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(54) Title: A METHOD FOR STENTING A VESSEL OF A RODENT AND FOR FACILITATING MONITORING OF THE EFFECT OF STENTING A VESSEL OF A RODENT

(57) Abstract: A method is provided for stenting a vessel of a rodent and for monitoring of the effect of a device, procedure or candidate biologically active agent on the vasculature of the rodent, where vasculature includes, without limitation arterial blood vessels. Procedures of interest include stent insertion, balloon angioplasty, drug treatment in conjunction with stent insertion and maintenance, and the like. Also provided are stents suitable for implantation in the vasculature of a rodent.

A METHOD FOR STENTING A VESSEL OF A RODENT AND FOR FACILITATING MONITORING OF THE EFFECT OF STENTING A VESSEL OF A RODENT

BACKGROUND OF THE INVENTION

Field of Invention

This invention relates generally to implantable stents, such as in an arterial blood vessel.

Description of Background

In the year 2000 over 1 million patients underwent percutaneous coronary intervention (PCI), with almost half including the placement of an intra-coronary stent. In general, a stent is a cylindrical device which serves to maintain the lumen within a blood vessel. Typically, stents are made of stainless steel. The stent is delivered to the target blood vessel, often an arterial blood vessel, via an expandable balloon catheter. Coronary artery disease manifests itself as symptoms such as chest pain and breathlessness due to a mismatch in oxygen supply and demand to the heart muscle. PCI is a well recognised treatment for stable coronary artery disease as well as unstable disease and evolving myocardial infarction. However, PCI has been limited by re-stenosis, occurring in 30% to 60% of patients receiving balloon angioplasty, and 20% to 40% of patients who in conjunction with balloon angioplasty also had an intra-coronary stent inserted.

In-stent re-stenosis leads to recurrence of symptoms such as breathlessness or chest pain. In-stent stenosis is the result of the body's natural healing process as a result of injury to the vessel wall from the metallic stent struts and from the expansion of the balloon to high pressures, which is used to expand the stent strut within the blood vessel. Numerous methods have been attempted to reduce in-stent stenosis. To prevent more site-specific drug therapy, avoiding administration of drugs to areas of the body not requiring treatment, stents may be coated with therapeutic agents by suspension from the surface of the stent strut from polymers such as silicones, polyurethanes, polyvinyl alcohol, poly(ethylene-co-vinyl alcohol), polyethylene, poly(lactide-co-caprolactone), hydrogels, substituted methacrylates, poly(ethylene-co-vinyl acetate), and hyaluronic acid. Examples of therapeutic agents include biocompatibles, antithrombotics, anticoagulants, steroids, antiinflammatory agents, cytostatic agents, cytotoxic agents, thrombolytics, monoclonal antibodies, and antifibrosis agents. The currently most utilised drug eluting stents target proliferation of smooth muscle cells. Although highly successful, there is uncertainty over the potential harm these drugs may have in the long term on the natural vessel wall healing. Importantly, there are several other biological pathways which may be investigated as novel molecular targets for limiting re-stenosis.

Experimental evidence from animals such as pig, canine and rabbit indicate that a number of biological factors are involved in the response to stent injury. Vascular injury by balloon angioplasty and the stent struts lead to disruption and loss of the endothelium, platelet aggregation and an acute inflammatory response. These cells release growth factors which activate vascular smooth muscle cells (VSMC). As the VSMCs migrate and proliferate they deposit extra-cellular matrix material, leading to narrowing within the stent within weeks or months after the PCI procedure. In other areas of vascular biology, great progress in understanding the molecular mechanisms surrounding re-stenosis has been made through the use of laboratory mice.

Advances in biotechnology which allow genes to be deleted (knocked out) or up-regulated have made mice the most widely used animals in vascular research.

Mice offer a number of advantages over larger animals. The entire mouse genome has been sequenced, allowing targeted deletion or up-regulation of specific genes. Established models of disease, such as atherosclerosis, are now available, as is the ability to track cells. For example, genetically modified mice which have reporter genes that are expressed specifically by the endothelium (vessel wall lining) or smooth muscle cells may be easily identified using a simple staining procedure. Furthermore, mice are easy to handle and breed, whilst also cost effective.

One example where genetic manipulations have been used as an advantage in mouse models of cardiovascular disease is in atherosclerosis. Genetically modified mice are available with a targeted deletion of the apolipoprotein-E gene, a key regulator of cholesterol metabolism. Such genetically modified mice are generally referred to as ApoE-KO mice. Wild type mice have very low cholesterol levels and are particularly resistant to the atherosclerosis. However, on standard rodent diet ApoE-KO mice have a total cholesterol >10mmol/L, which increases to more than 25mmol/L with a high fat diet. Thus, these mice are prone to the development of typical atherosclerotic lesions in weeks rather than months required by larger animal models. Validated reproducible measurements of atherosclerosis may be taken from the aortic sinus, braciocephalic artery and descending thoracic aorta.

However, because of their small size, and in particular, the small size of the vessels of the arterial, venous and cardiovascular systems of mice, and the difficulty of gaining access to the cardiovascular system of mice, due to the physical size of the heart in mice, it is difficult and in general impossible to successfully insert a stent in a vessel of the arterial and cardiovascular systems of mice.

Methods that permit utilization of small rodents such as mice in the testing and screening of cardiovascular devices and therapeutic agents are of great interest. The present invention addresses this issue.

SUMMARY OF THE INVENTION

Methods are provided for the insertion of a stent in a blood vessel of a rodent, *e.g.* mouse, rat, *etc.*, particularly a mouse. The method comprises inserting a vascular stent into a rodent blood vessel; and transplanting the stented rodent blood vessel into a recipient rodent, thereby constructing a functional interposition graft. By functional graft, it is understood that the graft allows the flow of blood through the lumen of the vessel. The stented rodent is useful in a variety of analytic tests and assays relating to the effects of balloon angioplasty and stenting, stent design and materials, elution of biologically active agents from stents, local and systemic treatments and the like. The invention is further directed towards a rodent having a stent inserted in a vessel thereof. In some embodiments, a stent suitable for insertion into rodent vasculature is provided.

Methods for inserting a stent in a vessel of a rodent comprise installing a stent in a first vessel of a donor rodent, and grafting the stented first vessel from the donor rodent into a second vessel of a recipient rodent, which is optionally immunologically compatible, although in some embodiments the donor and recipient are immunologically mismatched. In one embodiment of the invention the stent is installed in the first vessel prior to removal from the donor rodent, while in an alternative embodiment of the invention the first vessel is removed from the donor rodent prior to the stent being installed therein.

In one embodiment of the invention the second vessel is severed to produce two adjacent severed ends adjacent the severing, between which the stented first vessel is located, and the stented first vessel is grafted onto the respective severed ends of the second vessel.

The second vessel may be clamped at two spaced apart locations on respective opposite sides of the site at which the second vessel is to be severed prior to severing of the second vessel, and advantageously, the second vessel remains clamped at the respective two spaced apart locations until the stented first vessel has been grafted into the second vessel.

In one embodiment of the invention each severed end of the second vessel is passed through a central bore of a corresponding collar, and the respective portions adjacent the severed ends are folded back to embrace an outer periphery of the corresponding collars to form respective plug ends, and preferably, the portion adjacent each severed end embracing the outer periphery of the corresponding collar is secured thereto, and preferably, is secured by a first ligature extending around the portion adjacent the severed end and tied for securing the portion adjacent the severed end to the outer periphery of the collar.

In one embodiment of the invention the respective plug ends formed adjacent the severed ends of the second vessel are inserted into corresponding ends of the stented first

vessel and secured therein. Preferably, each plug end of the second vessel is secured in the corresponding end of the stented first vessel by a second ligature extending around the corresponding end of the stented first end adjacent the plug end and tied thereto for securing the corresponding end of the stented first vessel to the corresponding plug end of the second vessel.

Ideally, each collar is provided with a tab extending in a generally axial direction from one side thereof, and the corresponding portion of the second vessel adjacent the severed end is secured to the collar to form the plug end with the tab extending outwardly from the plug end intermediate the portion adjacent the severed end and a portion of the second vessel extending into the central bore of the collar so that the tab can be gripped by a gripping implement during insertion of the plug end of the second vessel into the corresponding end of the stented first vessel.

Preferably, the stent is installed in the first vessel intermediate the ends thereof, and preferably, spaced apart from the ends thereof, so that respective portions of the first vessel adjacent the ends thereof form respective sockets for receiving the corresponding plug ends of the second vessel creating a functional interposition graft, as known in the art, allowing flow of blood through lumen of the second vessel into the first vessel and back into the second vessel.

In one embodiment of the invention the first vessel is secured to the second vessel creating a functional interposition graft by securing the first vessel to the collar and subsequently securing the second vessel to the first vessel, which is already secured to the collar using ligatures. In another embodiment the ligatures are replaced by adhesive glue or sutures. In another embodiment the first vessel is secured to the second vessel directly by the use of adhesive glue or sutures.

In one embodiment of the invention the first vessel is provided by the thoracic aorta from the donor rodent, and preferably, the second vessel is provided by one of the carotid arteries of the recipient rodent. Advantageously, the stented first vessel is grafted into the carotid artery, and preferably, the common carotid artery. Preferably, the stented first vessel is grafted into the carotid artery intermediate the thoracic inlet and the bifurcation portion thereof.

The invention also provides a method for monitoring the effect of stenting in a vessel of a rodent, the method comprising installing a stent in a first vessel of a donor rodent, and grafting the stented first vessel from the donor rodent into a second vessel of a recipient rodent, so that the effect of stenting can be monitored.

In one embodiment of the invention a stent is provided, which stent is suitable for use with a mouse blood vessel. As used herein, the term stent is used as is known in the art, to refer to a prosthesis which can be inserted and held, when desired, in a lumen of a

vessel or organ in the body. Materials commonly used in stent construction include biologically compatible metals, *e.g.* stainless steel, titanium, tantalum, gold, platinum, copper and the like, as well as alloys of these metals; low shape memory plastic; a shape-memory plastic or alloy, such as nitinol; and the like. Also useful are polymeric biodegradable stents, anastomotic devices, and scaffolds, including synthetic biodegradable or bioerodible porous scaffolds.

A stent of the invention is at least about 1.5 mm in length, usually at least about 2.5 mm in length, and not more than about 5 mm in length, and may be around about 3 mm in length. The stent has a diameter prior to expanding of at least about 0.2 mm, usually around about 0.5 mm; and after expansion has a diameter of at least about 0.5 mm, usually at least about 1.0 mm, and not more than about 1.5 mm, usually not more than about 1.3 mm.

The stent is optionally crimped onto a balloon of length of the order of about 5 to about 10 mm, and having a diameter after inflating of at least about 0.5 mm and not more than about 1.25mm. The balloon may be inflated to a pressure of about 8 atmospheres, for expanding the stent to its expanded diameter. The balloon may be inflated to about 8 atmospheres for expanding the stent for a period in the order of 10 seconds.

In one embodiment of the invention the stent is coated with a medium, which may be a medium for minimising stenosis, or for delivery into the bloodstream or other stream passing through the vessel. In another embodiment the stent is covered with a polymer such as ethylene vinyl alcohol copolymer (EVOH); polybutylmethacrylate; poly(hydroxyvalerate); poly(L-lactic acid); polycaprolactone; poly(lactide-co-caprolactone); poly(lactide-co-glycolide); poly(hydroxybutyrate); poly(hydroxybutyrate-co-valerate); polydioxanone; polyorthoester; polyanhydride; poly(glycolic acid); poly(D,L-lactic acid); poly(glycolic acid-co-trimethylene carbonate); polyphosphoester; polyphosphoester urethane; poly(amino acids); cyanoacrylates; poly(trimethylene carbonate); poly(iminocarbonate); copoly(ether-esters); polyalkylene oxalates; polyphosphazenes; biomolecules; polyurethanes; silicones; polyesters; polyolefins; polyisobutylene and ethylene-alphaolefin copolymers; acrylic polymers and copolymers; vinyl halide polymers and copolymers; polyvinyl ethers; polyvinylidene halides; polyacrylonitrile; polyvinyl ketones; polyvinyl aromatics; polyvinyl esters; copolymers of vinyl monomers with each other and olefins, acrylonitrile-styrene copolymers, ABS resins, and ethylene-vinyl acetate copolymers; polyamides; alkyd resins; polycarbonates; polyoxymethylenes; polyimides; polyethers; epoxy resins; polyurethanes; rayon; rayon-triacetate; cellulose; cellulose acetate; cellulose butyrate; cellulose acetate butyrate; cellophane; cellulose nitrate; cellulose propionate; cellulose ethers; amorphous Teflon; and carboxymethyl cellulose or a combination thereof, but not limited to these polymers.

In a further embodiment of the invention the stent is a drug eluting stent. In another embodiment the stent is a drug coated stent. In another embodiment, a biologically active agent is administered locally or systemically in conjunction with the stent or angioplasty process.

Examples of drugs include, but are not limited to; antiproliferative, antineoplastic, antimitotic, antibiotic, anti-inflammatory, antiplatelet, anticoagulant, antifibrin, antithrombin, antiallergic and antioxidant substances. Antiproliferative substances include, but are not limited to, substances such as actinomycin D, trichostatin, retinoic acid or derivatives/analogs thereof (Sigma-Aldrich, Milwaukee). Antineoplastic or antimitotic substances include, but are not limited to, paclitaxel (TAXOL.RTM, Bristol-Myers Squibb Co., Stamford, Conn.), docetaxel (Taxotere.RTM., Aventis S. A., Frankfurt, Germany), methotrexate, azathioprine, vincristine, vinblastine, fluorouracil, doxorubicin hydrochloride (Adriamycin.RTM, Pharmacia & Upjohn, Peapack N.J.), and mitomycin (Mutamycin.RTM, Bristol-Myers Squibb Co., Stamford, Conn.). Antiplatelet, anticoagulant, antifibrin, and antithrombin substances include, but are not limited to sodium heparin, low molecular weight heparins, heparinoids, hirudin, argatroban, forskolin, vapiprost, prostacyclin and prostacyclin analogues, dextran, D-phe-pro-arg-chloromethylketone, dipyridamole, glycoprotein IIb/IIIa platelet membrane receptor antagonists, recombinant hirudin, and thrombin inhibitors such as Angiomax (Biogen, Inc., Cambridge, Mass.). Antiallergic substances include, but are not limited to, histamine antagonists, corticosteroids, cromoglicate sodium. Anti-inflammatory substances include, but are not limited to, corticosteroids, Non-steroidal anti-inflammatory drugs, Aspirin, Nuclear Factor Kappa B decoy, anti-chemokine agents, anti-tumor necrosis factor antibody, interleukin receptor antagonists and permirolast potassium. Agents which promote vascular healing and re-endothelialization include, but are not limited to, granulocyte macrophage colony-stimulating factor (GM-CSF), erythropoietin, estrogen, tetrahydrobiopterin, HMG-CoA reductase inhibitors, vascular endothelial growth factor, nitric oxide and nitric oxide donors to include, but are not limited to, Diethylamine NONOates, sodium nitroprusside, organic nitrates, S-nitroso-D,L-penicillaminamides, S-nitrosylated proteins or other S-nitrosothiols, hydroxynitrosohydrazino derivates, modified drugs with nitric oxide donor activity such as NO-aspirin (NO-ASA, NCX-4016) other COX-inhibiting nitric oxide donators (CINODs) or nitric oxide-generating enzyme systems, cofactors or substrates. Antioxidant substances include, but are not limited to, methytetrahydrofolate, ascorbic acid, glutathione, sulphur, lipoic acid, uric acid, carotenes, alpha-tocopherol and ubiquinone. Other substances include, but are not limited to, angiopeptin, angiotensin converting enzyme inhibitors such as captopril (Capoten.RTM. and Capozide.RTM, Bristol-Myers Squibb Co., Stamford, Conn.), cilazapril or lisinopril (Prinivil.RTM and Prinzide.RTM, Merck & Co., Inc.,

Whitehouse Station, N.J.), calcium channel blockers, colchicine, fibroblast growth factor (FGF) antagonists, fish oil (omega 3-fatty acid), anti-Platelet-Derived Growth Factor (PDGF) antibody, rapamycin, phosphodiesterase inhibitors, prostaglandin inhibitors, suramin, serotonin blockers, thioprotease inhibitors, triazolopyrimidines and thiazolidinediones. Examples of biologically active agents include, but are not limited to, antibodies, chimeric proteins or decoy inhibitors, genetic material delivered by agents such as viral gene transfer vectors or non-viral transfection methods, oligonucleotides or small interfering RNA molecules. The foregoing substances are listed by way of example and are not meant to be limiting. Other therapeutic substances which are currently available or that may be developed in the future are equally applicable.

In certain embodiments of the invention the recipient rodent is genetically modified, which genetic modification includes, without limitation, targeted deletion or upregulation of a gene, which gene may be involved in one or more cardiovascular pathways or pathologies. The modification may be conditional or constitutive, in either all cell types or in selected cell or tissue types. Genes of interest include, without limitation, the apolipoprotein-E gene, LDL receptor, angiotensin receptor, eNOS, iNOS, nNOS, and chemokines. For example, see Carmeliet and Collen (2000) J. Pathology 190:387-405 and Carmeliet, Moons and Collen (1998) Cardiovascular Research 39:8-33 and Svenson, Bogue and Peters (2003) J Appl Physiol 94:1650-1659, the contents of which are herein specifically incorporated by reference.

The recipient rodent is optionally genetically modified with targeted transgenic overexpression of a reporter gene, including, without limitation, LacZ, GFP, luc, *etc.* In another embodiment the recipient animal is a cell lineage specific knockout (Cre-Lox). In another embodiment the recipient animal genetic modification is inducible and is time and cell specific (eg. tetracycline inducible). Alternatively, one or both of the donor and recipient rodents may be wild-type rodents. In one embodiment of the invention the donor rodent is a mouse, and in another embodiment of the invention the recipient rodent is a mouse.

The recipient rodent and the donor rodent may be similarly genetically modified. Alternatively the recipient rodent and the donor rodent may be differently genetically modified. For example, to track endothelial or smooth muscle cell repopulation, a wild type donor stented vessel may be implanted into a LacZ, GFP, or luc expressing reporter recipient mouse. Conversely, to track endothelial or smooth muscle cell survival, LacZ, GFP, or luc expressing reporter donor mouse stented vessel may be implanted into a wild type recipient mouse. In another example, to determine the temporal relationship of the biological response to stenting, an inducible stented vessel may be implanted into a LacZ, GFP, or luc expressing reporter recipient mouse and induced at various time points or vice

versa. In another example, to determine the lineage of cells in stented vessels after implantation, a specific cell lineage could be flanked by lox p sites in the stented vessel and be implanted into a cre-recombinase expressing recipient mouse or vice versa.

Genetic manipulations have been used in mouse models of cardiovascular disease for cell tracking. Genetically modified mice are available with a targeted insertion of reporter genes that can be used to identify specific cell types. Examples of such genetically modified mice include, but are not limited to, beta-galactosidase (LacZ), green fluorescence protein (GFP) or luciferase (luc) strains. Specific cell types including, without limitation, expression in one or more endothelial cell types, such as the Tie2 gene and/or promoter, expression in one or more smooth muscle cell types, such as the SM22 gene and/or promoter; expression in various cells involved in inflammation, e.g. macrophages, monocytes, lymphocytes, dendritic cells, etc.; and the like. Markers can be readily identified using staining procedures or imaging modalities such as fluorescence microscopy or bioluminescence. Validated reproducible measurements of cell composition may be therefore taken.

In another embodiment either the recipient or donor animal receives transplanted bone marrow, bone-marrow derived cells, or other progenitor cells or stem cells either prior to or after the angioplasty or stent procedure. The transplanted or transferred cells may be derived from wild type or genetically modified animals, or may undergo prior treatment with drugs or gene delivery techniques and may be administered systemically or locally.

The invention also provides a method for monitoring of the effect of a procedure, device, genetic modification, or biologically active agent on a vessel of a rodent, the method comprising carrying out the procedure on a first vessel of a donor rodent, and grafting the first vessel having been subjected to the procedure from the donor rodent into a second vessel of a recipient rodent, so that the effect of the procedure can be monitored. In one such embodiment, the procedure is carried out in the first vessel prior to removal from the donor rodent, while in an alternative embodiment of the invention the first vessel is removed from the donor rodent prior to the procedure being carried out on the first vessel.

Procedures of interest include, without limitation, balloon angioplasty, installation of a stent, *etc.* which may be conducted in the presence or absence of biologically active agents, *e.g.* local or systemic pre-treatment of the animal with a drug of interest; local or systemic administration of a drug of interest following balloon angioplasty, and the like.

The invention further provides a method for testing the results of a procedure carried out on a vessel of a rodent, whereby a procedure is carried out on a first vessel of a donor rodent, and grafted into a second vessel of a recipient rodent according to the method of the invention for carrying out a procedure in a vessel of a rodent, the test method comprising removing the first vessel on which the procedure was carried out from the recipient rodent

after a predetermined test time period, ranging from brief intervals immediately post-procedure to long term effects observable months to years after the stenting procedure, for investigating the effect of the procedure on the first vessel.

Methods of assessment include, without limitation, histology, immunohistochemistry, angiography, computed tomography, intravascular ultrasound, optical coherence tomography, optical bioilluminescence, ultrasonography, doppler ultrasonography, magnetic resonance imaging, immunoblotting, real-time polymerase chain reaction, proteomic or metabolomic analysis, transcriptional and genomic analysis by microrarray or other high-throughput technology, fluorescence activated cell sorting, *etc.*

In one embodiment of the invention the preparation of the second vessel of the recipient rodent prior to grafting the first vessel from the donor vessel into the second vessel is similar to the preparation of the second vessel of the recipient rodent already described for grafting the stented first vessel into the second vessel of the recipient rodent.

The invention also provides a method for testing stenting in a vessel of a rodent whereby a stent is installed in a first vessel of a donor rodent and grafted into a second vessel of a recipient rodent according to the method according to the invention for inserting a stent in a vessel of a rodent, the test method comprising removing the stented first vessel from the recipient rodent after a predetermined test period for investigating the effect of stenting of the first vessel.

The invention also provides a recipient rodent having a stented first vessel grafted into a second vessel of the recipient rodent, and preferably, the stented first vessel is grafted into a carotid artery of the recipient rodent, and ideally, the stented first vessel comprises a portion of a thoracic aorta removed from a donor rodent, which preferably, is stented prior to being removed from the donor rodent.

The invention also provides a recipient rodent having a first vessel on which a procedure was carried out grafted into a second vessel of the recipient rodent, and preferably, the first vessel is obtained from a donor rodent; and the procedure is carried out in one embodiment of the invention prior to removal of the first vessel from the donor rodent, and in an alternative embodiment of the invention the procedure is carried out subsequent to removal of the vessel from the donor rodent.

In a further embodiment of the invention the first vessel is grafted into a carotid artery of the recipient rodent, and ideally, the first vessel comprises a thoracic aorta of the donor rodent. In one embodiment of the invention the procedure is a balloon angioplasty, and in another embodiment of the invention the procedure comprises the installation of a stent in the first vessel.

Brief Description of the Drawings

The invention will be more clearly understood from the following description of an embodiment thereof, which is given by way of example only, with reference to the accompanying drawings, in which:

Fig. 1 is a partly cutaway perspective view of a second vessel of a recipient rodent into which a first stented vessel from a donor rodent has been grafted by a method according to the invention for inserting a stent in a vessel of a rodent,

Fig. 2 is a perspective view of a portion of the second vessel of the recipient rodent of Fig. 1 during the carrying out of the method according to the invention,

Fig. 3 is a partly cutaway perspective view of the first stented vessel of Fig. 1,

Fig. 4 is a partly cutaway perspective view of the first vessel of Fig. 1 with a stent being inserted therein,

Fig. 5 is a perspective view of a collar used in the method according to the invention for inserting a stent in a rodent, and

Fig. 6 is a perspective view of a portion of the second vessel of Fig. 1 being engaged in the collar of Fig. 5.

Fig. 7 is a perspective view of the stent apparatus

DETAILED DESCRIPTION OF THE EMBODIMENTS

Referring to the drawings, there is illustrated a first stented vessel grafted into a second vessel of a recipient rodent by a method according to the invention for inserting a stent in a vessel of a rodent. The second vessel including the grafted first stented vessel are indicated generally by the reference numeral 1 in Fig. 1. In this embodiment of the invention the recipient rodent is a recipient mouse (not illustrated) which may be a wild mouse or may or may not be a genetically modified mouse, which may be an ApoE-KO mouse with a targeted deletion of the apolipoprotein-E gene, a mouse expressing reporter genes (LacZ, GFP, luc) an inducible mouse or a mouse expressing cre, in order to facilitate monitoring the effect of stenting in the vessel, and in particular, for monitoring the onset or otherwise of stenosis after stenting. Needless to say, the mouse if genetically modified may be otherwise genetically modified, depending on the tests or experiments to be carried out on the mouse. The first vessel in this embodiment of the invention is provided by a portion of a thoracic aorta 5, which has been removed from a donor mouse (not shown), which may be a wild mouse or may or may not have been genetically modified, and if genetically modified, may be similarly or differently genetically modified to the genetic modification of the recipient mouse, depending on the tests or experiments to be carried out on the recipient mouse. Prior to removal of the portion of the thoracic aorta 5, the thoracic aorta is stented, as will be described below, and is then removed from the donor mouse and grafted

into the second vessel, which in this embodiment of the invention is the common carotid artery 6 of the recipient mouse.

Prior to removal from the donor mouse, the thoracic aorta is severed from the heart. An incision is formed in the thoracic aorta adjacent the diaphragm of the donor mouse, and a stent 8, which is crimped onto a balloon 9 of a balloon catheter 10 is inserted into the thoracic aorta 5 either from the end of the thoracic aorta 5 which has been severed from the heart or through the incision. The balloon 9 with the stent 8 crimped thereon is located in the thoracic aorta so that the stent 8 is substantially centrally located between the severed end which has been severed from the heart, and the incision formed in the thoracic aorta 5 adjacent the diaphragm. This, thus, leaves end portions 11 of the thoracic aorta 5 free of the stent 8 to form respective sockets 12 at the respective opposite ends of the thoracic aorta 5 for facilitating grafting of the thoracic aorta 5, when removed from the donor mouse, into the carotid artery 6 of the recipient mouse, as will be described below. With the stent 8 centrally located in the thoracic aorta 5 by the balloon 9, the balloon 9 is inflated for expanding the stent 8 into the thoracic aorta 5.

In this embodiment of the invention the stent 8 is a stainless steel slotted tubular stent of approximately 2.5mm in length, having an expanded diameter of 1.3mm to 1.5mm. The stent 8 in the unexpanded state is of diameter of approximately 0.5mm when crimped onto the balloon 9. The balloon 9 is of length of approximately 10mm and of diameter when inflated of approximately 1.25mm. The balloon 9 is inflated to a pressure of approximately 8 atmospheres for approximately 10 seconds for expanding the stent 8 to its expanded diameter of between 0.5 mm and 1.5mm within the thoracic aorta 5, see Fig. 4.

With the stent 8 installed in the thoracic aorta 5, the balloon 9 is deflated and removed along with the balloon catheter 10. On completion of stenting, the thoracic aorta 5 is severed adjacent the incision which had been formed for accommodating the balloon catheter 10, and is removed from the donor mouse for grafting into the carotid artery 6 of the recipient mouse. By severing the stented thoracic aorta 5 at the incision which had been formed adjacent the diaphragm, the end portions 11 which are free of the stent 8 form the respective sockets 12 at the respective opposite ends of the removed stented thoracic aorta 5 for facilitating grafting of the removed portion of the thoracic aorta 5 into the carotid artery 6, as will be described below.

The recipient mouse is prepared for grafting of the stented thoracic aorta 5 into the carotid artery 6. An incision is made in the neck of the recipient mouse to expose the common carotid artery between its thoracic inlet and its bifurcation portion, and its carotid artery is clamped at the thoracic inlet and its bifurcation end to prevent blood flow there through. The clamping of the carotid artery 6 is not illustrated, but will be understood by

those skilled in the art. The carotid artery 6 is then severed intermediate the clamped portions to form respective severed ends 13.

A pair of collars 15 of polypropylene material each having an external diameter of 0.65mm and a central bore 16 extending there through are provided to form the respective severed ends 13 of the carotid artery into plug ends 17 for plugging into the corresponding sockets 12 formed in the stented thoracic aorta 5, as will be described below, see Figs. 2, 5 and 6. Initially, each severed end 13 is passed through the bore 16 of the corresponding collar 15 as illustrated in Fig. 6, and a portion 18 adjacent the corresponding severed end 15 is folded back over the corresponding collar 15, so that the folded portion 18 extends circumferentially around and embraces the collar 15, with the collar 15 effectively sandwiched between a portion of the carotid artery 6 extending through the bore 16 and the folded portion 18. The folded portion 18 adjacent each severed end 13 is secured to the corresponding collar 15 by a ligature, namely, a first suture 19 extending around the folded portion 18 and tied for securing the folded portion 18 tightly on an outer surface 20 of the collar 15 for forming the plug end 17. The collar 15 co-operates with the first suture 19 for retaining the folded portion 18 of the carotid artery 6 adjacent the corresponding severed end 13 securely on the collar 15. A tab 21 extends in a generally axial direction from each collar 15, and the folded portions adjacent the severed ends 13 are secured to the respective collars 15 with the tabs 21 extending rearwardly between the folded portions 18 of the corresponding severed ends 13 and the portions of the carotid artery 6 extending through the bore 16 for facilitating gripping of the plug end 17.

When the respective severed ends 13 of the carotid artery 6 have been secured to the corresponding collars 15 to form the respective plug ends 17, the stented thoracic aorta 5 is located between the severed ends 13 of the carotid artery 6 of the recipient mouse, and the plug ends 17 are engaged in the sockets 12 defined by the respective opposite ends of the stented thoracic aorta 5. The portions 11 forming the sockets 12 of the stented thoracic aorta 5 are then sealably secured to the plug ends 17 by second ligatures, in this embodiment of the invention second sutures 25 which extend around the portions 11 of the sockets 12 and are tied. With the stented thoracic aorta 5 grafted into the carotid artery 6, the clamps at the thoracic inlet and the bifurcation portion of the carotid artery 6 are released and the incision in the neck of the mouse is sutured.

The recipient mouse is now ready to be subjected to predetermined feeding regimes, exercising regimes, and other regimes required to carry out desired tests in order to monitor the effect of stenting on the recipient mouse. The mouse is subjected to such regimes for a predetermined time period, and at the end of the predetermined time period the stented aorta 5 is removed from the carotid artery 6 to facilitate tests and investigations to be carried out in order to determine the effect of stenting of the stented thoracic aorta 5.

These include, but are not limited to, imaging of stented vessels using high throughput imaging technologies such as intravascular ultrasound, optical coherence tomography, computed tomography, and optical bioilluminscence.

Typically, a number of recipient mice are prepared with stented thoracic aortas from donor mice grafted into the common carotid arteries of the respective recipient mice. Additionally, in order that comparative tests may be carried out on the mice which have been subjected to stenting of the thoracic aorta, and mice which have been subjected to balloon angioplasty of the thoracic aorta, and also in order that comparative tests may be carried out on control mice, recipient mice having thoracic aortas which have been subjected to balloon angioplasty are prepared using the method of the invention, and control mice are also prepared using the method according to the invention.

To prepare the mice with thoracic aortas subjected to balloon angioplasty, the thoracic aortas of donor mice are subjected to balloon angioplasty in similar manner as the manner in which the thoracic aortas of donor mice are stented. The thoracic aorta of each donor mouse is severed from the heart of the mouse, and an incision is formed in the thoracic aorta adjacent the diaphragm. The thoracic aorta is then subjected to balloon angioplasty by inserting a balloon on a balloon catheter into the thoracic aorta either through the severed end or through the incision. After subjecting the thoracic aortas of the donor mice to balloon angioplasty, the respective thoracic aortas are removed from the donor mice, and grafted into the recipient mice as already described with reference to Figs. 1 to 7.

In the preparation of the control mice, the thoracic aorta of each donor mouse is removed by severing the thoracic aorta adjacent the heart and adjacent the diaphragm. The removed thoracic aorta is then grafted into the common carotid artery of the respective recipient mice as already described with reference to Figs. 1 to 7.

The following is a description of experiments which have been carried out on mice using the method according to the invention, whereby stented donor thoracic aortas were grafted into the carotid arteries of some recipient mice to determine the time course of the vascular injury response to stenting, stented vessels. To investigate the biological utility of the model stented donor thoracic aortas and donor thoracic aortas which were subjected to balloon angioplasty were grafted into the carotid arteries of other recipient mice. In the description of the following experiments, the effect of stenting donor thoracic aortas grafted into the carotid arteries of recipient wild mice and recipient genetically modified mice has been investigated, and comparisons have also been made to demonstrate the efficacy of stenting as opposed to balloon angioplasty.

In one experiment stented donor thoracic aortas were grafted into the common carotid arteries of a number of wild mice using the method of the invention described with

reference to Figs. 1 to 7, and harvested 1 day, 7 days, 14 days or 28 days post-operatively to determine the time course of vascular injury response to stenting in rodents using this technique. Stented thoracic aortas were harvested and embedded into a plasticizing resin and sectioned using a diamond tip rotary blade. Although this is a technically complex method of histology, it has the advantage of allowing sectioning of the stent struts, thus maintaining exact vessel architecture.

An evaluation of the sectioned pieces revealed that balloon deployment of the stent provided symmetrical stent expansion and uniform stretching of the donor thoracic aorta around the stent struts leading to the formation of a clear intima producing moderate in-stent stenosis. Stent expansion increased the total vessel wall area by approximately 50% compared with reference aorta. It is possible to appreciate the tissue architecture including an endothelial layer and a cellular neointima.

Neointimal hyperplasia increased progressively over the 28 day time period as evidenced by measures of both neointimal thickness and neointimal area (Table 1). Neointimal thickness also increased during the 28 day period, following a similar pattern to the measurements of neointimal area (Table 1). Total medial area remained consistent through the 4 time points and a progressive increase in the neointima to media ratio over time was also observed (Table 1). Consistent with these findings, luminal area decreased between day 1 and day 28, equating to in-stent stenosis of 1.3±0.2, 10.8±1.5, 15.6±1.4 and 32.9±2.0 percent on days 1, 3, 7 and 28 respectively (Table 1). Stent expansion was not different between the various time points, indicating that in-stent stenosis was the result of neointimal hyperplasia. Furthermore, injury scores were not different amongst the time intervals (Table 1).

Histological analyses of stented vessels harvested at the four time points are shown in Table 2. One day following stent implantation, the luminal surface of the stented vessel was covered with a monolayer of leucocytes (Table 2), and a thin adherent monolayer of platelets (Table 2). Small clusters of proliferating cells were identified within the media. Monocyte/ Macrophage staining was negative. By 7 days, mural thrombi surrounded the stent struts (Table 2). Leucocytes and platelets were present on the injured surface and lined the organizing thrombus/neointima, which also contained some macrophages and a high proportion of proliferating cells (Table 2). At 14 days, the localized thrombus was no longer present, and a defined neointima (Fig. 2), which stained positively for SMC α-actin was seen. Masson trichrome staining revealed that a proportion of the neointima was composed of collagen and elastin (Table 2). Monocyte/macrophage staining increased and was predominant in the peri-strut regions (Table 2). Furthermore, the total number of proliferating cells at 14 days was similar to 7 days, yet as a proportion of the neointima markedly decreased (Table 2). Platelet staining was absent. Twenty eight days following

the procedure a marked neointima was present leading to moderate in-stent stenosis. The proportion of the neointima which stained positive with α-actin increased, as did the collagen and elastin content of the neointima (Table 2). Monocyte/ macrophage and cell proliferation staining both decreased compared with 7 and 14 days.

Based on these observations, a further experiment was carried out to address two important questions that would demostrate similarity in a model to human PCI: firstly, to determine the effect of hypercholesterolemia and atherosclerosis on stenting by comparing wild type mice to genetically modified hypercholesterolemic atherosclerotic ApoE-KO mice, and secondly, to compare the effects of stenting, balloon angioplasty and grafting alone (control mice) on wild mice and ApoE-KO mice.

In this experiment stented donor thoracic aortas were grafted into the common carotid arteries of a number of wild mice using the method of the invention described with reference to Figs. 1 to 7, and donor thoracic aortas which had been subjected to balloon angioplasty were grafted into the common carotid arteries of a number of other wild mice, also using the method of the invention described with reference to Figs. 1 to 7. Twenty-eight days later the stented thoracic aortas and the thoracic aortas which had been subjected to the balloon angioplasty were harvested and embedded into a plasticizing resin and sectioned using a diamond tip rotary blade.

Three weeks after birth ApoE-KO mice and wild mice were weaned onto normal rodent diet and at eighteen to twenty-two weeks some of the mice underwent stenting using the method of the invention described with reference to Figs. 1 to 7, others underwent balloon angioplasty also using the method according to the invention described with reference to Figs. 1 to 7, and the remainder of the mice had thoracic aortas from donor mice which had not been subjected to any procedures grafted into the remaining recipient mice using the method according to the invention described with reference to Figs. 1 to 7, in order to provide control mice. Accordingly, recipient ApoE-KO mice and wild mice were provided with stented thoracic aortas, ApoE-KO mice and wild mice were produced having thoracic aortas which had been subjected to balloon angioplasty, and ApoE-KO mice and wild mice were provided which had been subjected to grafting of donor thoracic aortas which had not been subjected to any procedure to provide control mice.

The stented thoracic aortas, the thoracic aortas which had been subjected to balloon angioplasty, and the grafted only thoracic aortas (control) were harvested four weeks later and processed for histology as already described.

From the histological sections several parameters were quantified to compare the groups. Intimal area defined as the area inside the internal elastic lamina, lumen area, vessel area, defined as the area inside the external elastic lamina and stent expansion, the area inside the stent struts.

In a first study the effects of stenting in wild-type mice were compared against atherosclerotic ApoE-KO mice. Four weeks postoperatively, histological analysis in stented vessels of wild type control mice revealed that stent expansion created clear neointimal hyperplasia. However, in atherosclerotic ApoE-KO mice, neointimal hyperplasia was increased. Intimal area in ten mice was quantified for comparing wild type to ApoE-KO. Despite equal stent expansion, intimal area was increased by 30% in ApoE-KO group. In addition it was observed that both stent expansion and intimal hyperplasia were highly reproducible. These findings suggest that hypercholesterolemia and atherosclerosis in the ApoE-KO mice significantly increase in-stent stenosis, demonstrating this technique can utilize genetic modification to investigate the role of specific genes in re-stenosis.

Due to the results of the first study, a second study was carried out in order to compare the process of stenting to balloon angioplasty or grafting only (control) in wild-type mice versus ApoE-KO mice. Stenting was carried out using the method according to the invention described with reference to Figs. 1 to 7, and balloon angioplasty was carried out also using the method according to the invention described with reference to Figs. 1 to 7. Donor thoracic aortas which were not subject to any procedure from donor mice were grafted into the common carotid arteries of recipient mice using the method according to the invention described with reference to Figs. 1 to 7 to provide the control mice.

Four weeks postoperatively, histological analysis of the grafted only thoracic aortas (control) revealed minimal intimal hyperplasia with appearance identical to the native mouse thoracic aorta in both wild-type mice and ApoE-KO mice. Balloon angioplasty had little effect in wild-type mice, however, in ApoE-KO mice after balloon angioplasty there was significant intimal hyperplasia. Despite the balloon stretch, there was no difference in total vessel area compared to control. Consequently, the intimal response to balloon angioplasty caused significant luminal narrowing in ApoE-KO mice but not in wild-type mice. In stented thoracic aortas, intimal hyperplasia was increased compared to balloon angioplasty. However, stenting greatly increased total vessel area. As a result, lumen area remained significantly enlarged despite intimal hyperplasia.

In summary, therefore, it has been found that balloon angioplasty and stenting in the mice using the method according to the invention described with reference to Figs. 1 to 7:
results in morphometric vessel changes similar to human PCI.
results in increased in-stent stenosis in the genetically modified atherosclerotic apolipoprotein-E knockout mice compared with unmodified wild type control mice.

The method according to the invention provides an opportunity impossible in other animal models to directly investigate the role of other key genes in re-stenosis using knockout and transgenic mice.

Using the method according to the invention it is also possible to track endothelial cell loss and re-population using reporter genes specific to endothelial cells, and investigate the role of endothelial progenitor cells in this process, which has previously been unexplored, and it will also be possible to investigate drug eluting stents (including polymers, biologics, drugs, and materials), and although this may be done in other animal models, the method according to the invention provides the opportunity to conduct pre-clinical animal trials quickly (one month) as with mice with ApoE-knockout gene develop atherosclerosis in weeks compared to months required in larger animal models. Using the method according to the invention it is also possible to perform high throughput genomic studies using whole genome microarray of the stented vessel. Furthermore it is also possible to perform high throughput genomic studies on specific cell types within the stented vessel using laser capture microdissection. It is further possible to understand the biological response of the artery wall, with regard to specific cell types, to current drug eluting stents. It is also possible to use temporal and cell-specific inducible transgenics to invesitigate critical timepoints in the response to vascular healing after balloon angioplasty and stenting. It is further possible to investigate the biological origin of neointimal cells in in-stent restenosis using cre-lox technology.

While the method according to the invention has been described in conjunction with mice, it will be readily apparent to those skilled in the art that the method according to the invention may be carried out on rats or any other rodent. It is also envisaged that other donor vessels besides the thoracic aorta may be stented and grafted into other vessels of recipient rodents besides the carotid artery. However, the advantage of stenting the thoracic aorta is that it is the largest arterial vessel in a rodent, and thus is more suitable for stenting. The advantage of grafting the stented vessel into the carotid artery is that the carotid artery is a relatively easily accessible vessel.

While the methods according to the invention have been described in conjunction with wild-type mice and genetically modified mice, namely, ApoE-KO mice, the methods according to the invention may be carried out on mice which are otherwise genetically modified. Additionally, while the stenting and balloon angioplasty has been carried out on the donor thoracic aortas while still in the donor mice, it will be readily apparent to those skilled in the art that the stenting and balloon angioplasty of the donor thoracic aorta could be carried out after removal of the thoracic aorta from the donor mice.

While in the method described for evaluating the effects of stenting and balloon angioplasty on the thoracic aortas which were harvested from the recipient mice, the harvested thoracic aortas were embedded into a plasticizing resin and sectioned using a diamond tip rotary blade, the plastizising resin may be removed after sectioning, and this

allows for immunohistochemical staining to identify specific cells, antigenic epitopes or molecules.

The invention is not limited to the embodiment hereinbefore described, which may be varied in construction and detail.

Table 1: **Histomorphometric characteristics of neointimal response to stenting in wild type mice (time course).**

	Day 1	Day 7	Day 14	Day 28
Total vessel area, mm^2	1.01±0.06	0.95±.04	0.97±0.02	1.08±0.03
Neointimal Thickness, μm	5.6±1.8	31±8.5*	48±10	110±4.1*†
Neointimal area, mm^2	0.01±0.00	0.07±0.01*	0.10±0.01	0.26±0.01*†
Medial Area, mm^2	0.12±0.02	0.13±0.01	0.11±0.01	0.18±0.01
N/M ratio	0.09±0.02	0.58±0.10*	0.93±0.10	1.52±0.11*†
Lumen Area, mm^2	0.74±0.05	0.59±0.03	0.57±0.03	0.54±0.04‡
% Stenosis	1.3±0.2	10.8±1.5*	15.6±1.4	32.9±2.0*†
Stent Expansion, mm^2	0.77±0.05	0.73±0.03	0.75±0.03	0.80±0.02
Injury Score	1.02±0.25	1.11±0.45	1.08±0.18	1.30±0.33
Inflammation Score	0.18±0.08	1.52±0.09*	2.41±0.11*	1.00±0.09*†

† denotes P<0.0001 across all time points (ANOVA). ‡ denotes P<0.01 across all time points (ANOVA). * denotes P<0.05 compared with previous time point (Bonferroni's Multiple Comparison Test). Data represent the mean of 6-9 animals per group.

Table 2: Cellular composition of neointimal response to stenting in C57BL/6 mice (time course).

	Day 1	Day 7	Day 14	Day 28
Vessel cell density x 100/mm^2	3.90±0.37	26.1±4.54*	41.7±2.36*	55.24±9.67*†
Surface-adherent leucocytes (cells per x200 high power field)	14.4±1.74	32.8±5.27*	7.60±2.37*	0.40±0.25*†
Platelets, (% lumen circumference)	100±0.00	100±0.00	0*	0†
Macrophage, (% neointima)	0	22.6±6.32*	39.3±8.93*	8.8±1.2*†
Cell proliferation, (% neointima)	0	27.4±8.68*	8.89±3.84*	2.13±0.84*†
SMC, (% neointima)	0	14.2±4.34*	44.2±8.33*	73.2±9.43*†
Collagen, (% neointima)	0	0	3.34±2.12*	12.3±4.62*†

† denotes P<0.01 across all time points (ANOVA). * denotes P<0.05 compared with previous time point (Bonferroni's Multiple Comparison Test). Data represent the mean of 6-9 animals per group.

What is claimed is:

1. A method for stenting a vessel of a rodent, the method comprising:

inserting a vascular stent into a first rodent blood vessel;

transplanting the stented rodent blood vessel into a recipient rodent, thereby constructing a functional interposition graft with a second blood vessel.

2. The method of Claim 1, wherein said inserting step is performed in vivo.

3. The method of Claim 1 wherein said inserting step is performed ex vivo.

4. The method of Claim 1, wherein said inserting step utilizes balloon angioplasty.

5. The method of Claim 1, wherein the interposition bypass graft is constructed using collars that aid in securing the first blood vessel to the second blood vessel.

6. The method of Claim 1, wherein the interposition bypass graft is constructed with an adhesive glue.

7. The method of Claim 1, wherein the interposition bypass graft is constructed with sutures.

8. The method of Claim 1, wherein the rodent is a mouse.

9. The method of Claim 8, wherein the first blood vessel is obtained from a first, donor mouse and the second blood vessel is obtained from a second, recipient mouse, and, wherein the donor mouse and the recipient mouse are genetically identical.

10. The method of Claim 8, wherein the first blood vessel is obtained from a first, donor mouse and the second blood vessel is obtained from a second, recipient mouse, and, wherein the donor mouse and the recipient mouse are genetically non-identical.

11. The method of Claim 9 or Claim 10, wherein at least one of the donor or recipient mouse is altered from wild type by incorporation of cell-specific reporter genes or tags.

12. The method of Claim 9 or Claim 10, wherein the donor mouse is altered from wild type by incorporation of inducible genes allowing the donor stented vessel to generate a biologically active agent.

13. The method of Claim 1 wherein the first blood vessel and the second blood vessel are arteries.

14. The method of Claim 1 wherein the stent is a coated or drug eluting stent.

15. A rodent comprising a vascular stent in a functional interposition graft.

16. The rodent of Claim 15, wherein said the stent is introduced into the rodent by the method set forth in any one of Claims 1-15.

17. The rodent of Claim 15 or 16, wherein the stent is at least about 1.5 mm in length and not more than about 5 mm in length, and has a diameter after expansion of at least about 0.5 mm and not more than about 1.5 mm.

18. A method of testing the effects of a device, procedure, genetic modification, or biologically active agent on the vasculature of a rodent, the method comprising: contacting the rodent of any one of Claims 15-17 with said device, procedure or candidate biologically active agent and determining the effect of the device, procedure or candidate biologically active agent on the vasculature of the rodent.

19. The method of Claim 18, wherein the effects of a device are tested and wherein the device is a stainless steel stent, a metal alloy stent, or a biodegradable stent.

20. The method of Claim 18, wherein the effects of a procedure are tested and wherein the procedure is balloon angioplasty.

21. The method of Claim 18, wherein the effects of a procedure are tested and wherein the procedure is stenting.

22. The method of Claim 18, wherein the effect of a systemically administered agent is tested.

23. The method of Claim 18, wherein the effect of a locally administered agent is tested.

24. The method of claim 18 wherein the effects of an agent are tested, and where the agent is bone marrow or bone marrow derived cells.

25. The method of claim 18 wherein the effects of an agent are tested, and where the agent is progenitor or stem cells.

26. The method of Claim 24 or 25, wherein cells undergo pretreatment with drugs or genes.

27. The method of Claim 18, wherein the effect is determined by histology, immunohistochemistry, angiography, computed tomography, intravascular ultrasound, optical coherence tomography, optical bioilluminscence, ultrasonography, doppler ultrasonography, magnetic resonance imaging, immunoblotting, real-time polymerase chain reaction, proteomic or metabolomic analysis, transcriptional and genomic analysis by microrarray or other high-throughput technology.

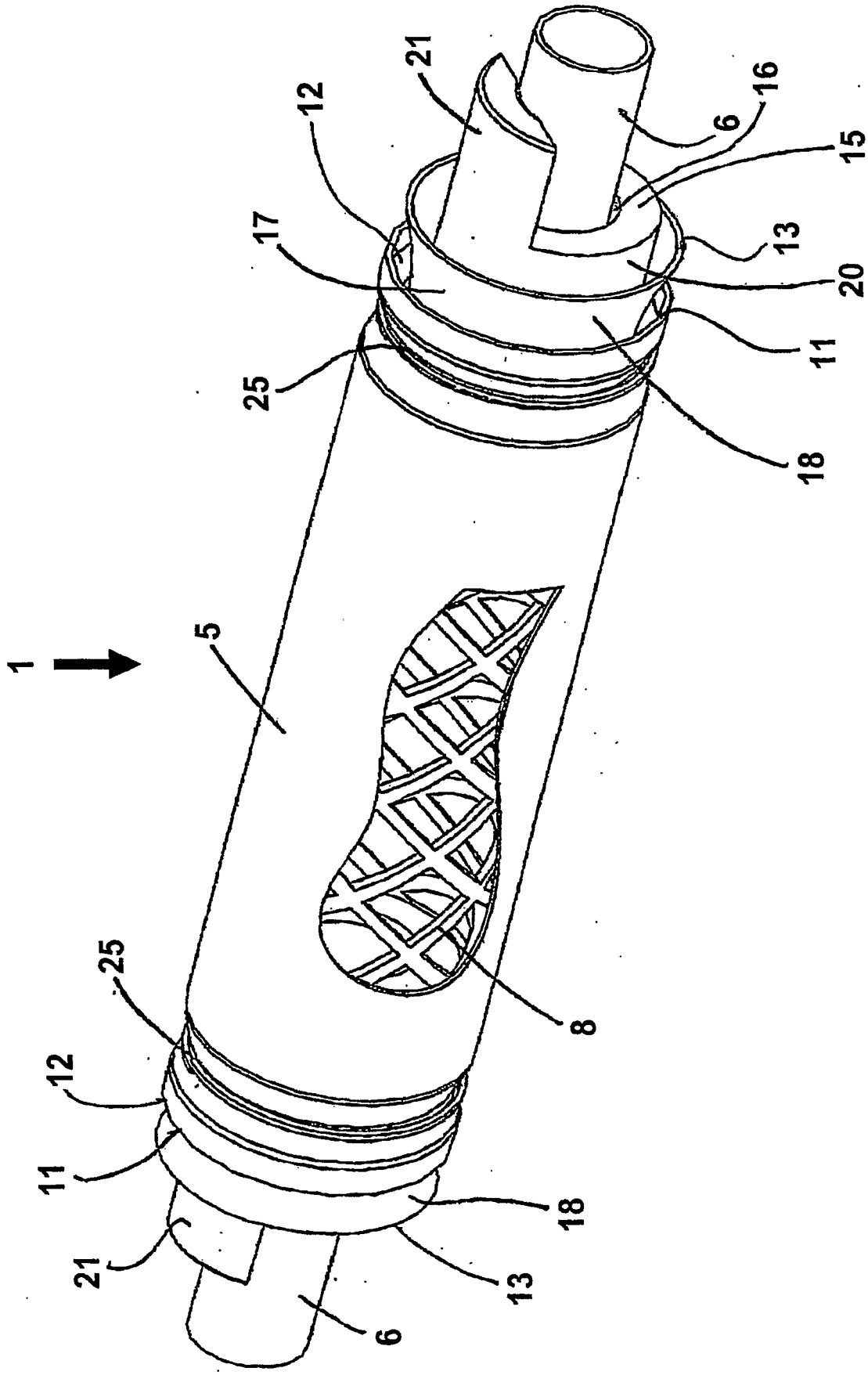

Figure 1

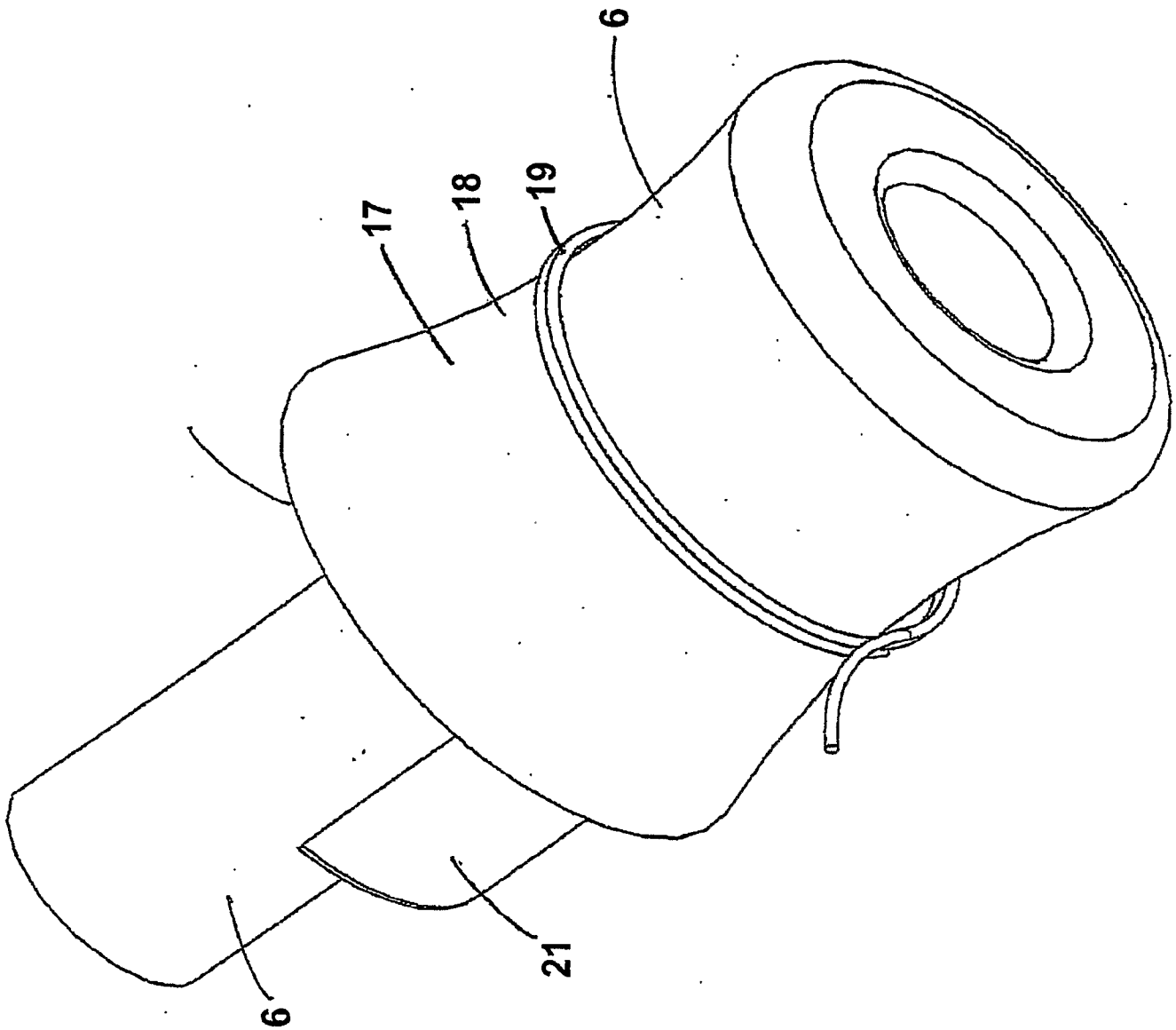

Figure 2

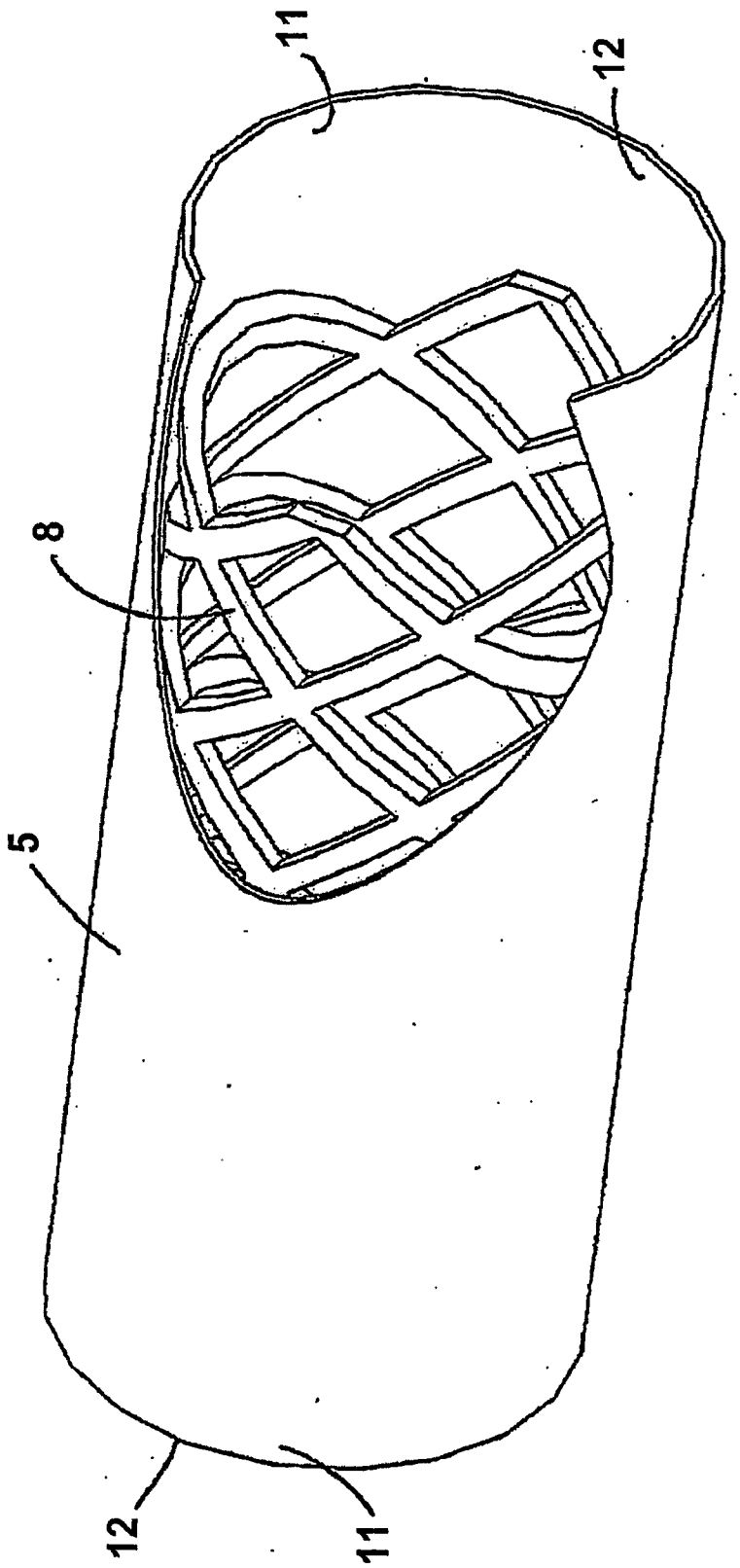

Figure 3

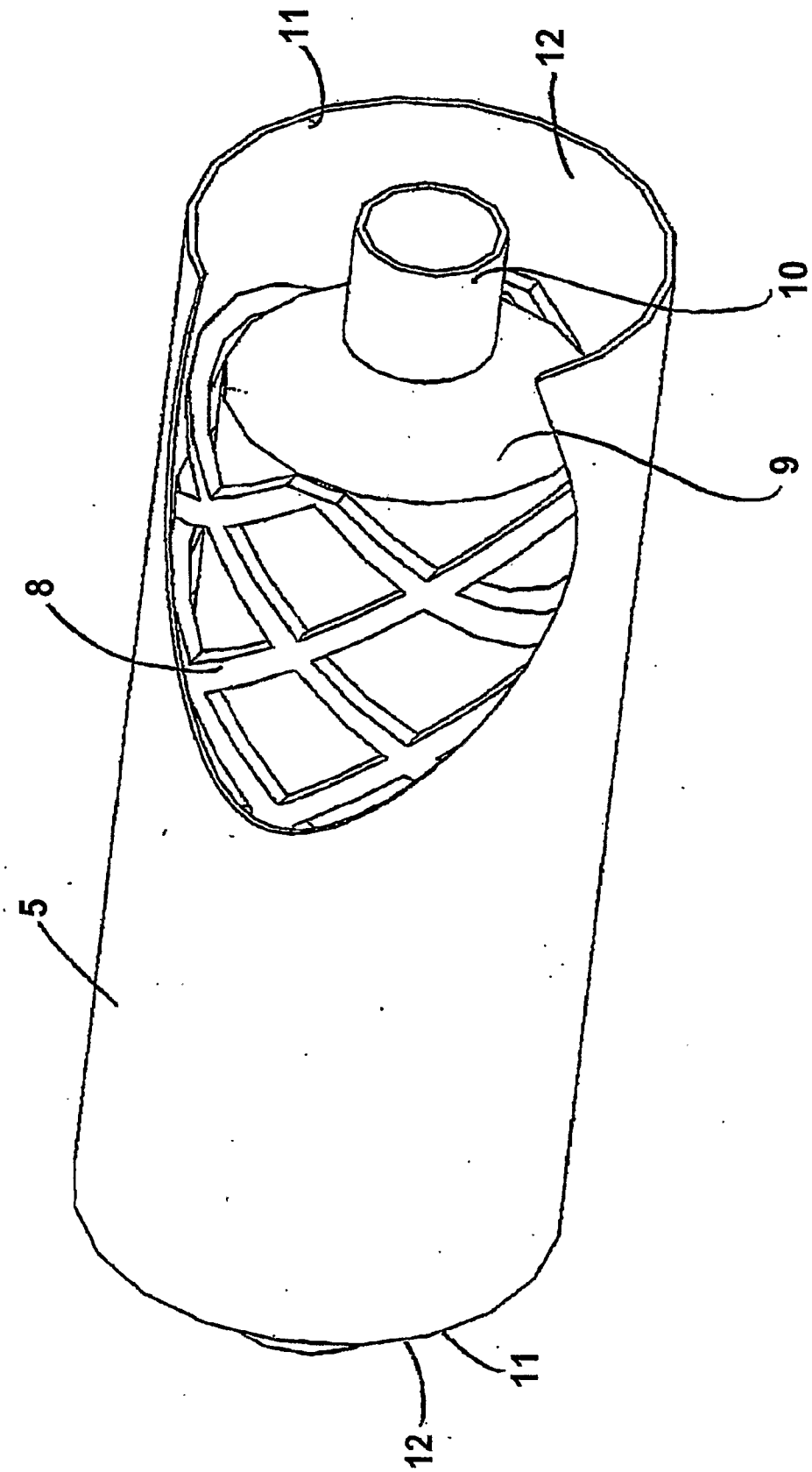

Figure 4

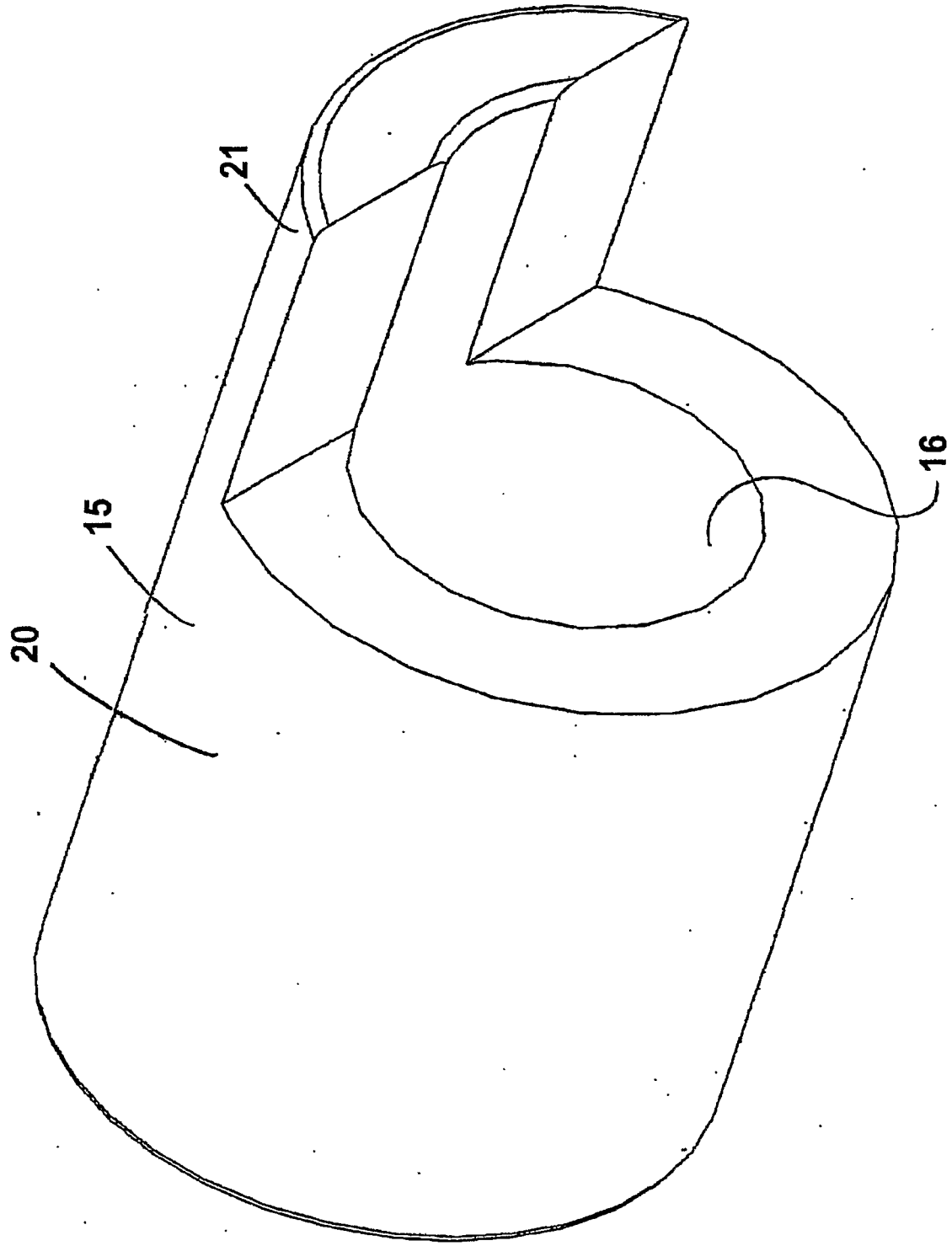

Figure 5

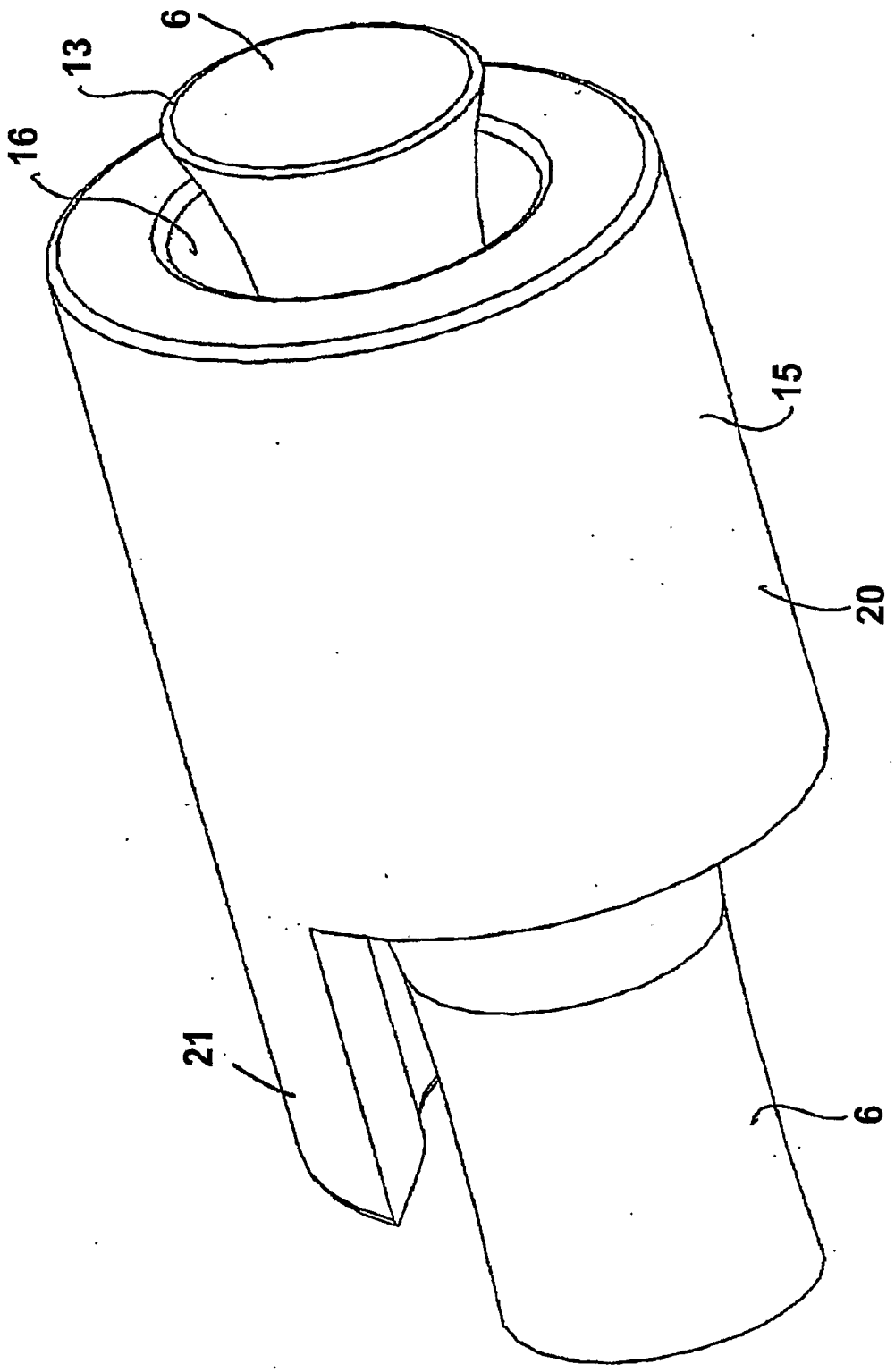

Figure 6

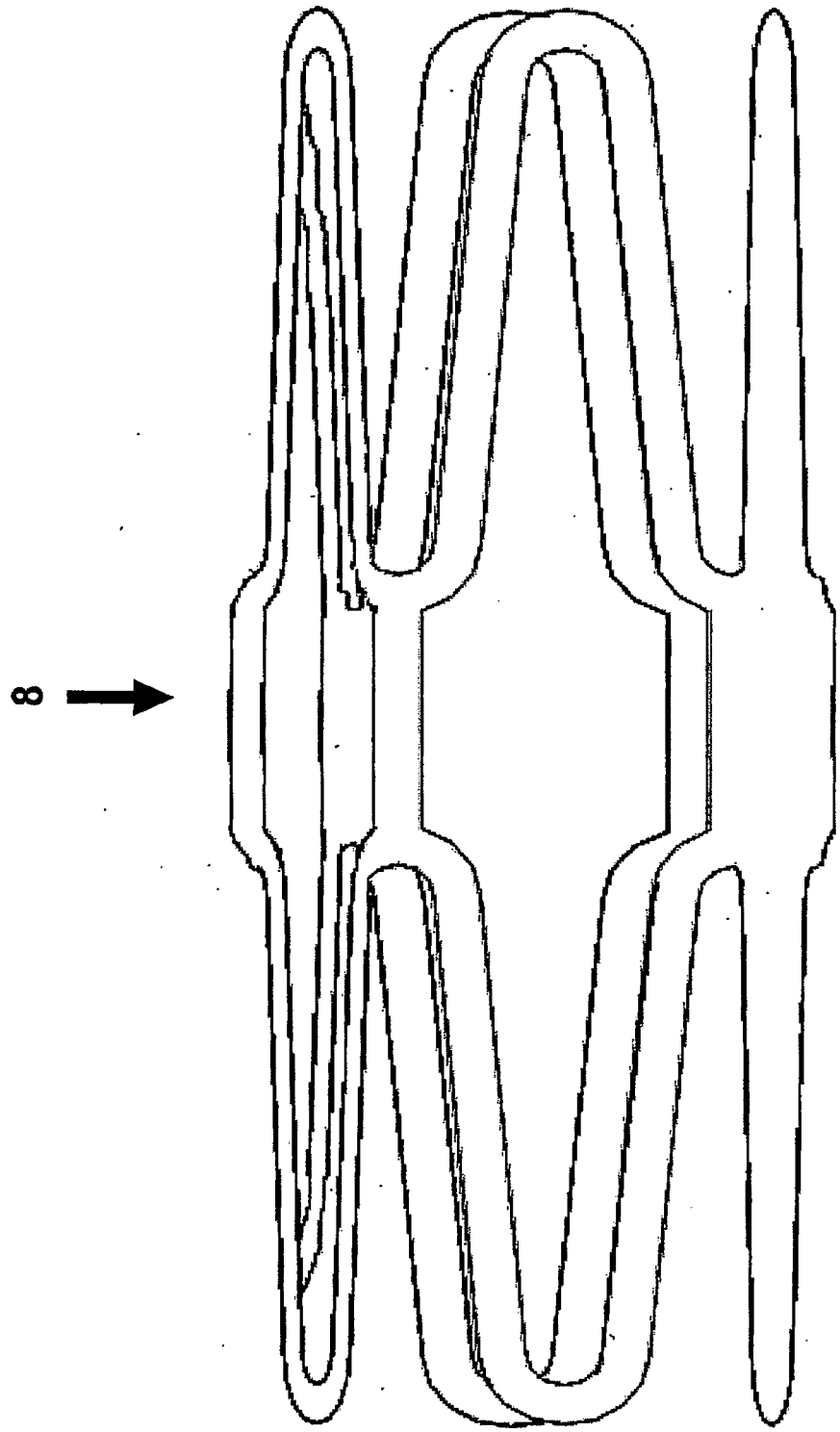

Figure 7