface is not coated with cells prior to implantation and

wherein said genetically altered endothelial progenitor cells are captured from the circulating blood and bind *in vivo* to said medical device to form a confluent endothelium in situ and express and secrete said at least one therapeutic gene product.

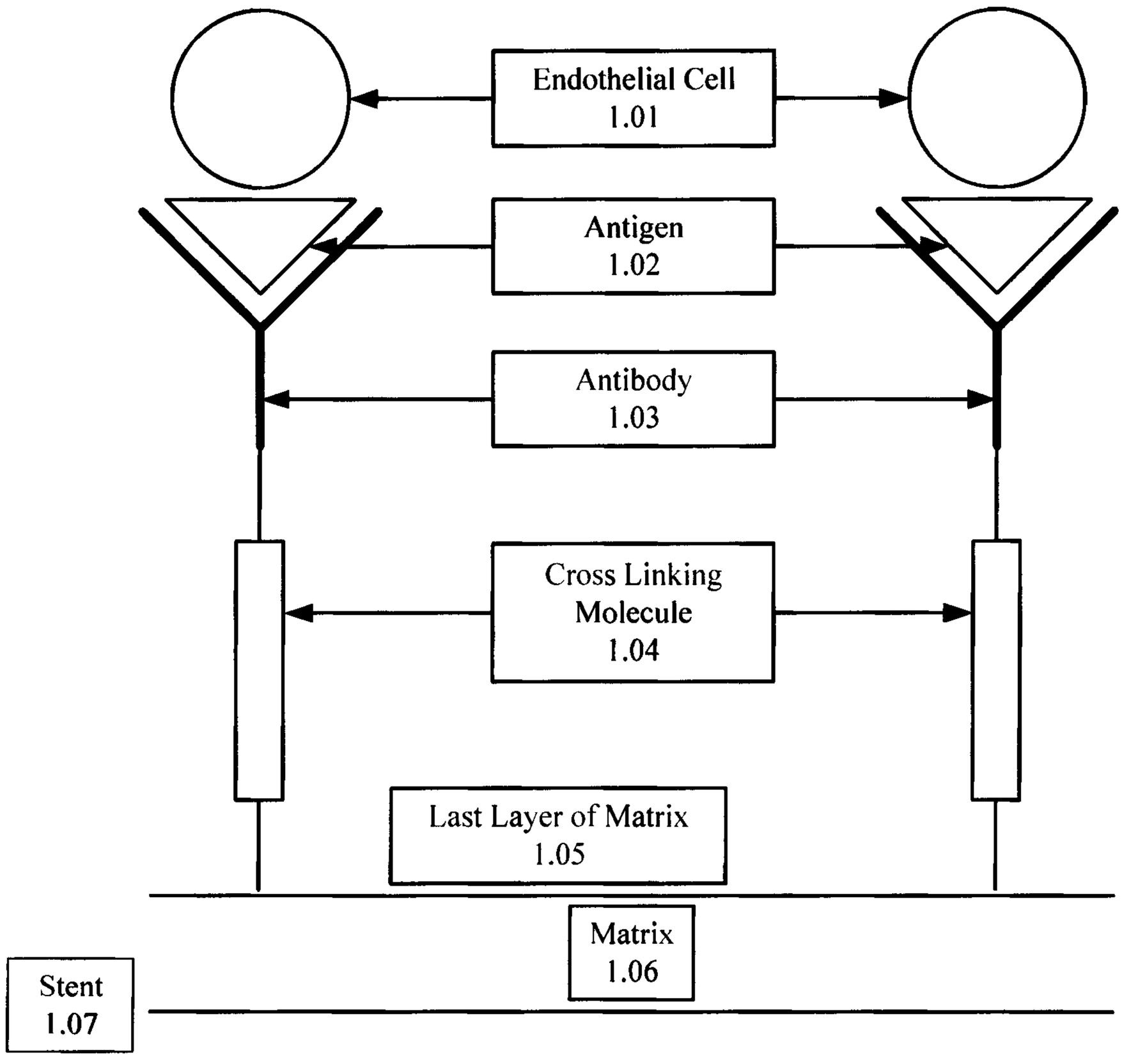

Endothelial Cell
1.01
Antigen
1.02
Antibody
1.03
Cross Linking
Molecule
1.04
Last Layer of Matrix
1.05
Matrix
1.06
Stent
1.07

FIG. 1A

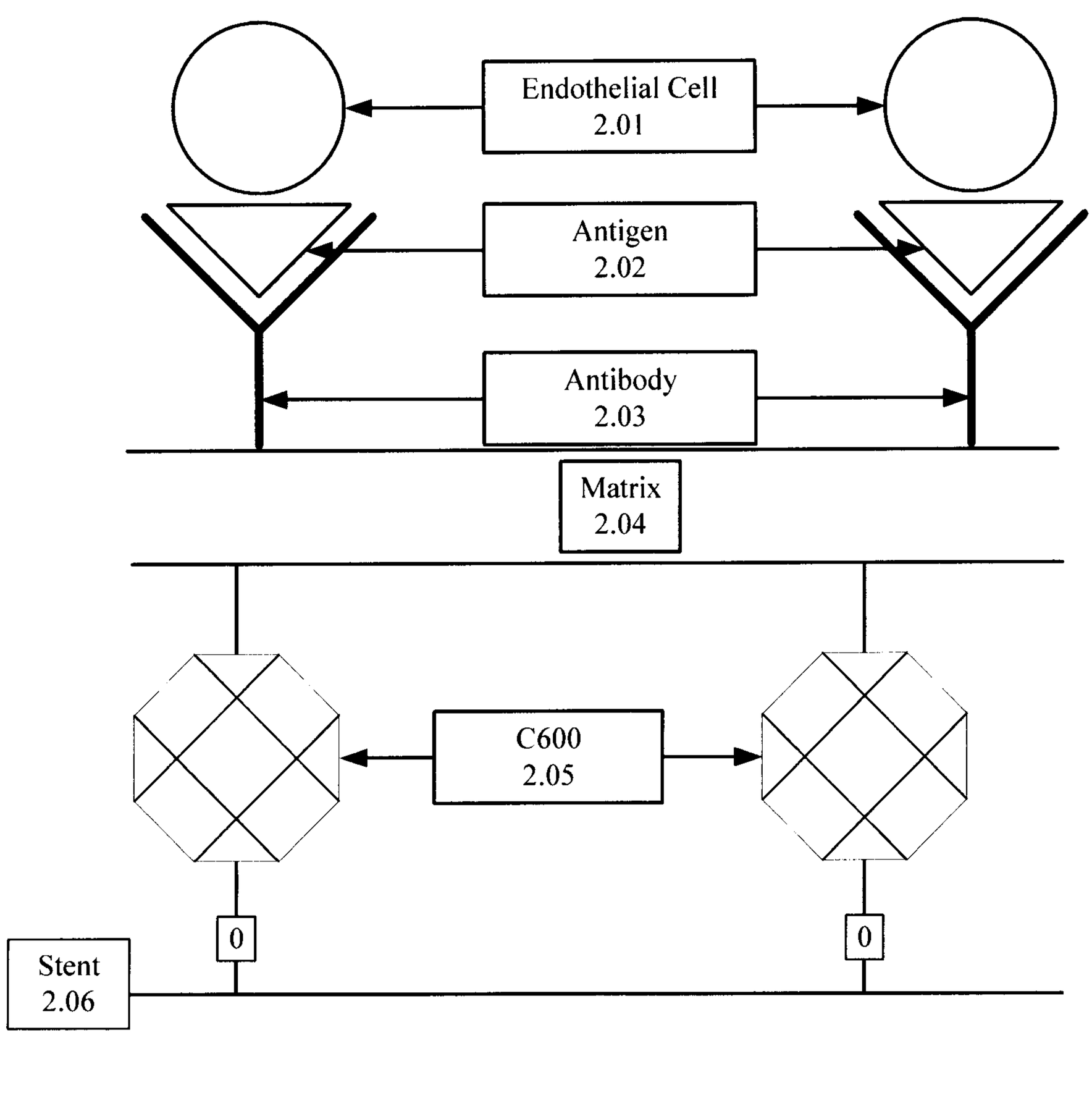

Endothelial Cell
2.01
Antigen
2.02
Antibody
2.03
Matrix
2.04
C600
2.05
0
0
Stent
2.06

FIG. 1B

FIG. 1C

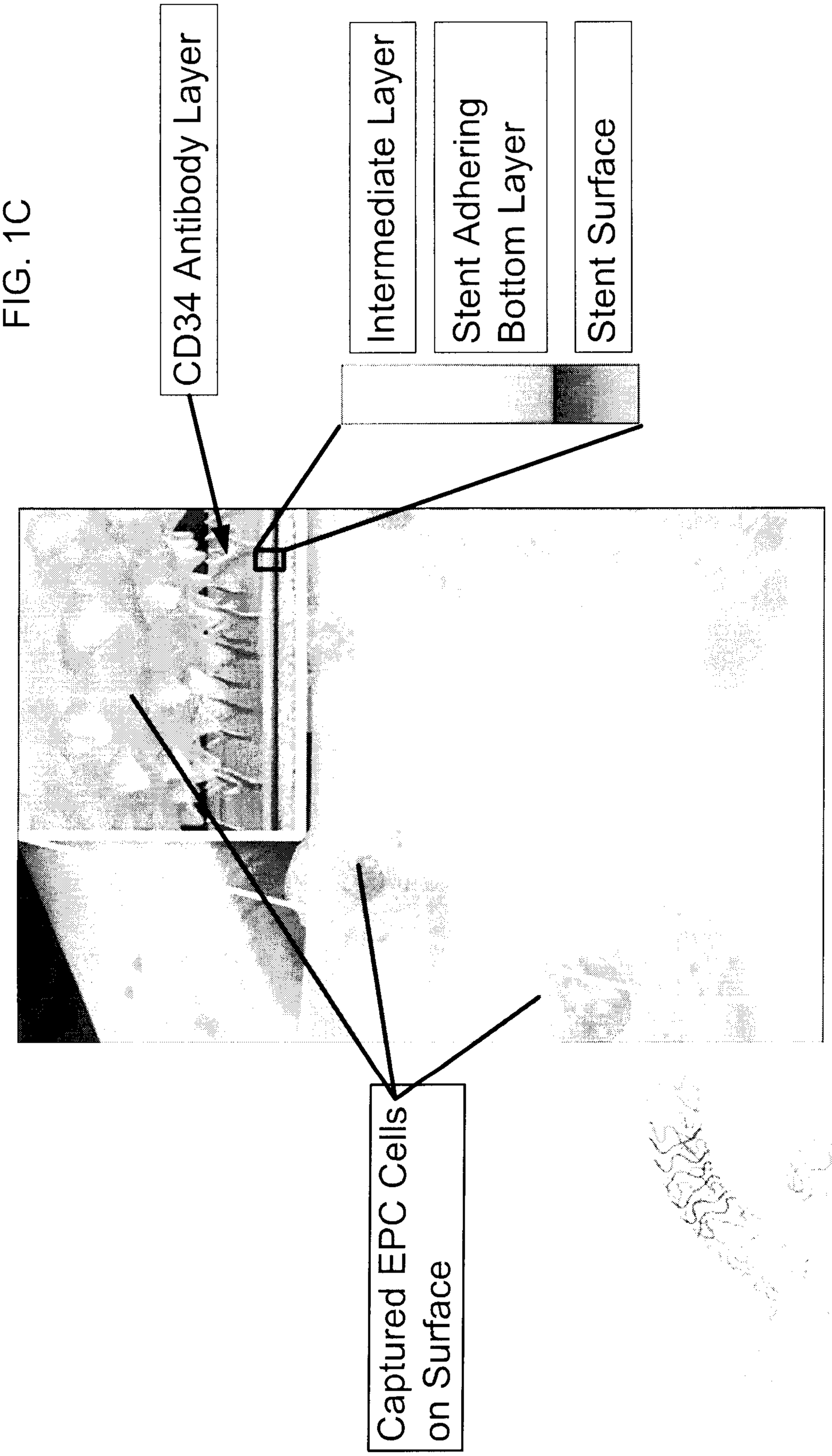
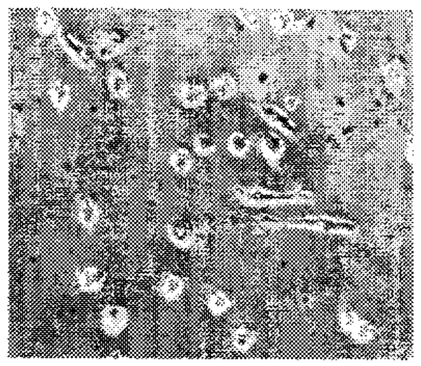
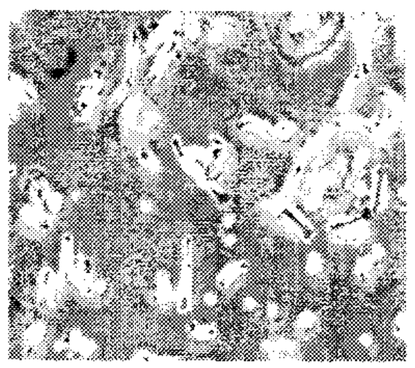

CD34 Antibody Layer
Intermediate Layer
Stent Adhering Bottom Layer
Stent Surface
Captured EPC Cells on Surface

FIG. 2A

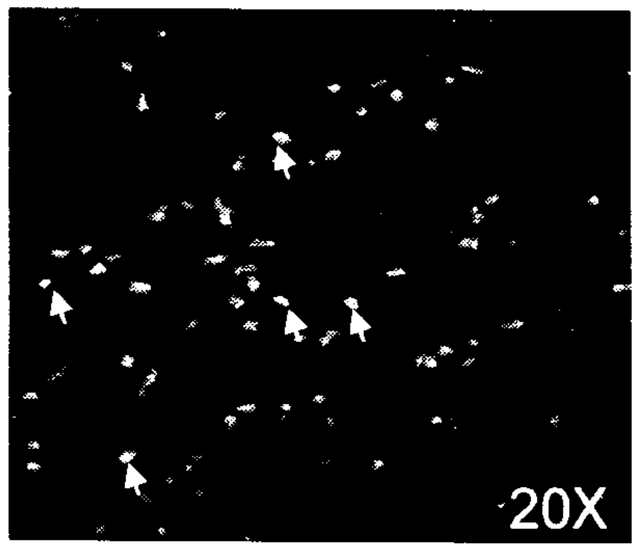


Enriched Medium Isolation

FIG. 2B



CD34 Bead Isolation



White Arrow – VEGFR-2(+)

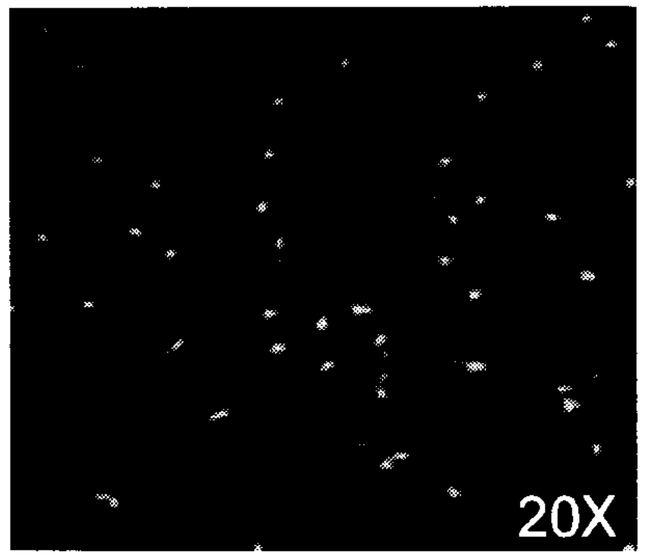
Blue Arrow – VEGFR-2(-)

24 h EPC cultures stained with anti-VEGFR-2 antibody

FIG. 2C

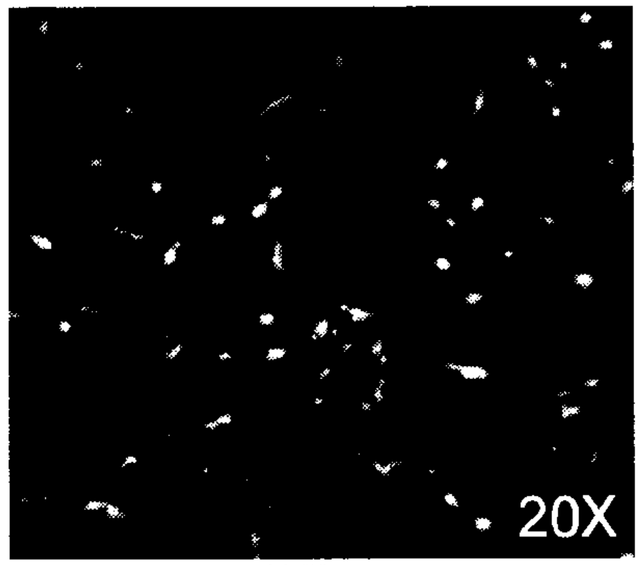
EPC Phenotyping

FIG. 2D



PI Nuclear Staining

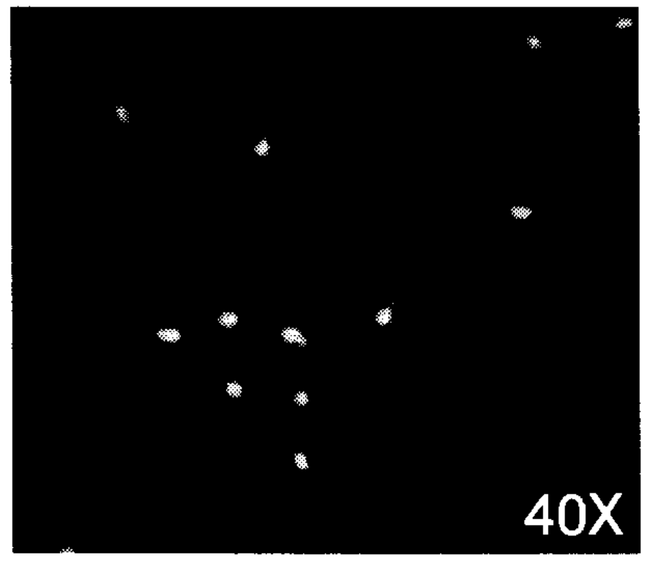
FIG. 2E



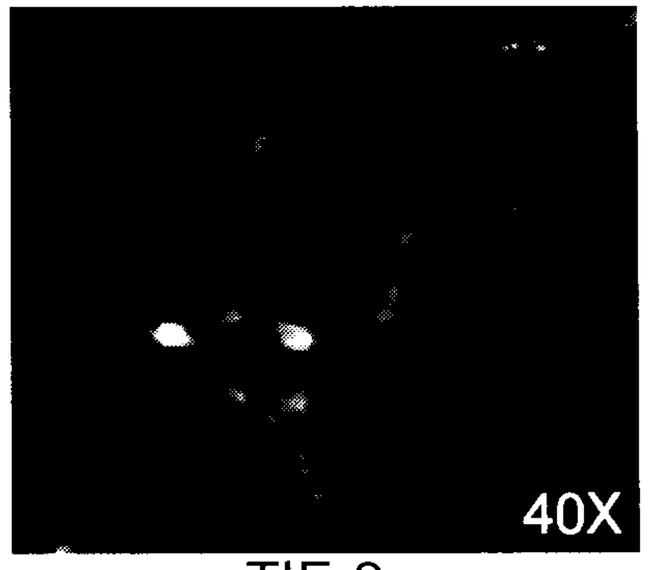
TIE-2

7-Day EPC cultures stained with anti-Tie-2 antibody

FIG. 2G



PI Nuclear Staining



TIE-2

FIG. 2F

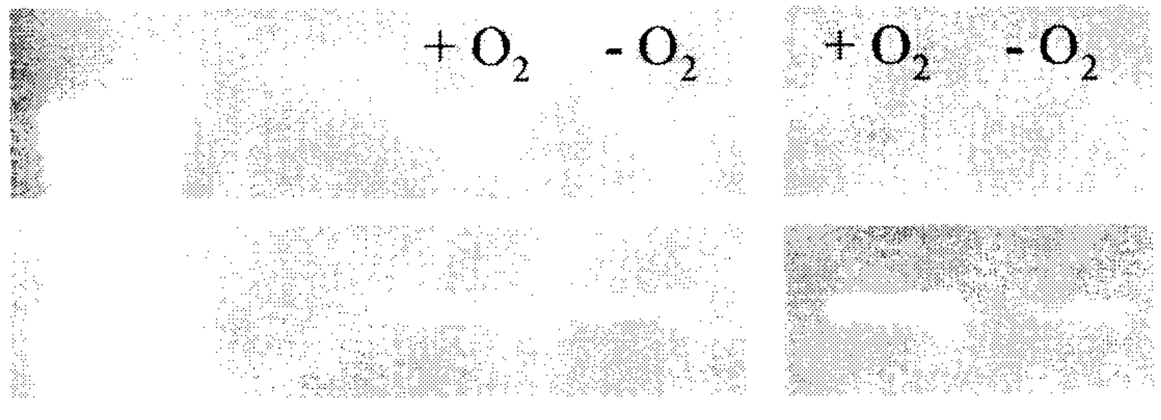


FIG. 3A

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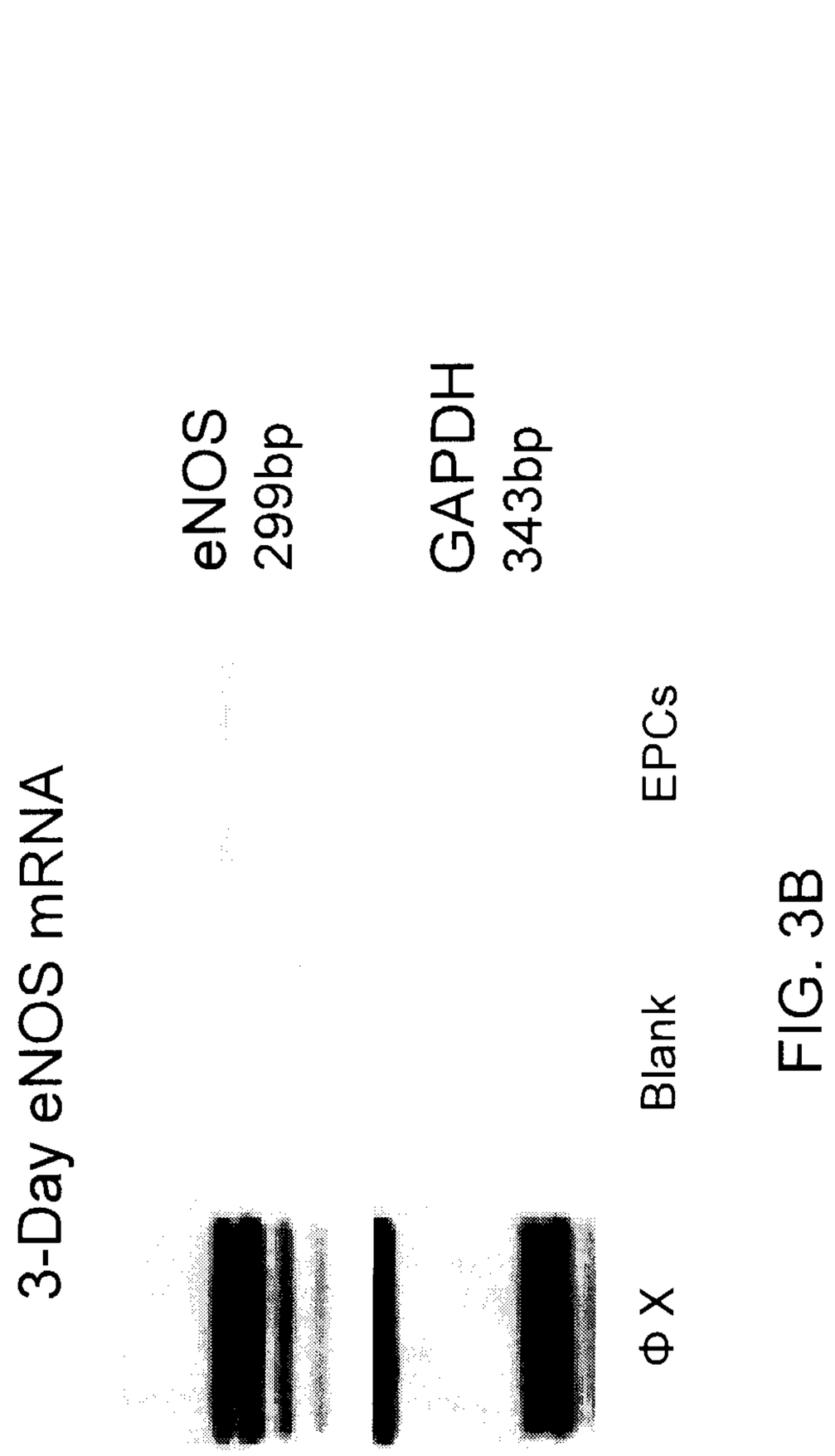
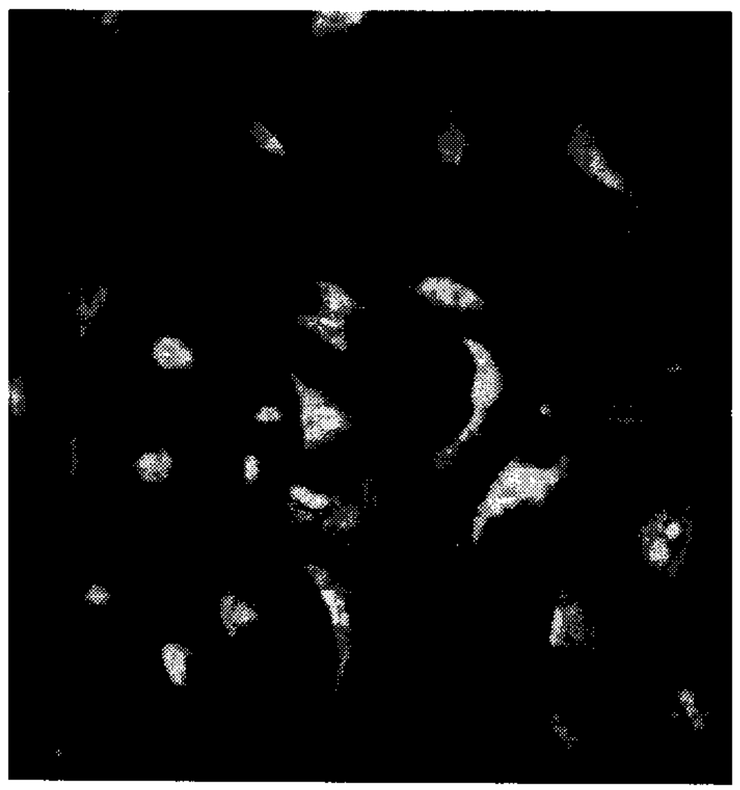


FIG. 3B

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HUVEC Staining

FIG. 4A



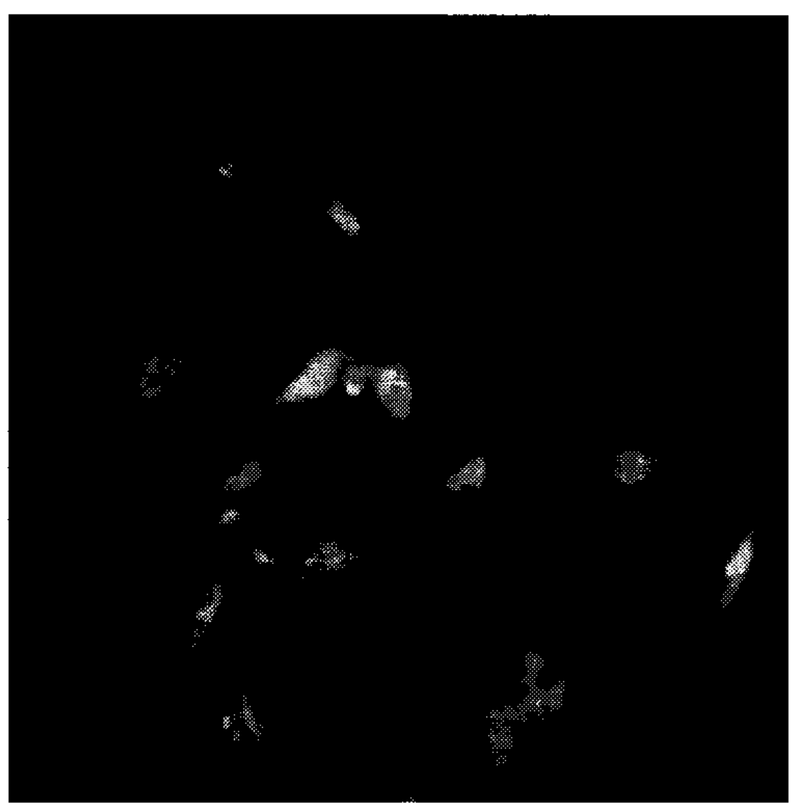
CMDx +Ab

FIG. 4B

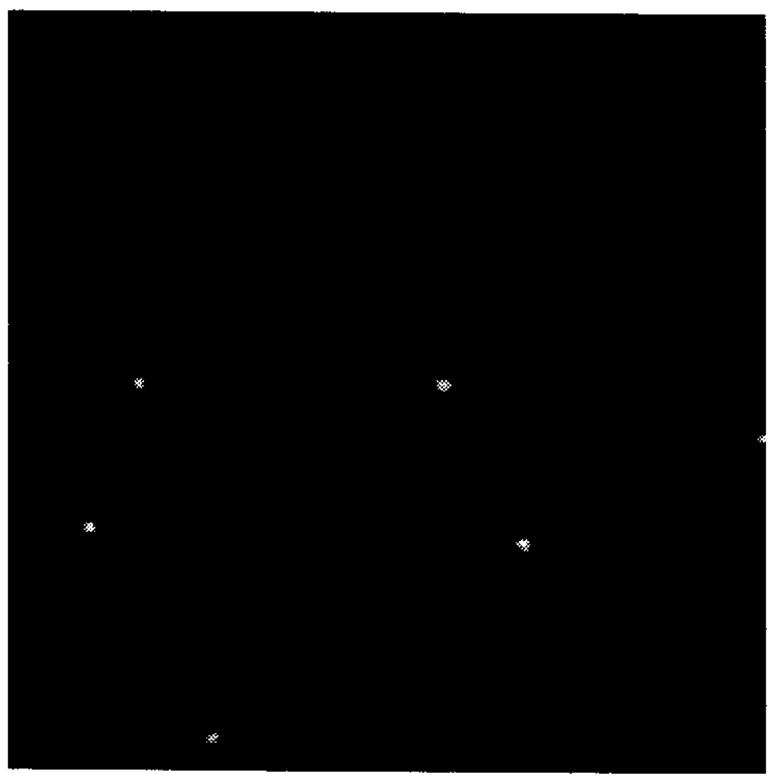


Gelatin + Ab

FIG. 4C

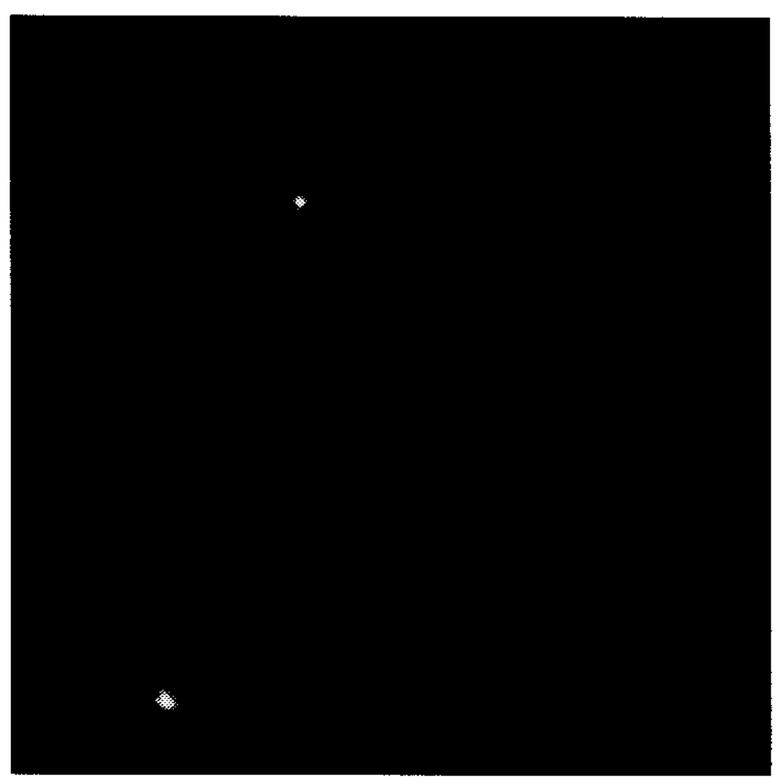


Bare SST



CMDx

FIG. 4D



Gelatin

FIG. 4E

All images at 20X

FIG. 5A

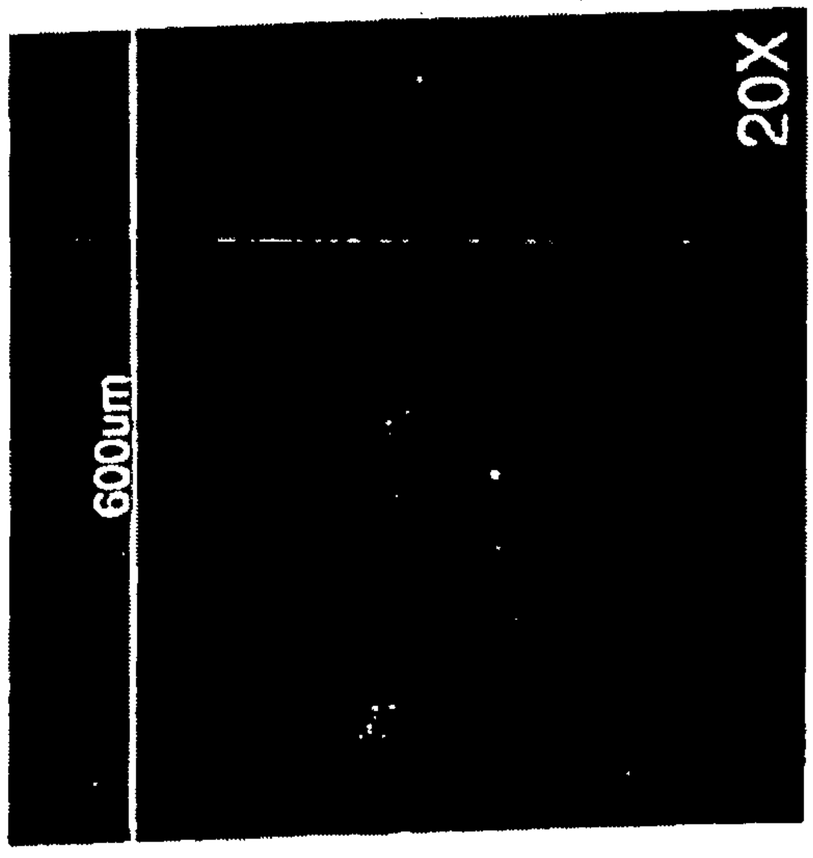


FIG. 5B



FIG. 5C

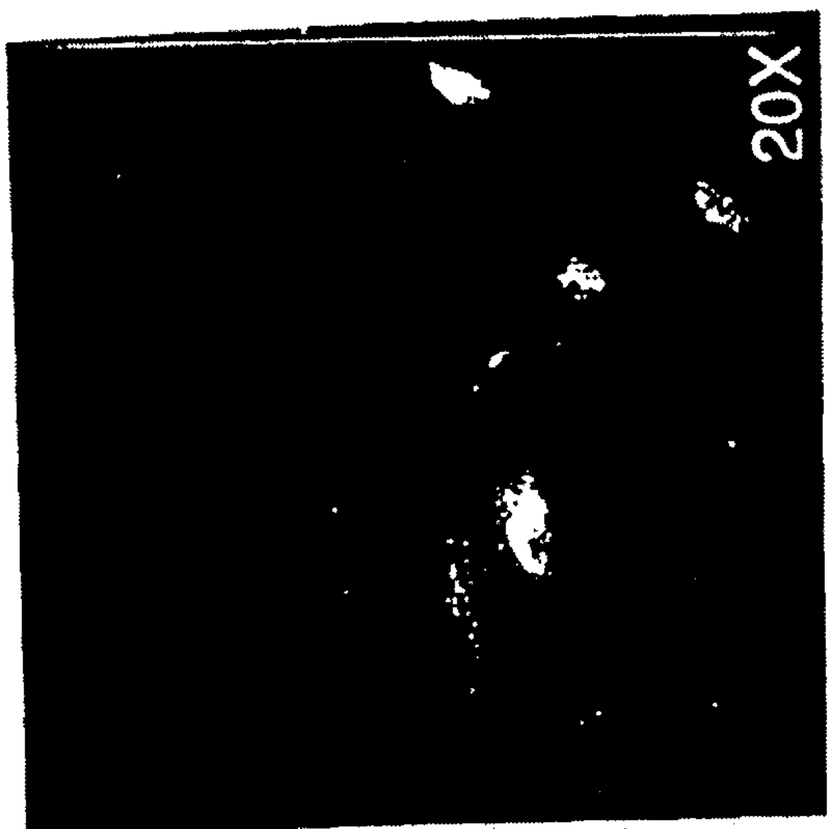


FIG. 5D

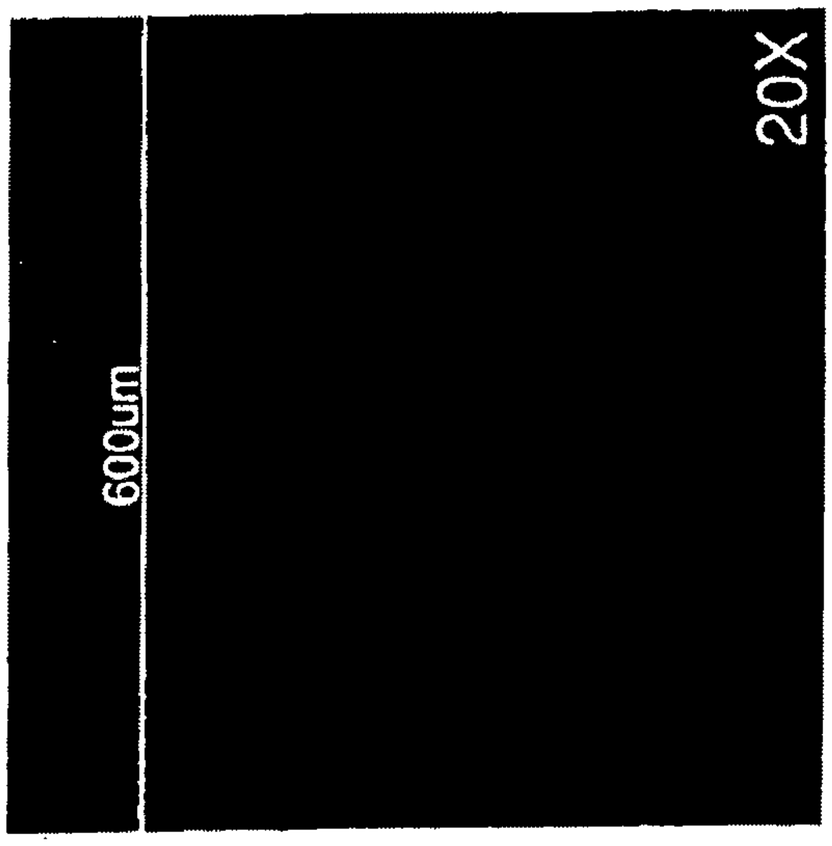


FIG. 5E

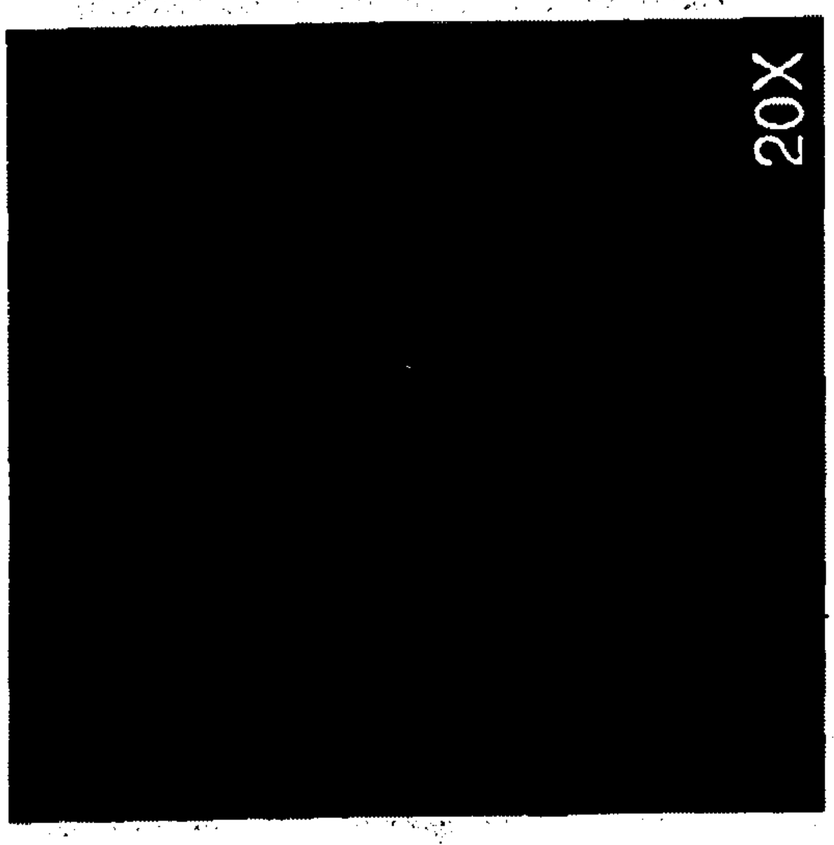


FIG. 5F

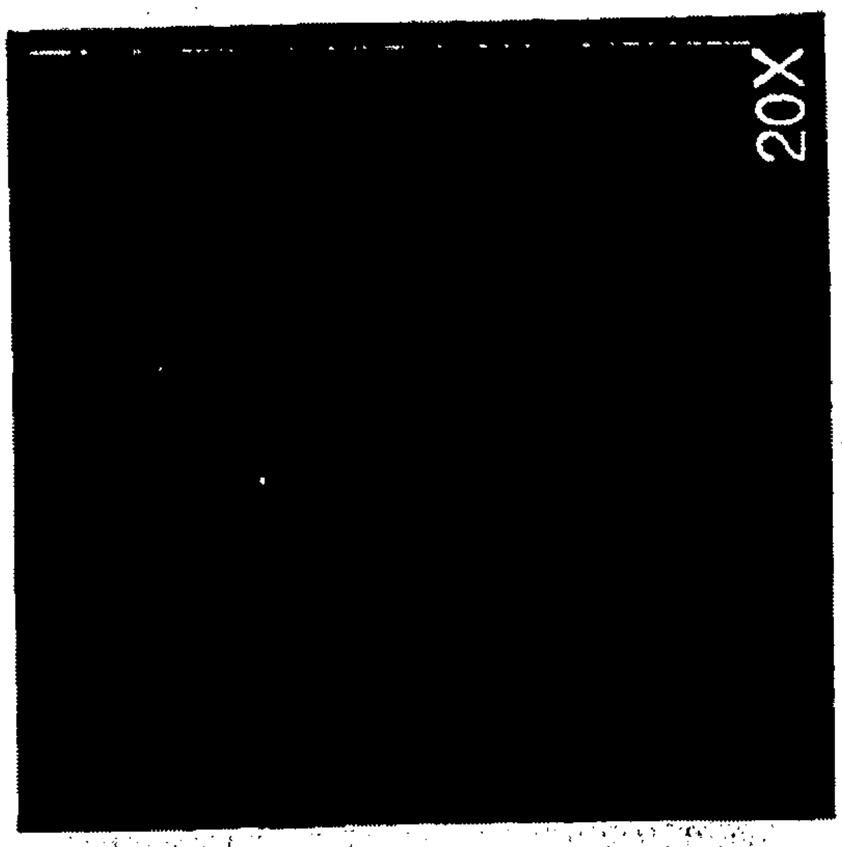


FIG. 6A

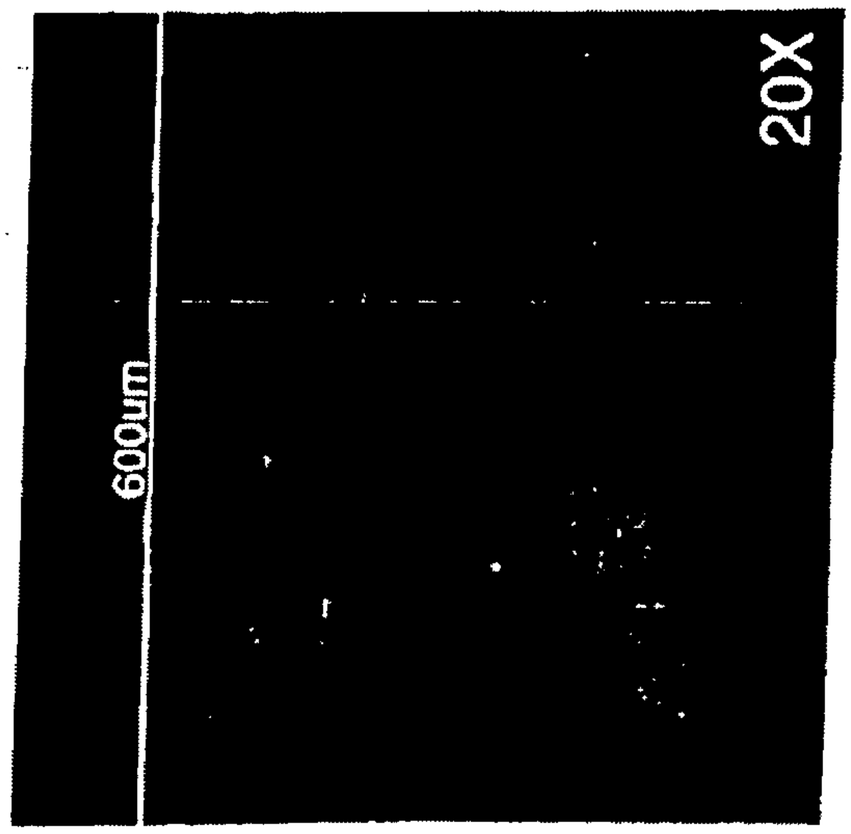


FIG. 6B

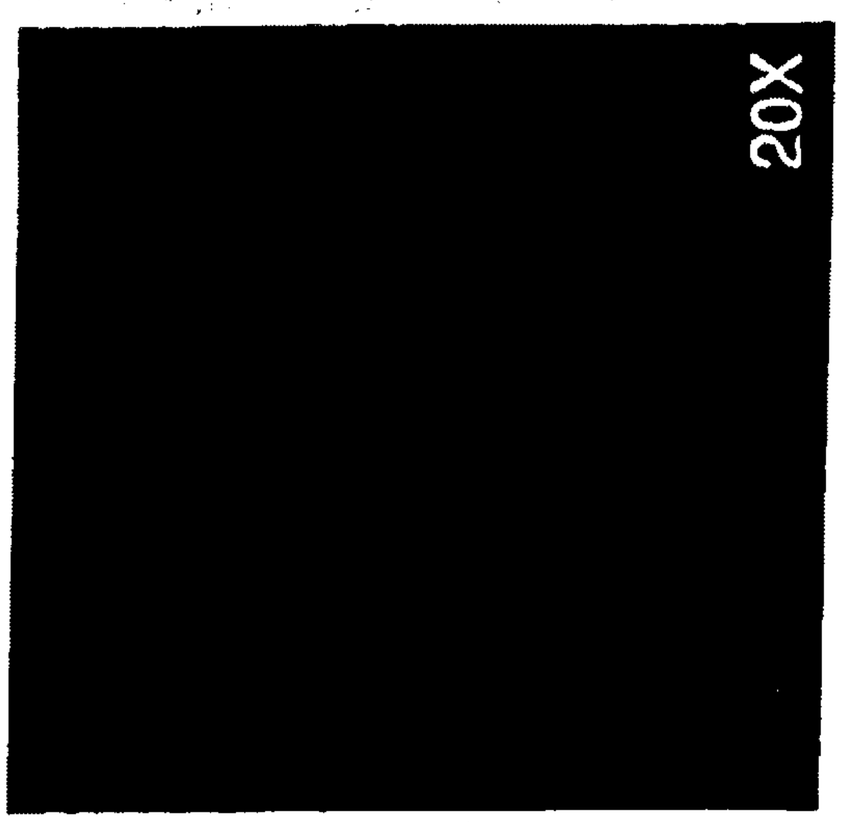


FIG. 6C



FIG. 6D

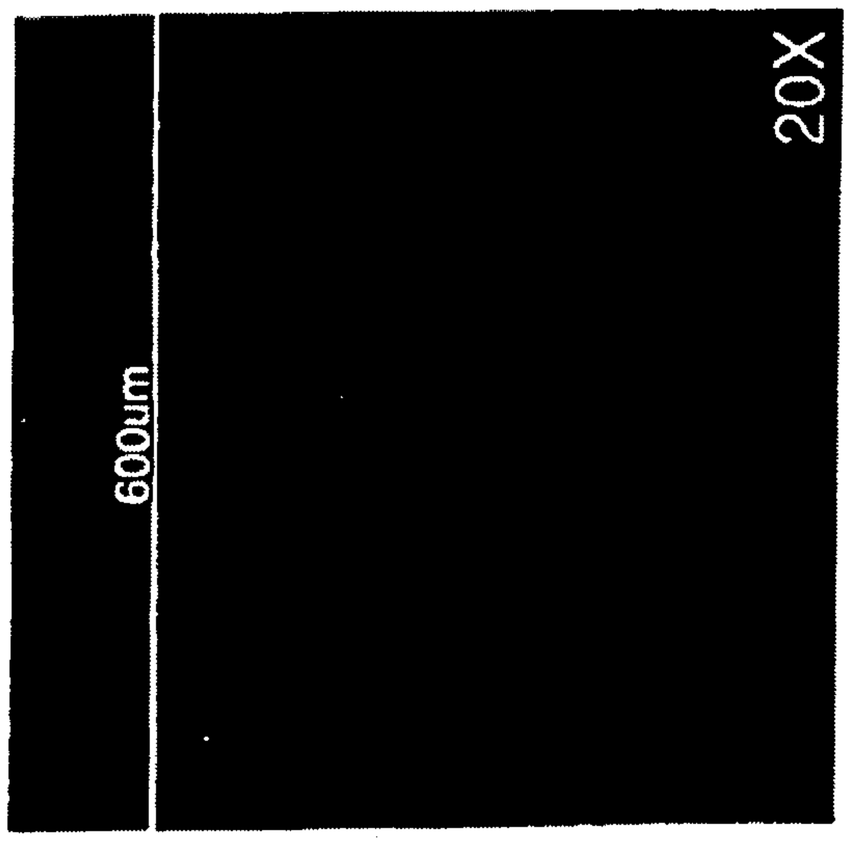


FIG. 6E



FIG. 6F



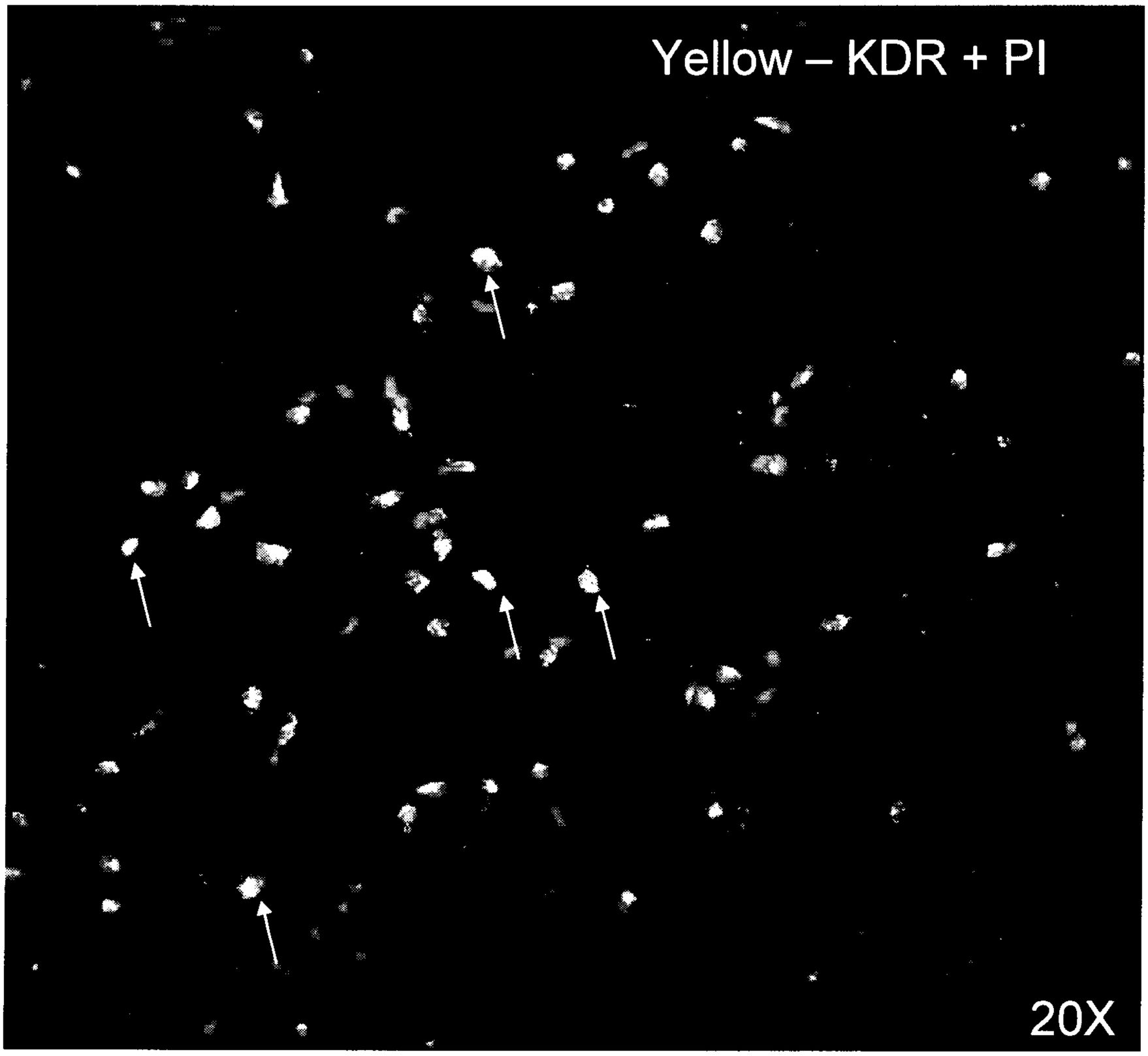


FIG. 7

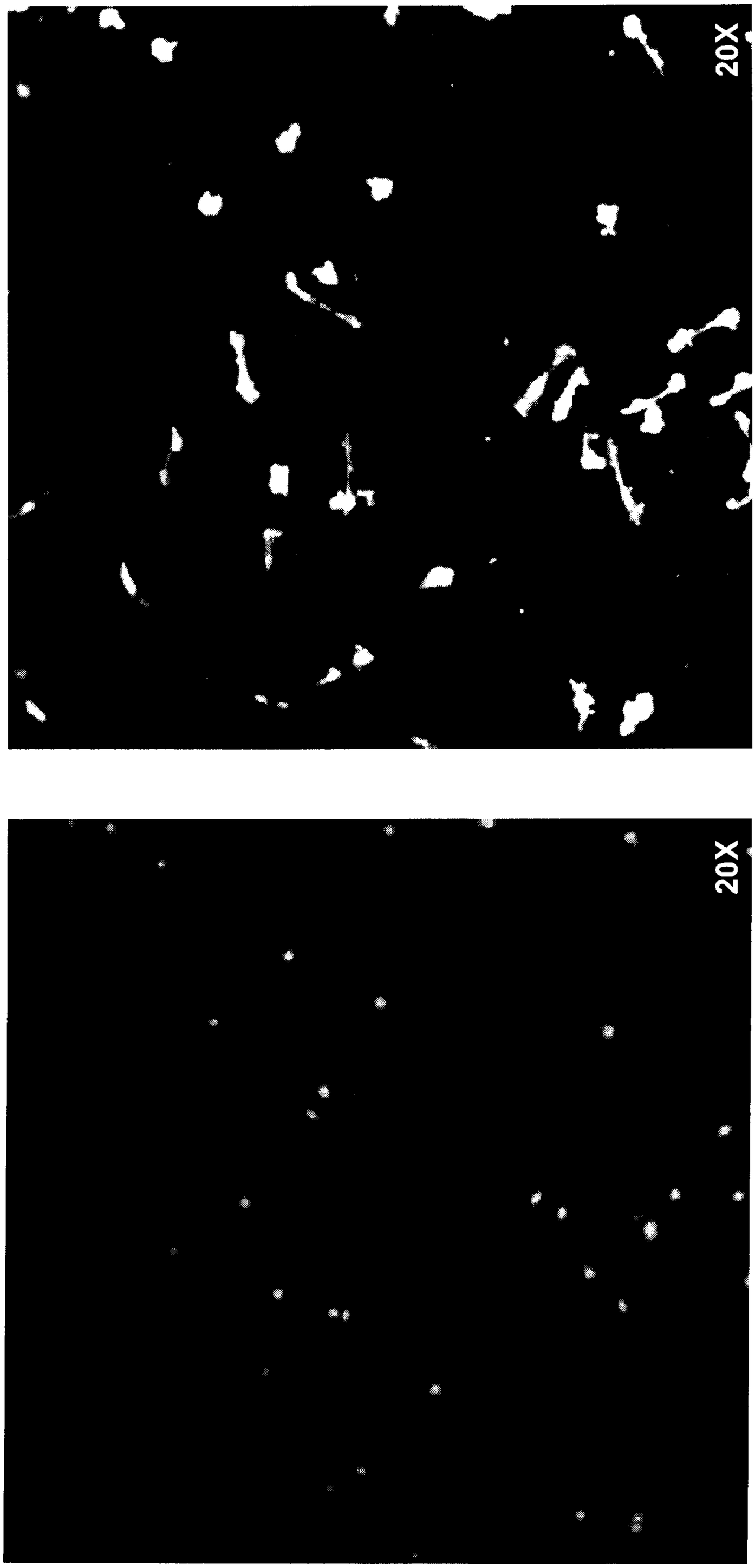

20X
PEC Staining – 7 day (KDR)
20X
PI Nuclear Staining

FIG. 8A

20X
KDR with FITC secondary
20X
PI Nuclear Staining

FIG. 8B

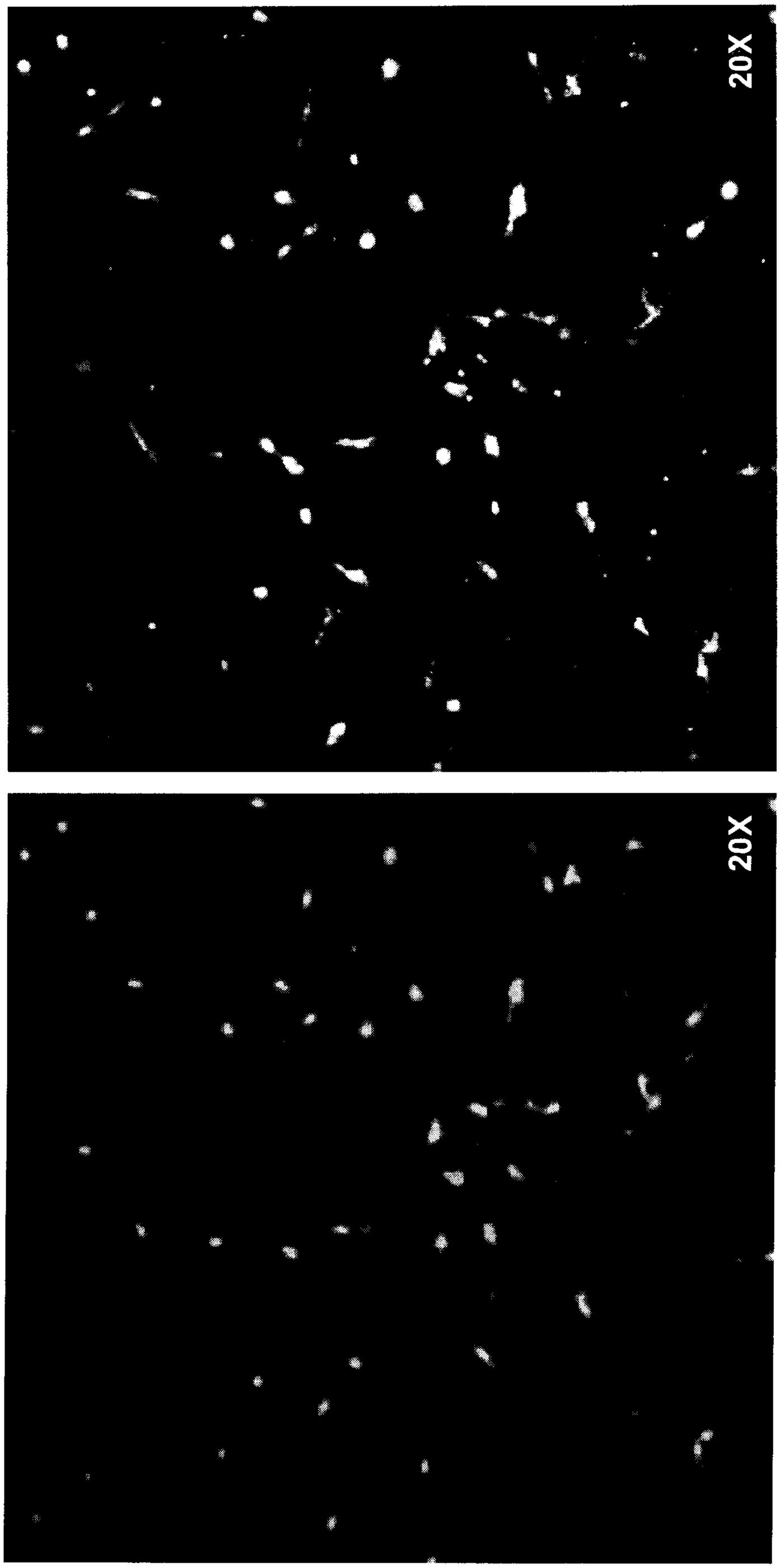
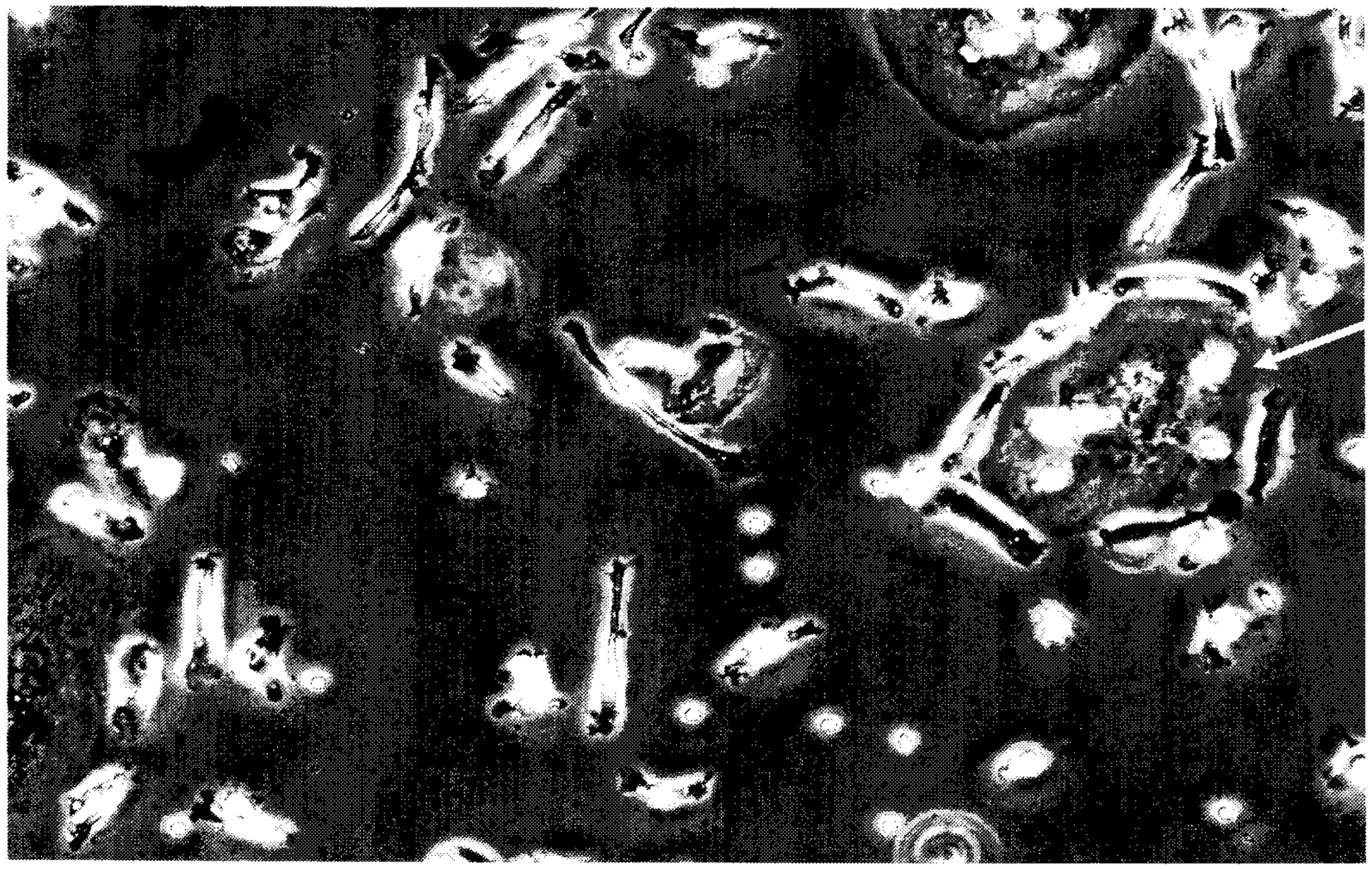

20X
TIE-2 with FITC secondary
20X
PEC Staining – 7 day (TIE-2)
PI Nuclear Staining

FIG. 9B

FIG. 9A

EC tube formation



Clear evidence of ECs forming tubes

FIG. 10A

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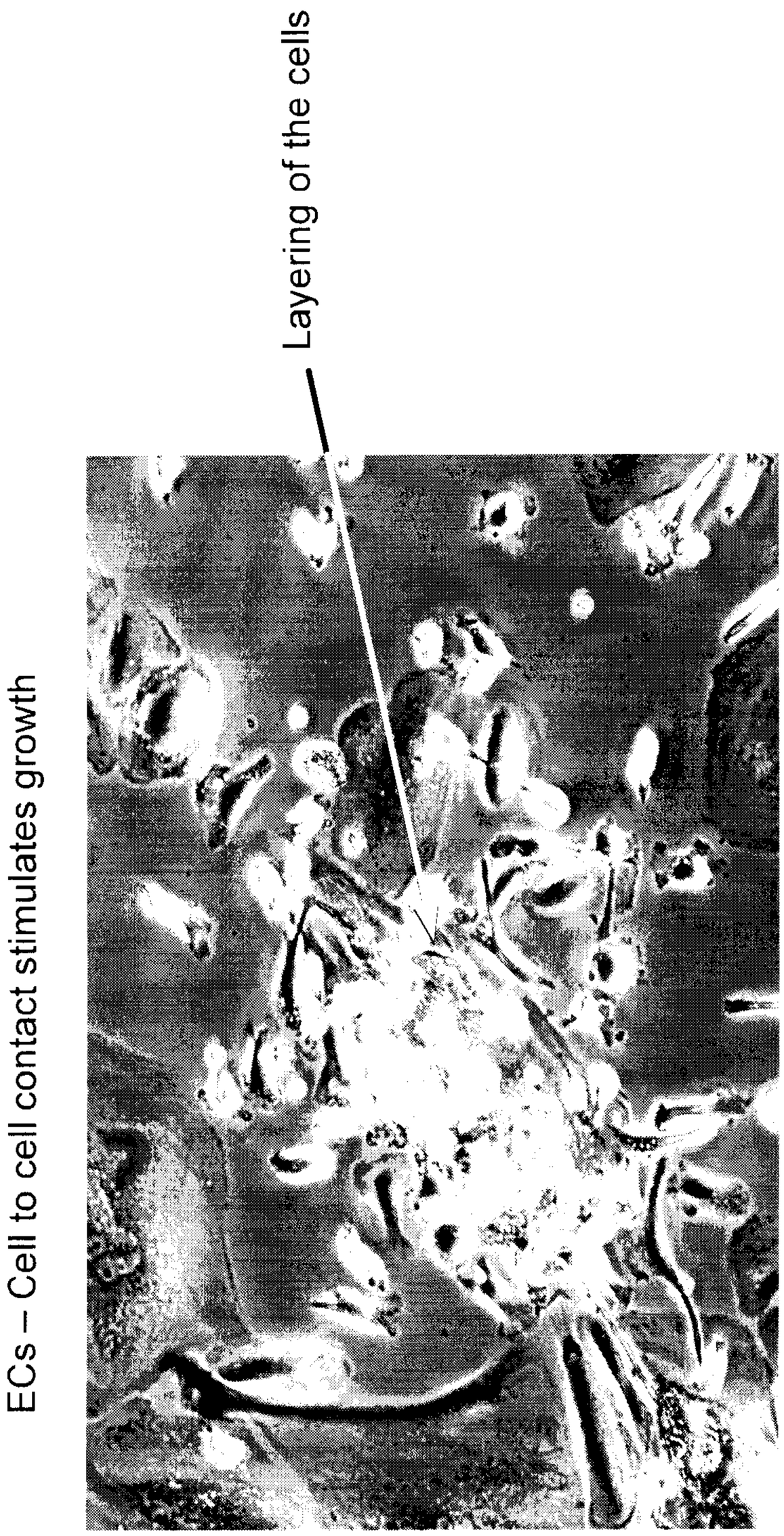

Layering of the cells
ntact stimulates growth
ECs – Cell to cell

FIG. 10B



picture of Ecs in culture

FIG. 10C

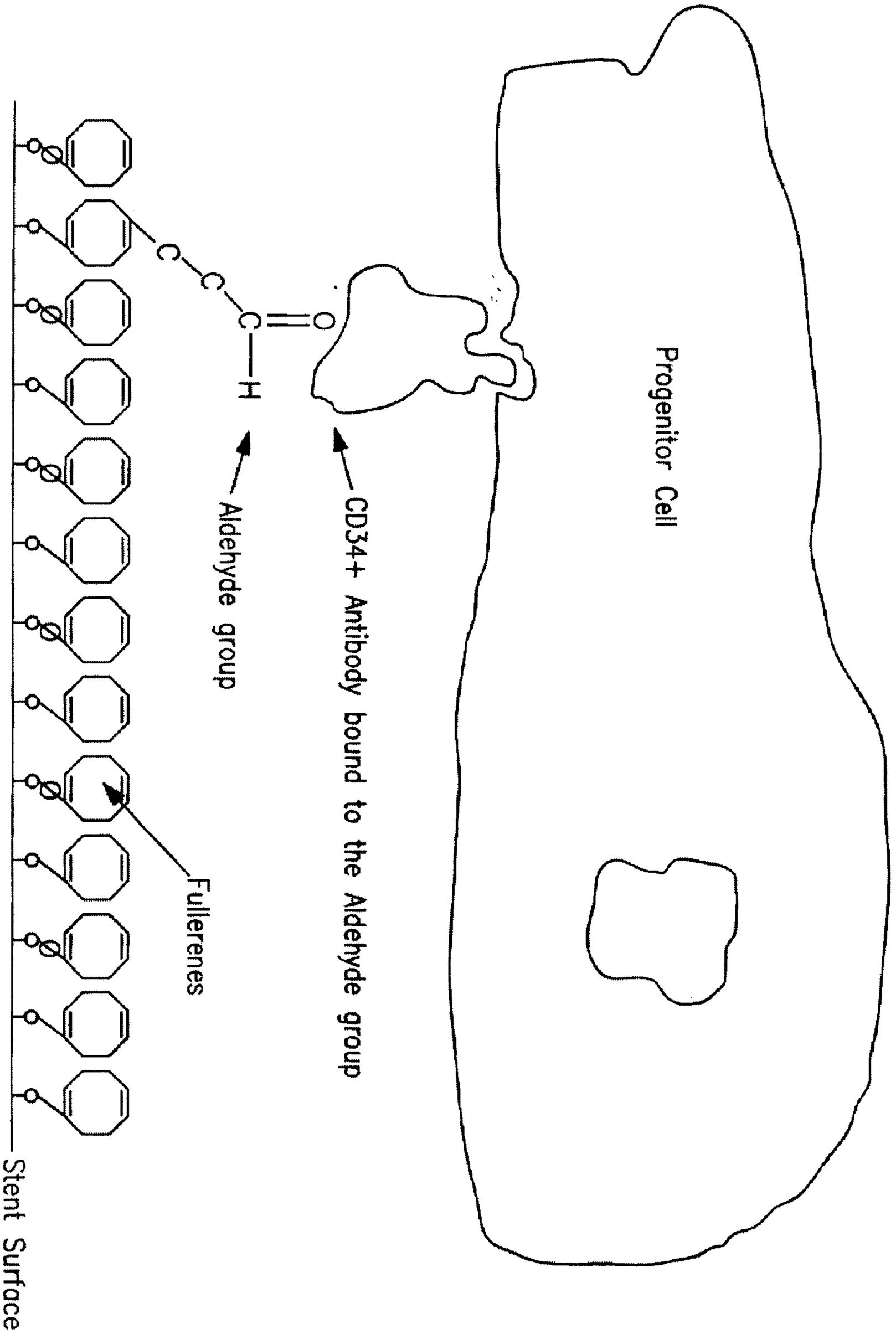

Progenitor Cell
CD34+ Antibody bound to the Aldehyde group
Aldehyde group
Fullerenes
Stent Surface

FIG. 11

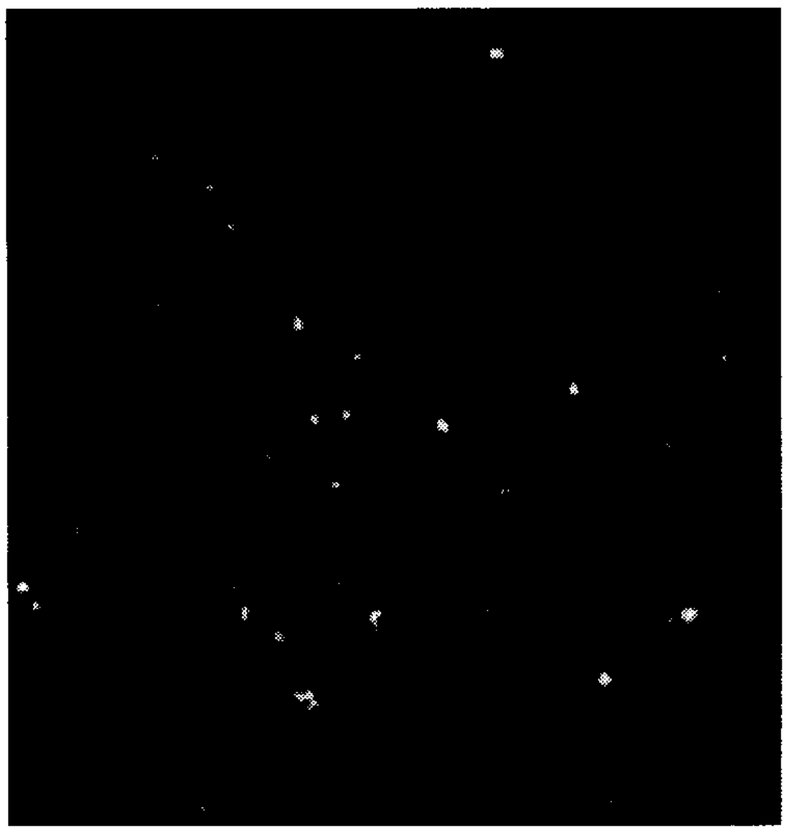


FIG. 12B

FIG. 12A

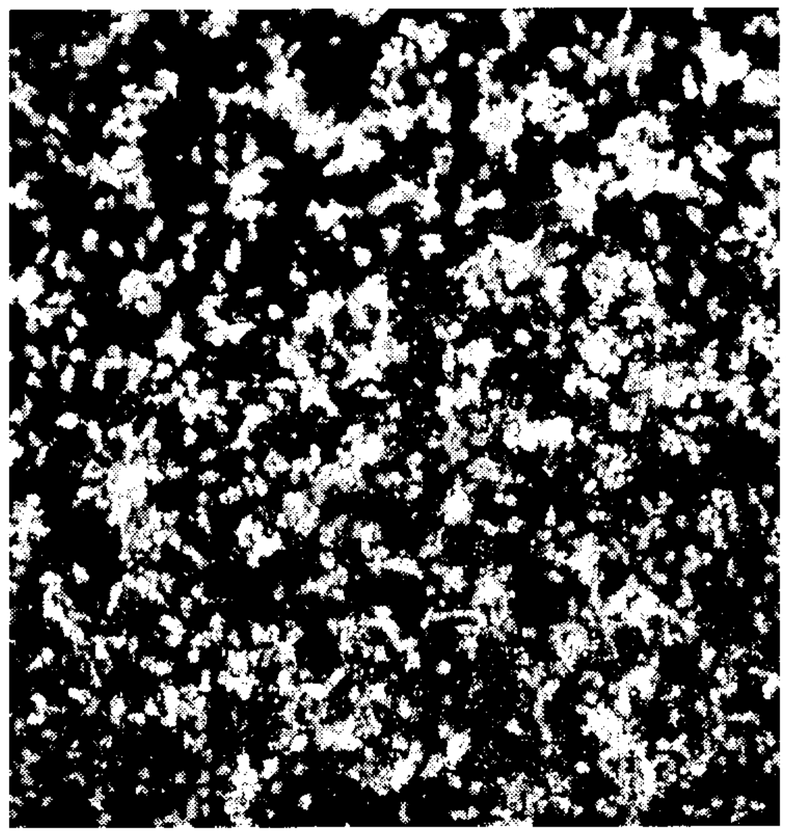
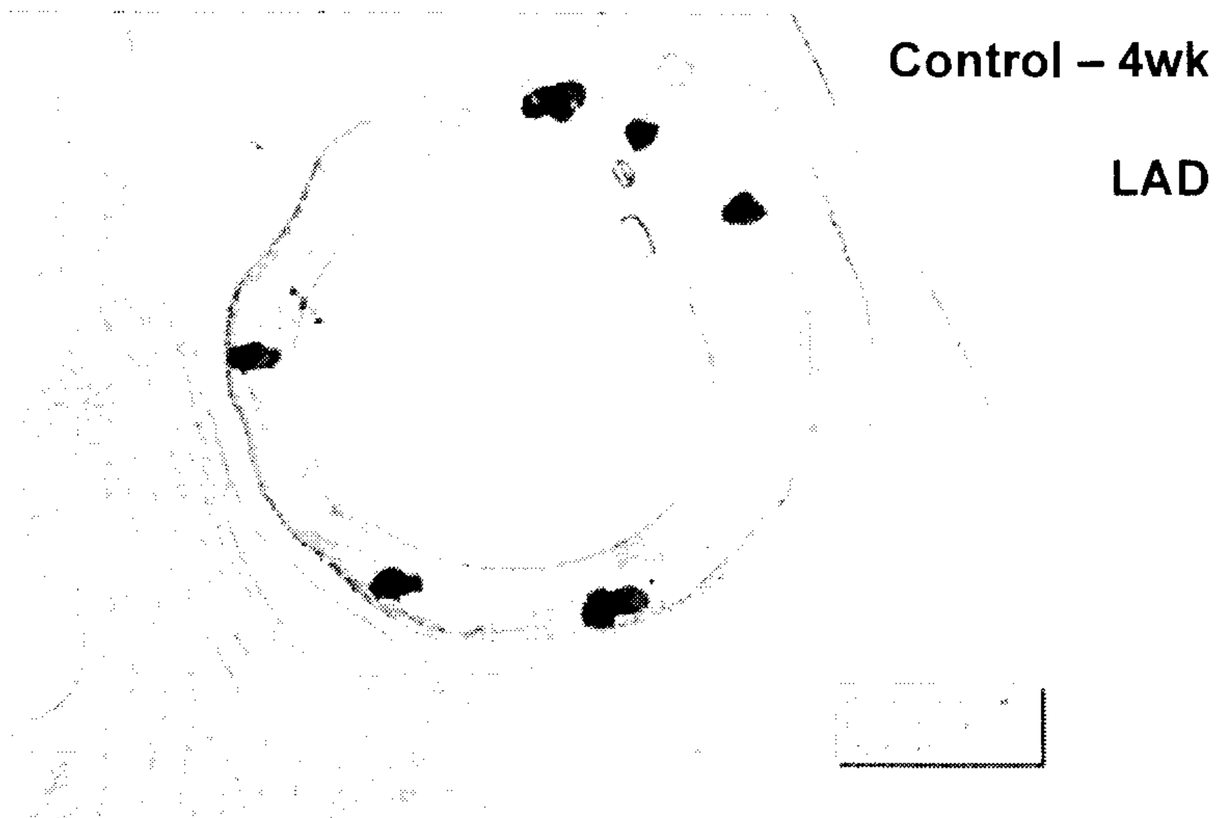


FIG. 12D

FIG. 12C

Intimal Hyperplasia in Stented Artery Segments

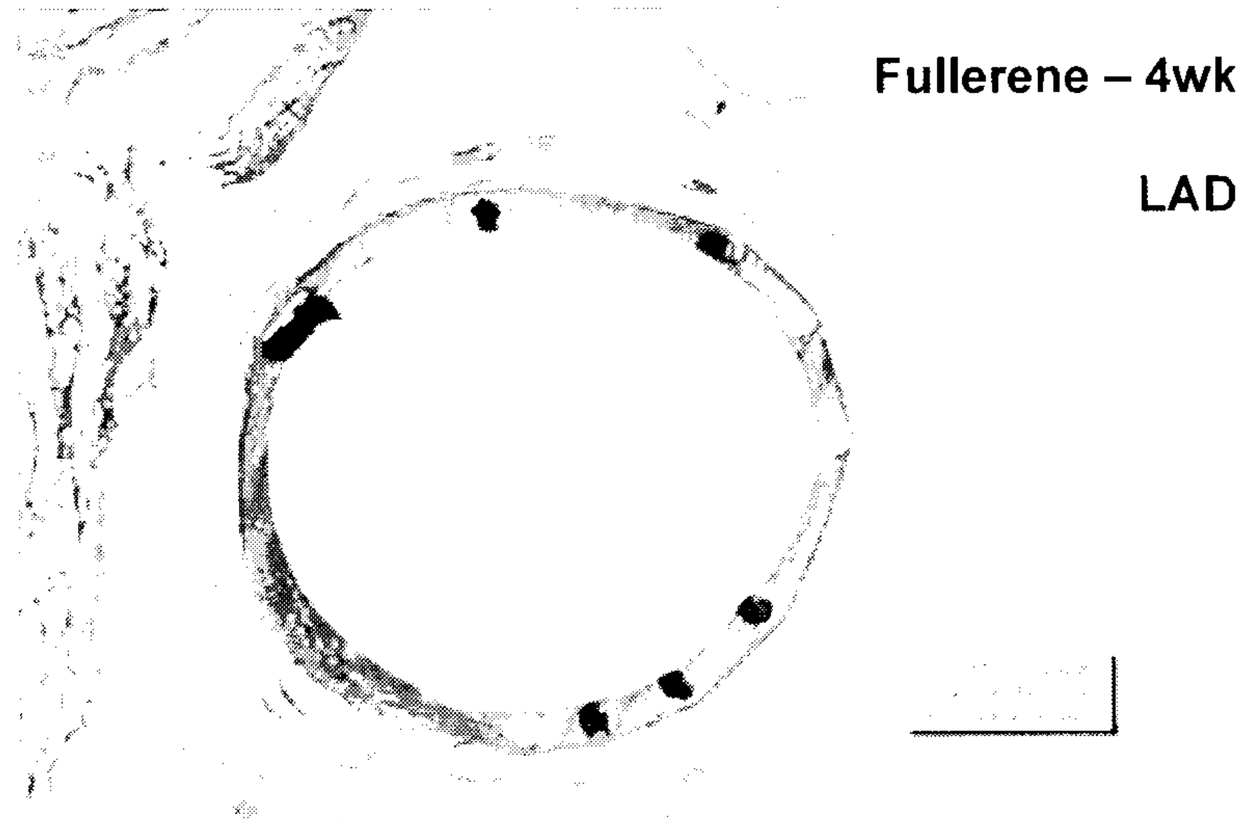
FIG. 13A



Control – 4wk

LAD

FIG. 13B



Fullerene – 4wk

LAD

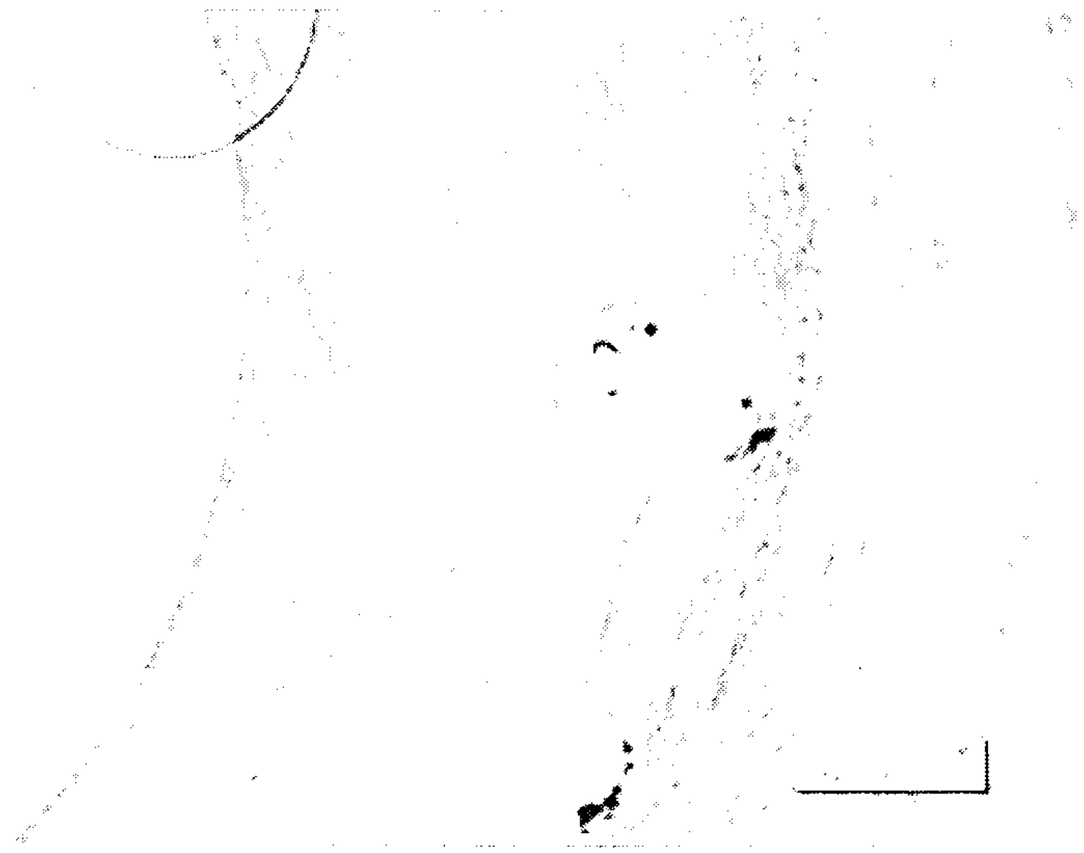


FIG. 13C

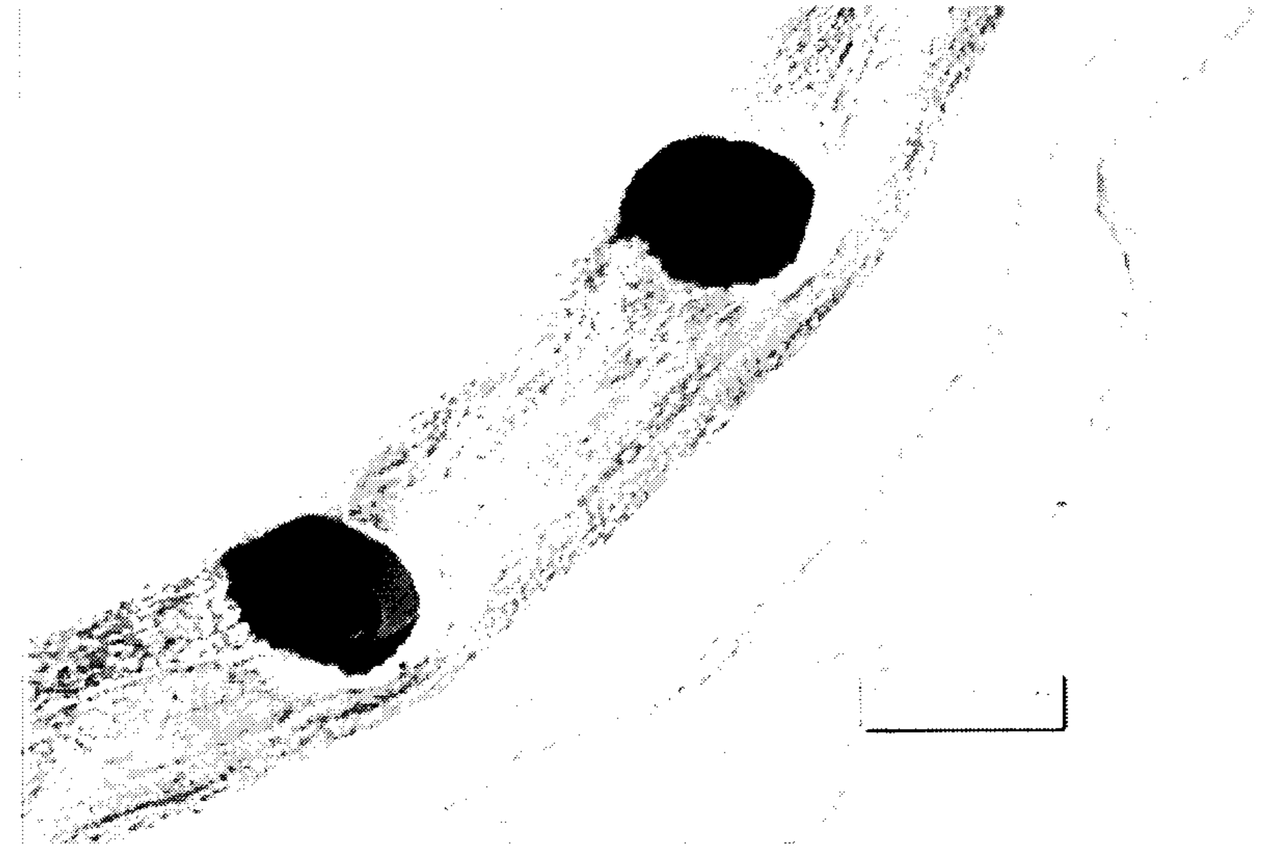
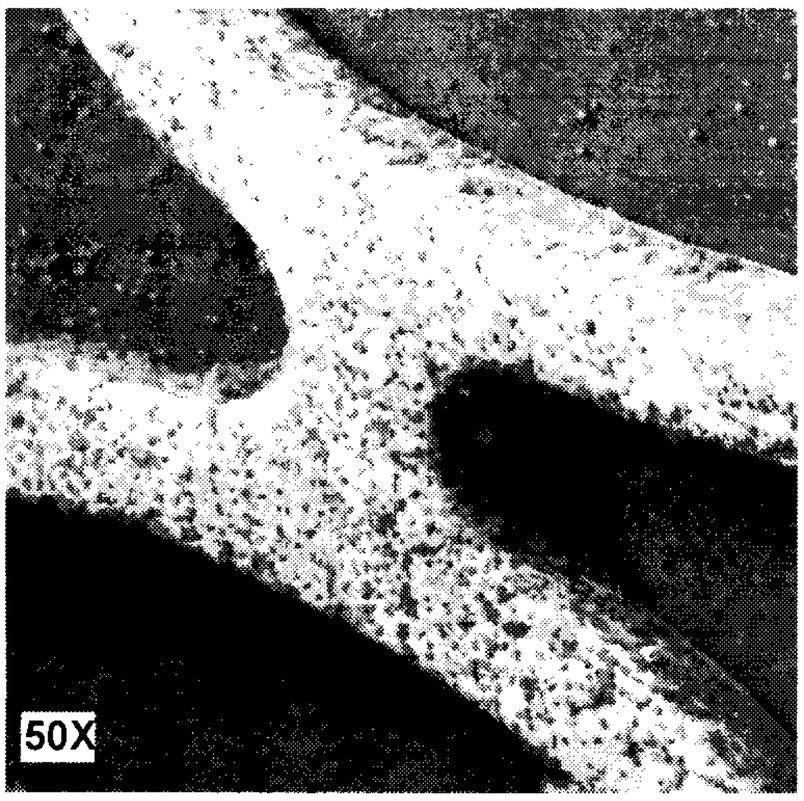


FIG. 13D

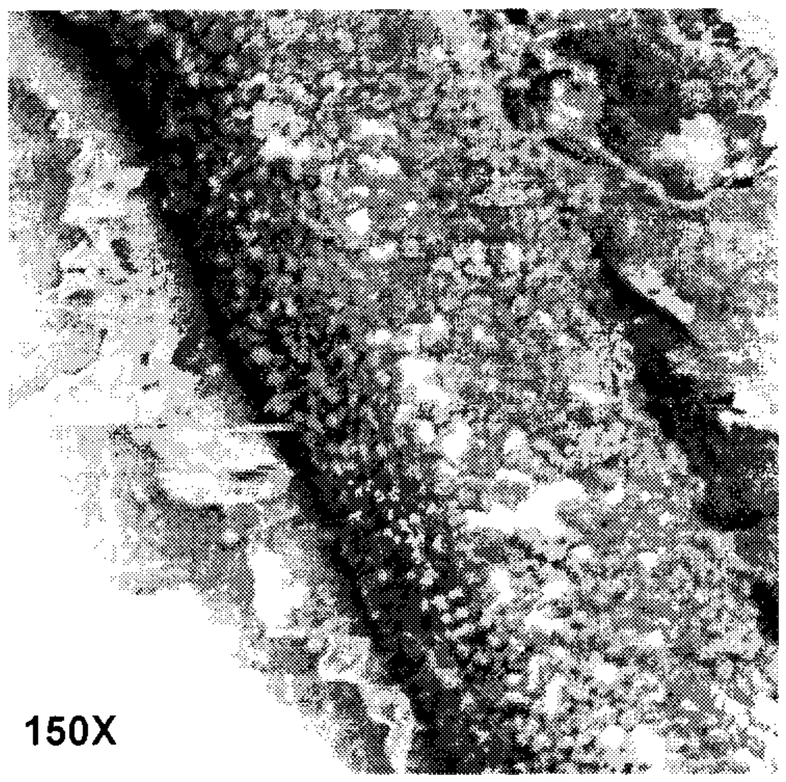
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In Vivo EPC Capture – 1 Hour Explant



Dextran coated

FIG. 14A

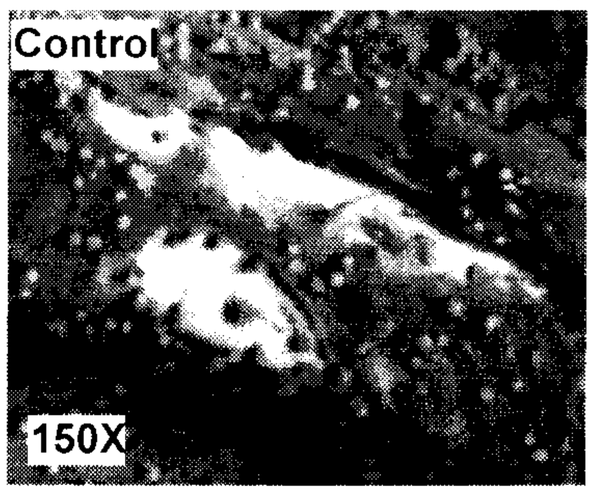


Dextran + Ab coated

FIG. 14B

In Vivo EPC Capture – 48 Hours

FIG. 14C



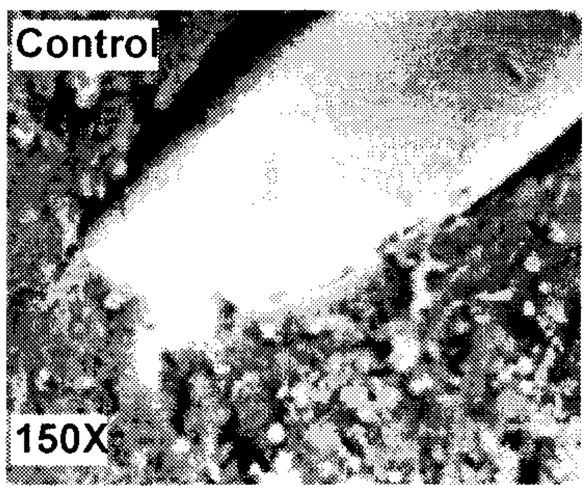


FIG. 14D

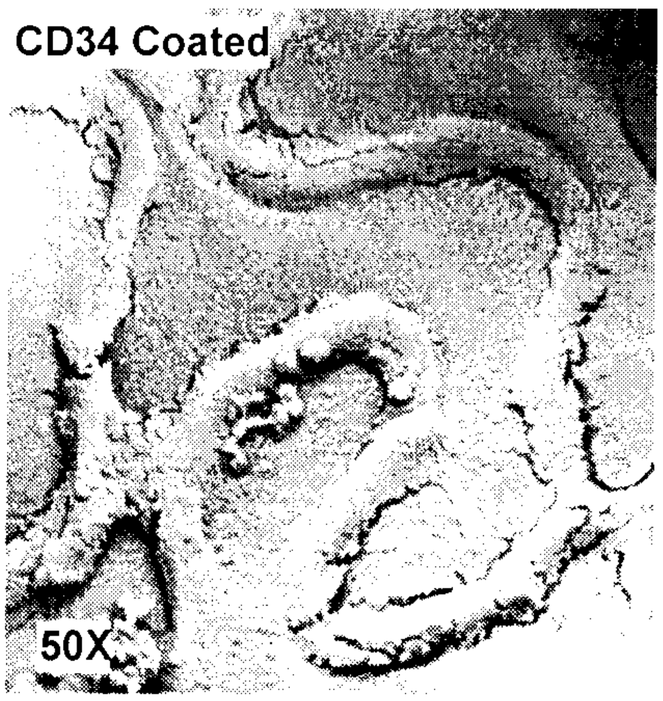


FIG. 14E

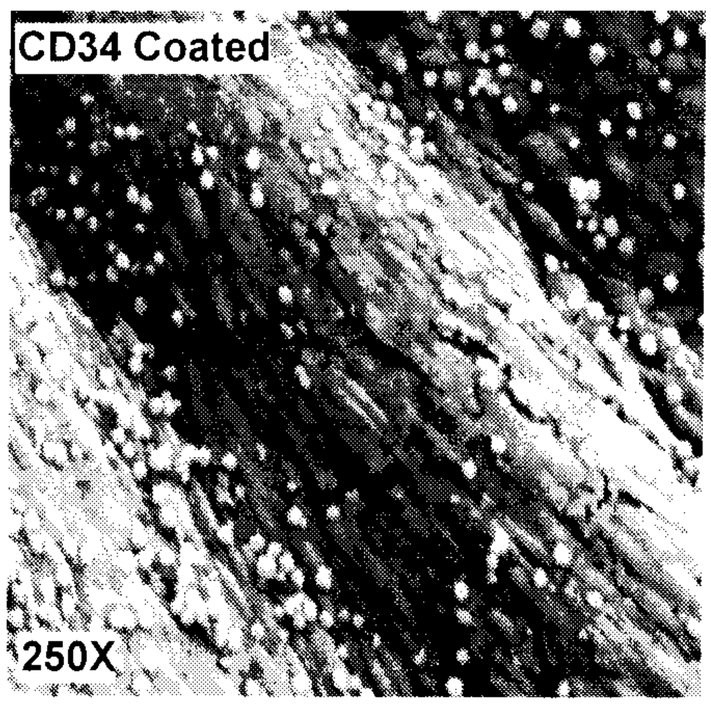


FIG. 14F

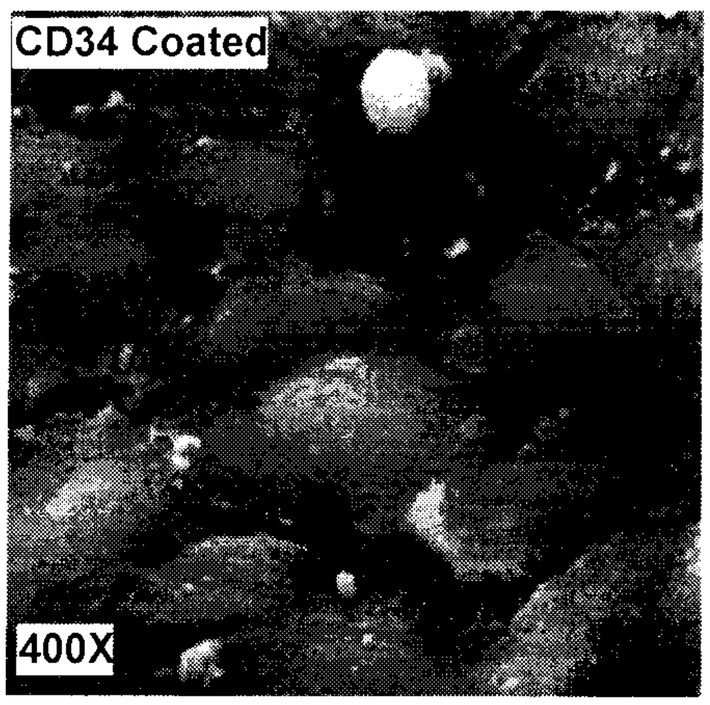


FIG. 14G

In Vivo EPC Capture – 28 Day Histology

FIG. 14H

Bare SST
Control – 4wk

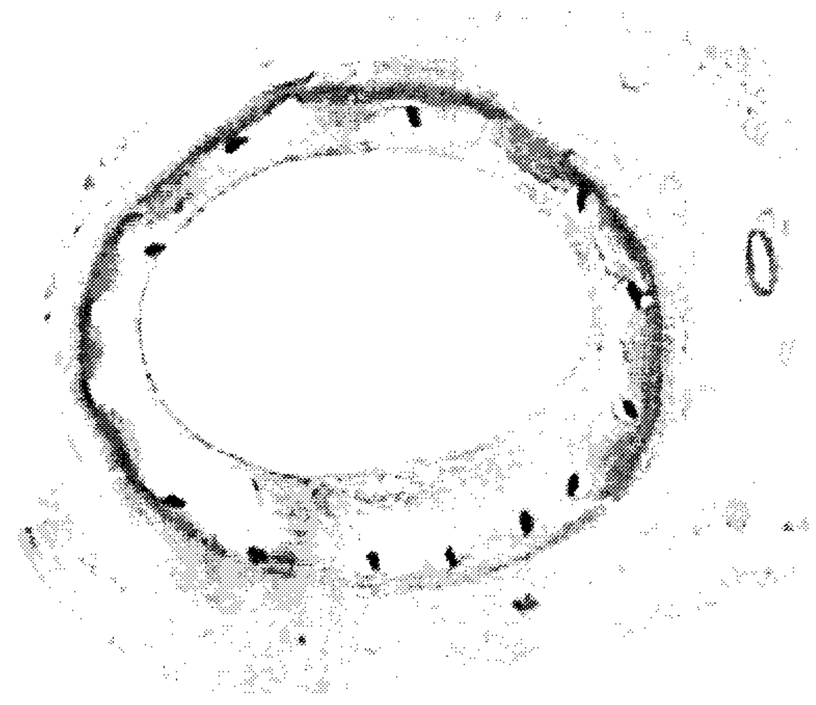


FIG. 14J

Dextran Base Coating -
4wk

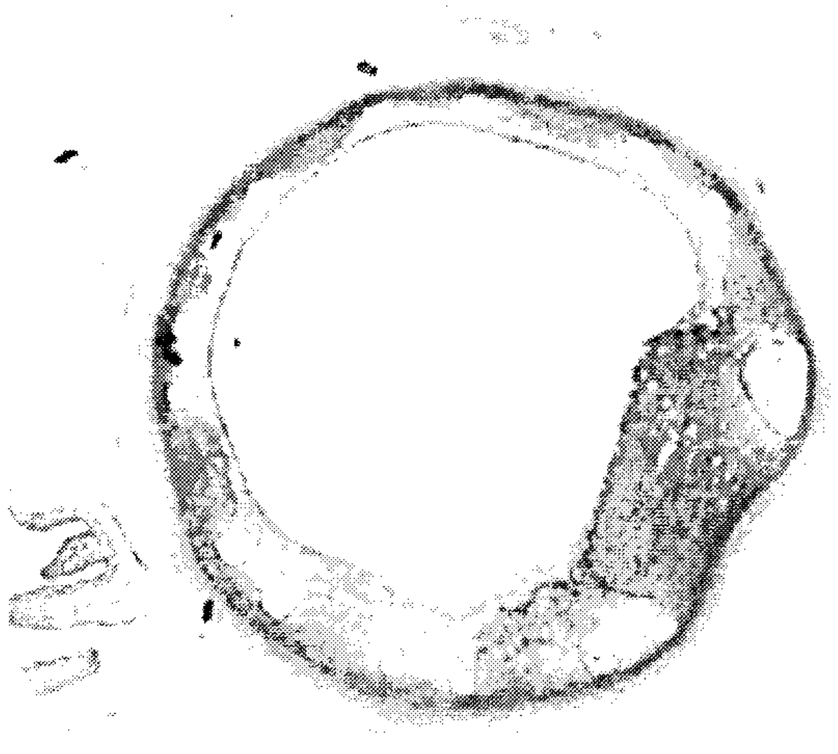
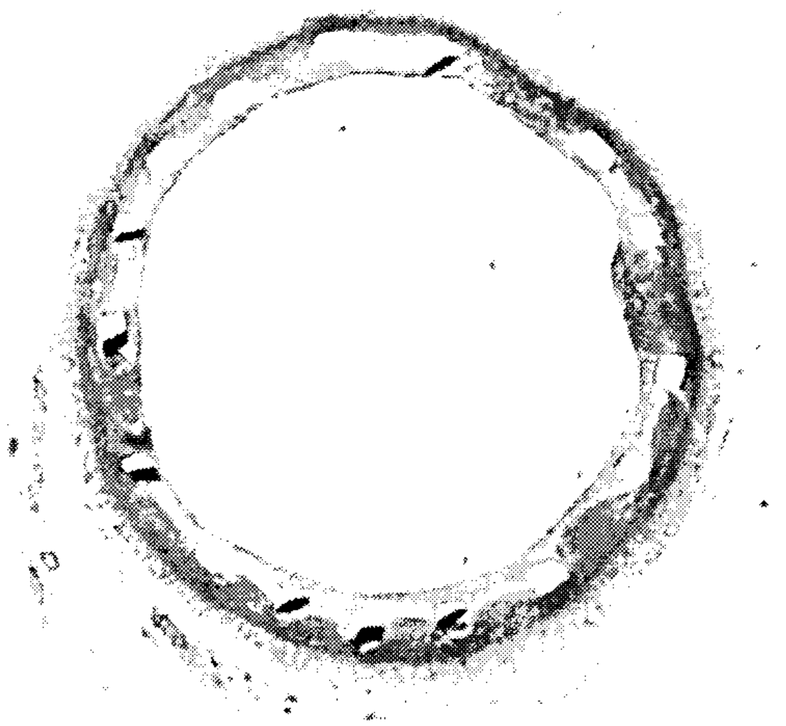


FIG. 14L

Dextran w/ CD34 Ab – 4wk



Bare SST
Control – 4wk

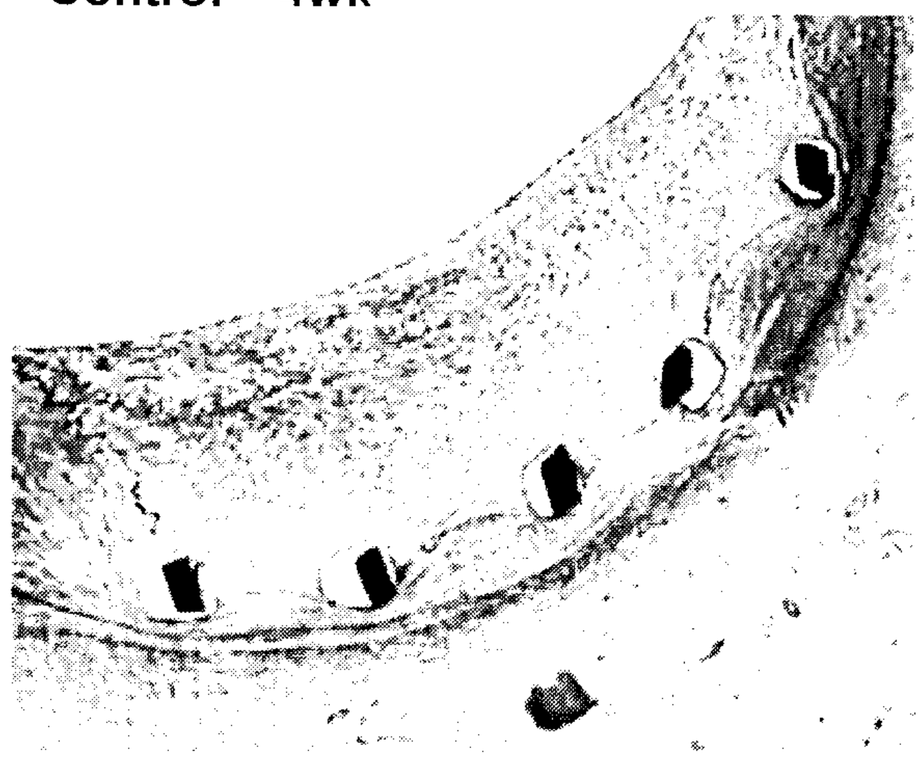


FIG. 14I

Dextran Base Coating -
4wk

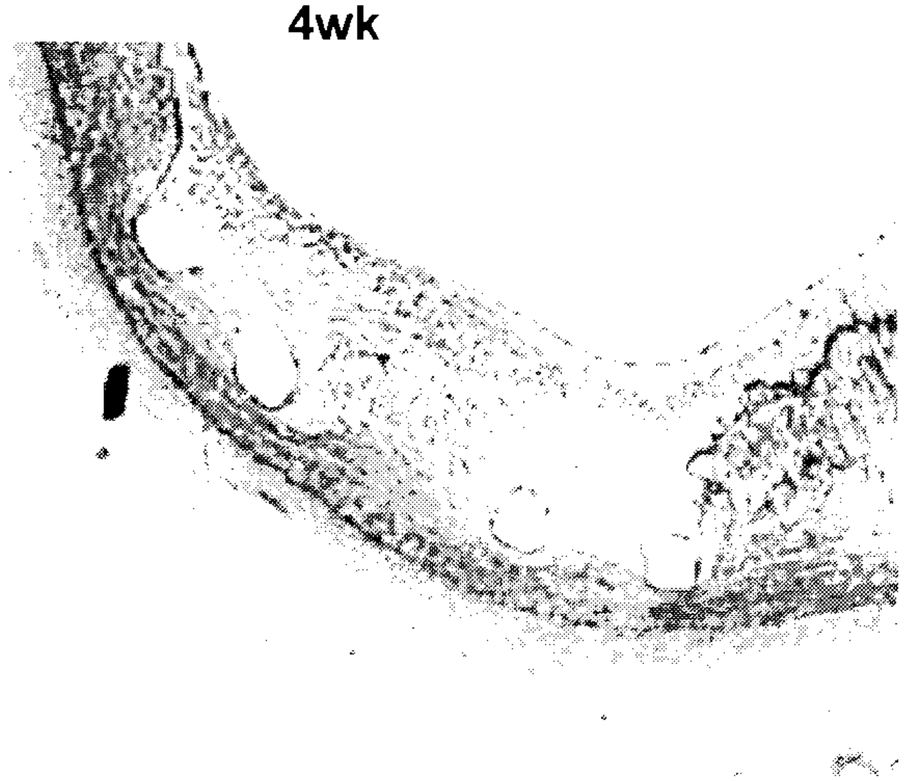


FIG. 14K

Dextran w/ CD34 Ab – 4wk

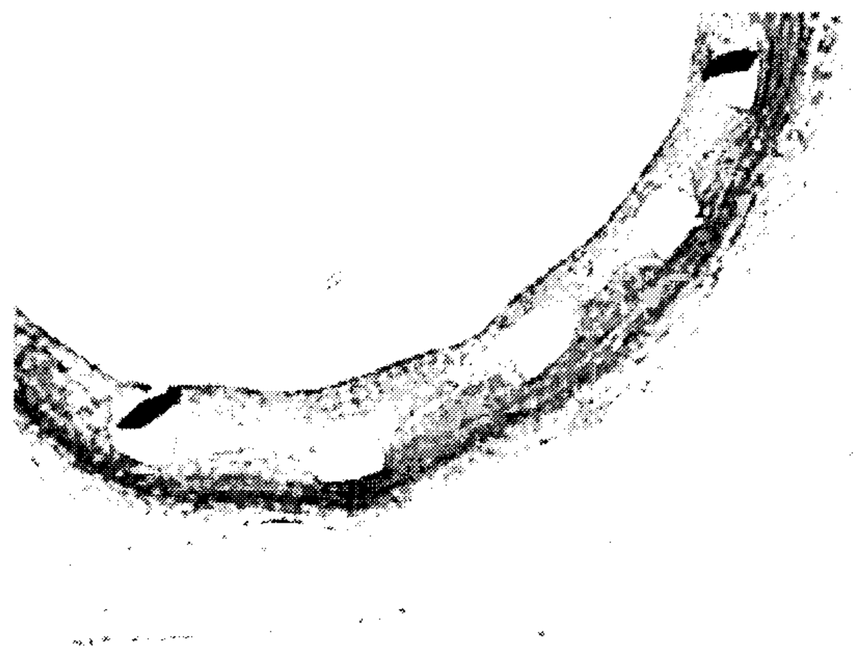


FIG. 14M

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In Vivo EPC Capture

FIG. 15A



3.0 mm R stents with Antibody
48 hours post implant
Stained with anti VERGFR-2

FIG. 15B

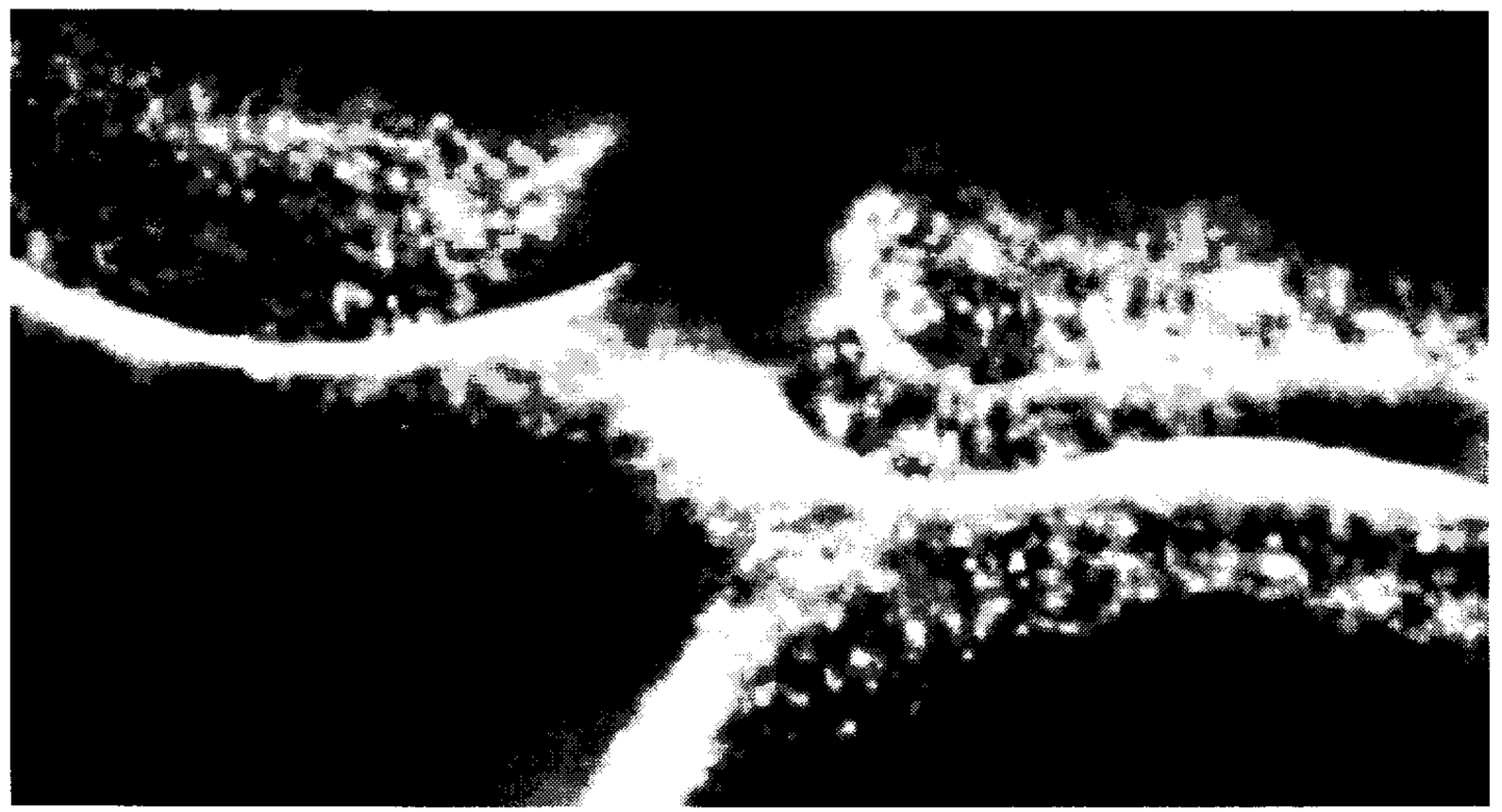
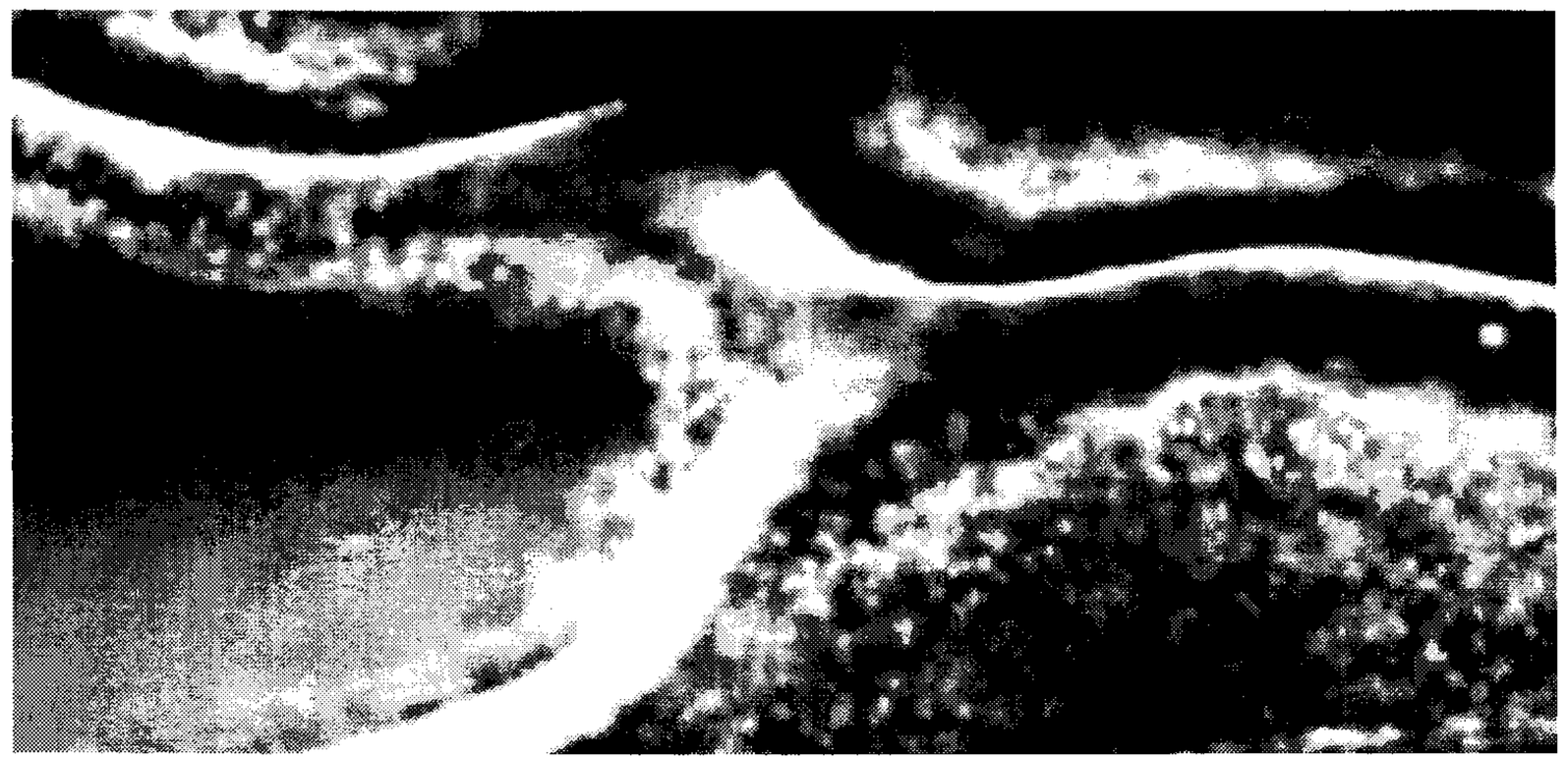
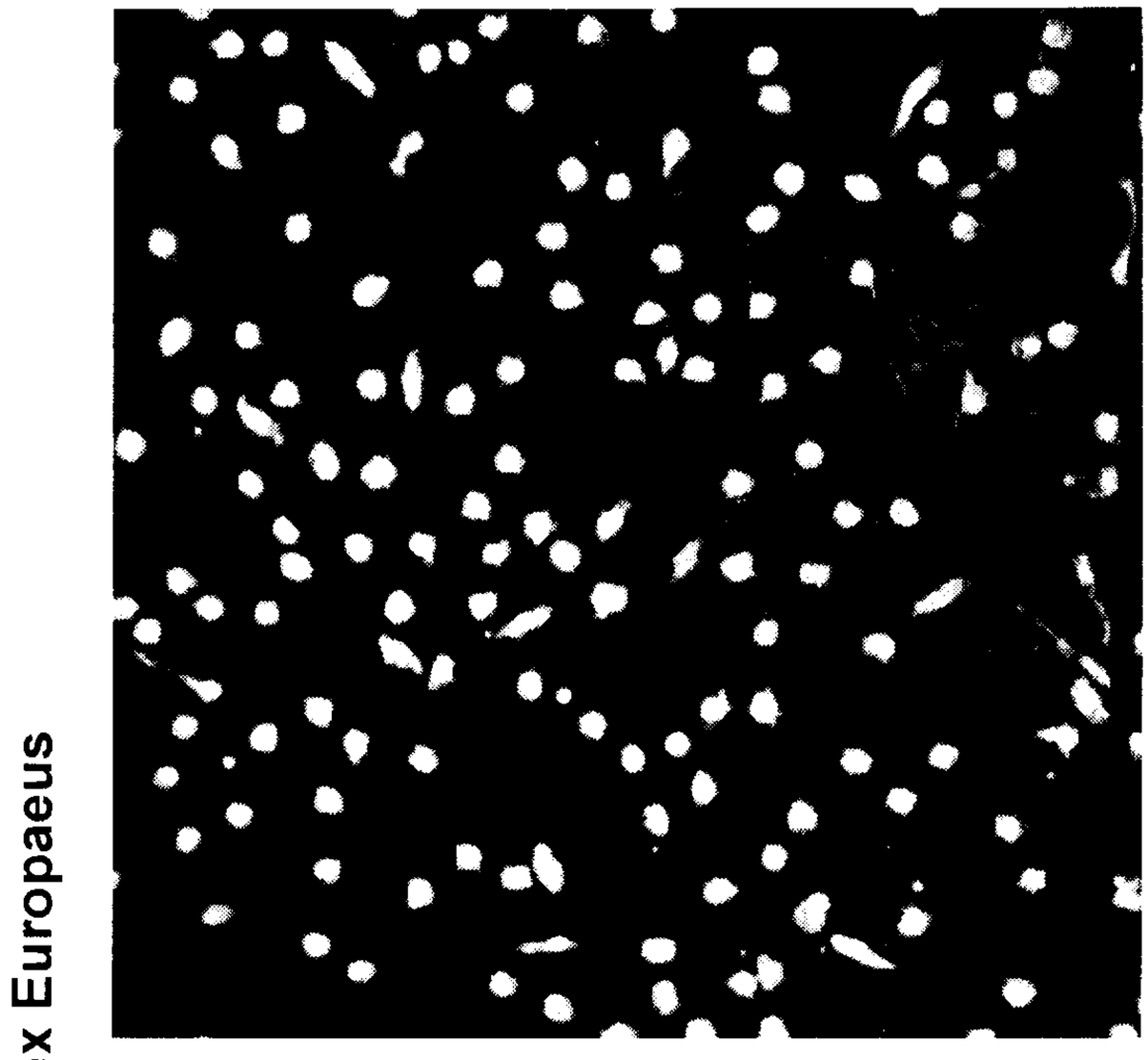


FIG. 15C

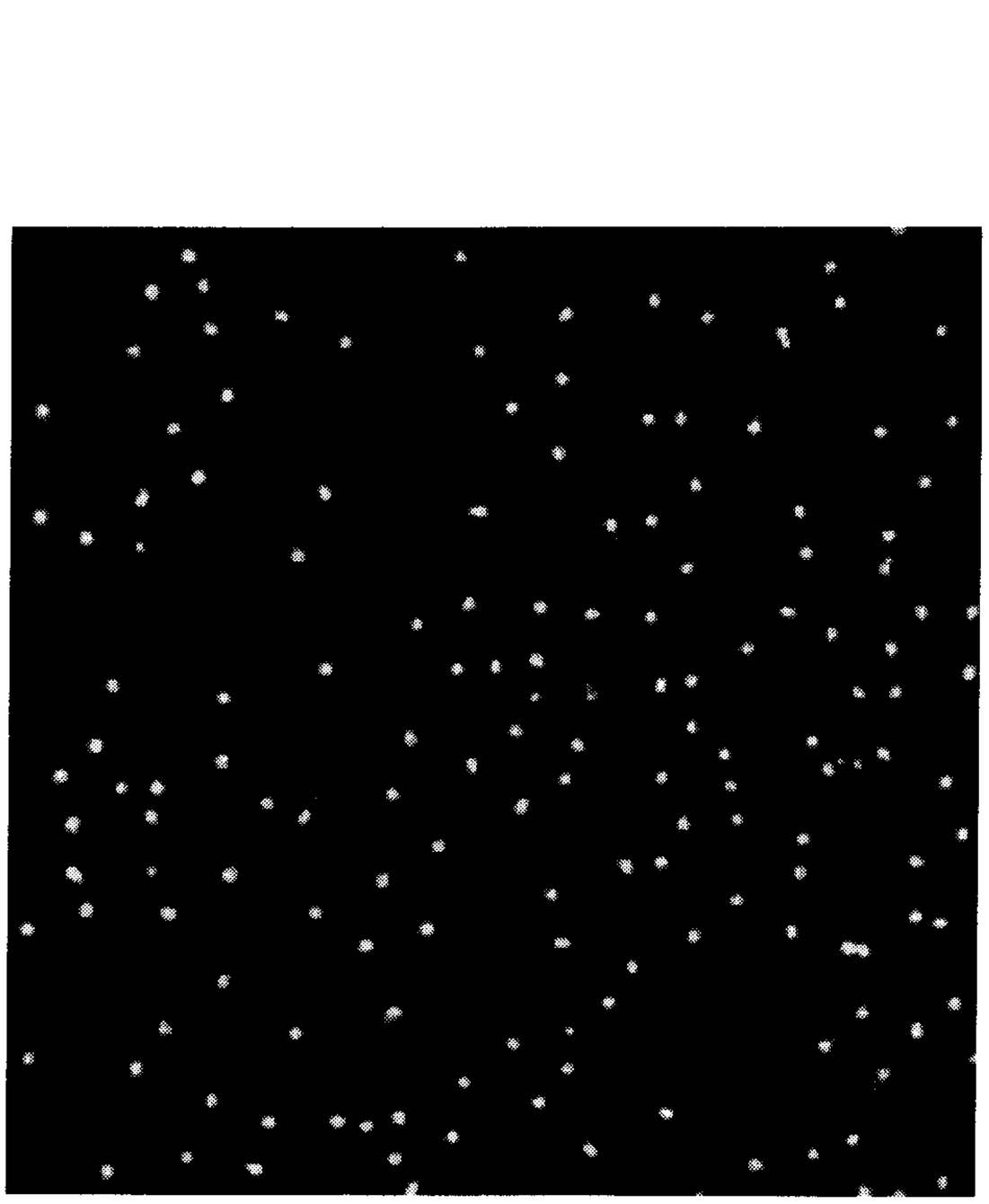


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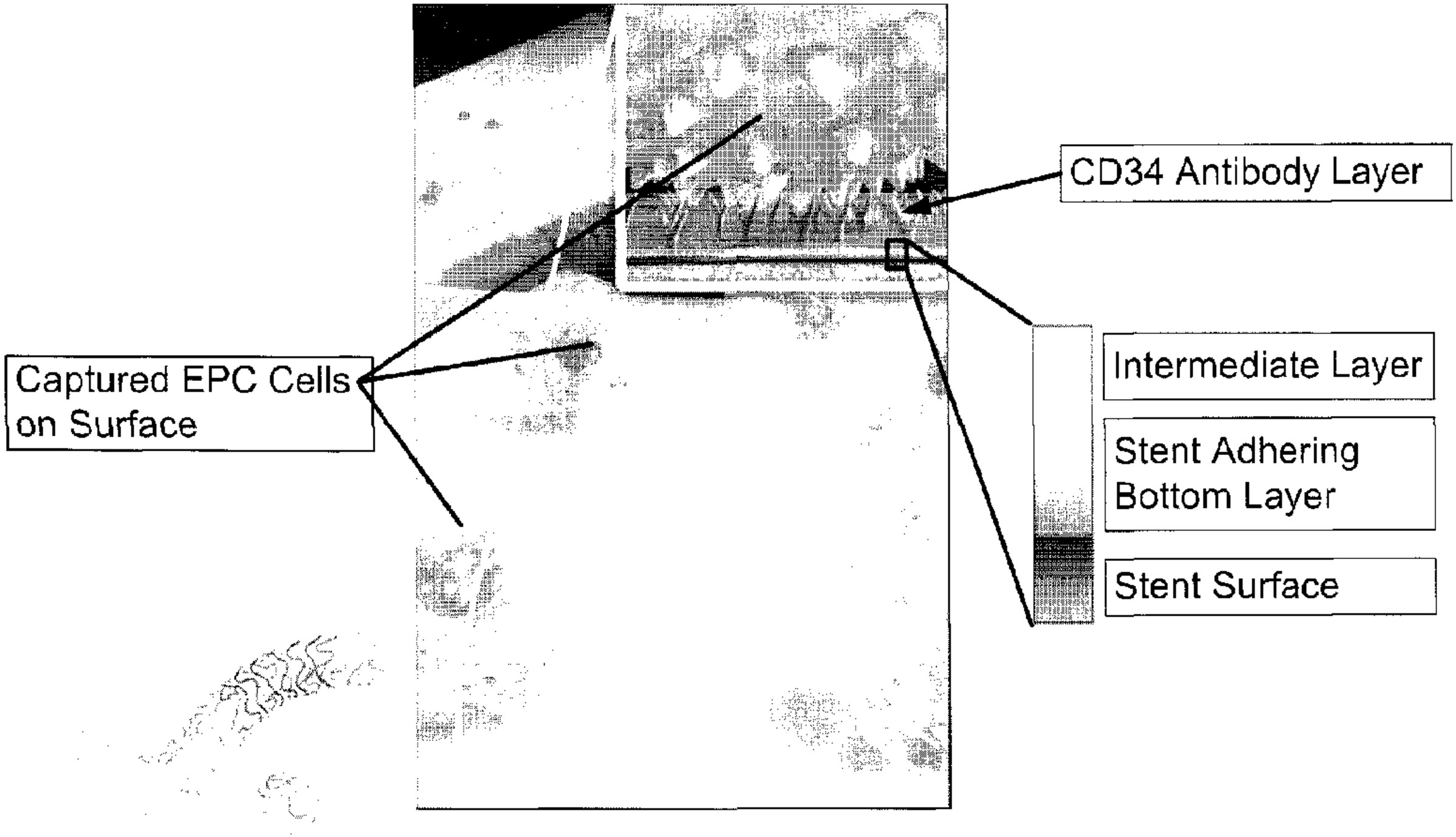
EPCs stained with Ulex Europaeus Lectin/FITC

FIG. 16B



Propidium Iodine

FIG. 16A


CD34 Antibody Layer
Captured EPC Cells on Surface
Intermediate Layer
Stent Adhering Bottom Layer
Stent Surface