(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau

(43) International Publication Date
29 December 2004 (29.12.2004)

PCT

(10) International Publication Number
WO 2004/112863 A1

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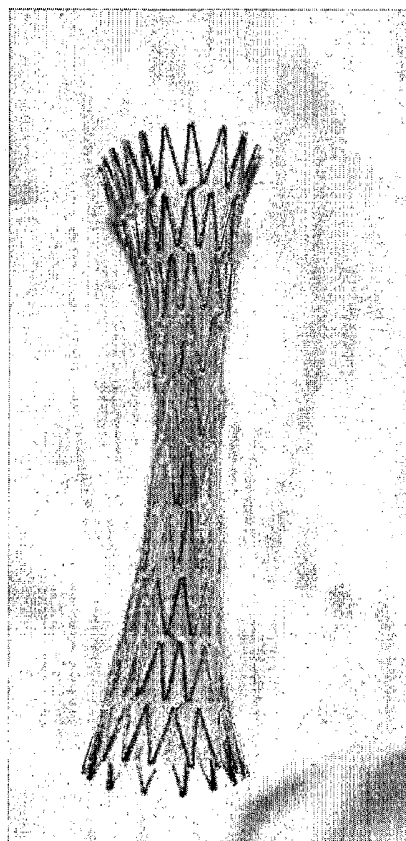
(54) Title: BIODEGRADABLE MEMBRANE-COVERED IMPLANT COMPRISING CHITOSAN

(57) Abstract: The present invention relates to medical implants such as stents, which can be coated with a biodegradable membrane containing or not an active ingredient or medicament, their method of preparation and their use. The present invention provides for a biodegradable membrane covered endovascular device. The device is coated with a solution comprising chitosan and chitosan plasticizer or cross-linker, the solution which when dried thereon, upon rehydration, forms a flexible polymeric membrane on the device, or which when freeze-dried thereon, forms upon rehydration a porous flexible polymeric membrane.

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GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

BIODEGRADABLE MEMBRANE-COVERED IMPLANT COMPRISING CHITOSAN

TECHNICAL FIELD

[0001]   The present application relates to the field of medical implants such as stents, which can be coated with a biodegradable membrane containing or not an active ingredient or medicament.

BACKGROUND OF THE INVENTION

[0002]   Membrane-covered-stents are used or investigated in the treatments coronary diseases such as aneurysm, pseudoaneurysm, large dissection of the vascular wall during angioplasty and thrombus containing lesions (Baldus S, et al., *Catheter Cardiovasc Interv*; **53**: 1-4, 2001). In particular, covered-stents may be useful to stabilize the friable plaque in saphenous vein graft diseases. This may in turn reduce the risk of distal embolization following angioplasty of such vessels. Their uses have also been questioned in the treatment of peripheral diseases (Stockx L. *Eur J Radiol*; **28**:182-188, 1998). The lack of long term biocompatibility of the synthetic polymers such as PTFE, Dacron and polyurethane currently used as covering material remains however a major limitation of these devices. To circumvent these limitations, biological polymers-based membrane covered stents have been proposed using exogenous fibrin, collagen, or bovine pericardium for instance (Goodwin SC, et al., *Invest Radiol*; **35**:420-425, 2000; Cloft HJ, et al., *Radiology*; **214**:557-562, 2000; Gaspar J, et al., *Arch Cardiol Mex*; **71** :286-294, 2001; and McKenna CJ, et al., *J Am Coll Cardiol*; **31**:1434-1438, 1998). While some promising results have been reported, a completely engineered membrane would present obvious advantages. Chitosan is a biocompatible, non-immunogenic and biodegradable polymer with bioadhesive, wound healing, and antimicrobial properties. Chitosan blend with various polymers have been widely investigated to improve the mechanical properties of chitosan. In particular, chitosan-polyethylene oxide (PEO)/polyethylene glycol (PEG) interpenetrated networks display excellent mechanical properties with improved ductility in comparison to chitosan alone (Kolhe P, and Kannan RM. *Biomacromolecules*; **4**:173-180, 2003; and Budtova T, et al., *J Appl Polym Sci*; **84**: 1114-1122, 2002).

[0003]   Chitosan is also widely used in drug delivery application and promotes absorption of drugs, peptides and proteins through biological tissues (Singla AK, and Chawla M. *J Pharm Pharmacol*; **53**:1047-1067, 2001). In revascularization procedures, neointimal hyperplasia within the stent struts remained however a consistent cause of failure. Systemic pharmaceutical strategies have been up to date mainly unsuccessful. Local drug/gene delivery conjugated with stent implantation yields the hope to eliminate clinical restenosis (Fattori R, and Piva T. *Lancet*; **361**:247-249, 2003). In particular, antiproliferative drug-eluting stents have been shown to significantly reduce restenosis. The long term safety and efficiency as well as the cost/efficiency of these therapeutic strategies remain however questioned.

[0004]   It would be highly desirable to be provided with a new biodegradable membrane-covered endovascular device and a method for making same.

SUMMARY OF THE INVENTION

[0005]   One aim of the present invention is to provide a chitosan-based membrane covered device, such as a stent, with properties that could be tailored to specific applications of such endoprosthesis.

[0006]   Another aim of the present invention is to provide a new method for preparing the device of the present invention.

[0007]   In accordance with the present invention there is provided a biodegradable membrane covered endovascular device, said device being coated with a solution comprising chitosan and chitosan plasticizer or cross-linker dried on the device, forming a flexible polymeric membrane on the device upon rehydration.

[0008]   In one embodiment of the invention, the solution is freeze-dried on the device, forming a porous flexible polymeric membrane on the device, upon rehydration.

[0009]   The chitosan plasticizer or cross-linker can be for example (without limitation) polyethylene oxide (PEO), cellulose, keratin, collagen, anionic polysaccharide, glutaraldehyde or poly(α-hydroxy acid).

[0010]   The membrane so produced has a further commercial potential as it can be used for treating various diseases when an active ingredient is coupled to the membrane. In such case, the active ingredient can simply be added at the time of forming the membrane, to the solution of chitosan/chitosan plasticizer or cross-linker prior to coating the device. Active ingredients that can be useful and that are thus envisioned in the present invention are for example, without limitation, growth factors, anti-proliferative drugs, anti-inflammatory drugs, nitric oxide donor compounds, radionuclides, nucleic acids, peptides, and proteins●.

[0011]   In a preferred embodiment of the present invention, the device is a stent, a stent graft or a coil, having preferably a metallic scaffold made of stainless steel, NiTi alloy, titanium alloys, Conichrome, Phynox, MP35N, cobalt-based alloys, tantalum titanium-zirconium-niobium alloys, titanium-aluminum-vanadium alloy, platinum, tungsten, tantalum, or a combination thereof.

[0012]   In a further preferred embodiment, the solution is a 70:30 weight to weight chitosan:polyethylene oxide solution.

[0013]   The term chitosan used herein is meant to include chitosan, chitosan blends or composites complex with a biocompatible synthetic or natural polymer.

[0014]   Still in accordance with the present invention, there is provided a method for producing a biodegradable membrane-covered device, said method comprising the steps of:

  a) preparing a solution containing chitosan and a chitosan plasticizer or cross-linker;

  b) contacting a device to be covered with the solution of step a); and

c) allowing the solution to dry onto said device.

[0015]   In another embodiment of the invention, the solution in step c) above is freeze-dried onto the device, forming a porous biodegradable membrane-covered device.

[0016]   In a further embodiment, the method may also comprise after step c) the steps of:

d) rinsing the device of step c) with an alkaline solution;

e) rinsing the device of step d) with a physiological buffer;

f). allowing the device of step e) to dry.

[0017]   In one embodiment of the invention, the solution of step a) above is prepared by dissolving in solution chitosan in an acidic medium and the chitosan plasticizer or cross-linker in another acidic medium and mixing the solutions of chitosan and chitosan plasticizer or cross-linker together to form the solution used in step a).

[0018]   Further in accordance with the present invention, there is also provided a method for treating aneurysm comprising the steps of implanting a device as defined above in a blood vessel of a patient suffering from an aneurysm, such that the device is implanted over the opening of the aneurismal sac in the blood vessel, the chitosan/polyethylene oxide coated device allowing cells to adhere to the device, thereby closing the aneurismal sac.

[0019]   In fact, the device of the present invention should not be limited to stent and restenosis, but should also include devices that need to be implanted and that need to release a medicament or an active ingredient at the site of implantation. For example, the device of the present invention can also be used for treating a vasculature-related disease such as stenosis or restenosis, or for treating a disease located at the surface of a vessel, such as a blood vessel or the esophagus. The device of the present invention can also be used for

treating tumors or cancer, such as esophagus tumors or cancer, for treating vein graft–induced diseases.

[0020]   The versatility of the fabrication method describes here allows to design membranes with different thickness, structure, and porosity.

[0021]   For the purpose of the present invention the following terms are defined below.

[0022]   The terms " bioactive drug", "active drug" and "medicament", all used interchangeably, are intended to mean any compound able to stimulate or inhibit cellular events. Examples of such drugs are, without limitation, an anti-proliferative or anti-inflamatory drug, nitric oxide donor compounds, and proteins, DNA, and radionuclides able to stimulate/inhibit cellular events. In a preferred embodiment, an anti-proliferative or anti-inflamatory drug is loaded into the membrane. For exemple, anti-proliferative drugs can include paclitaxel, sirolimus, tacrolimus, everolimus, dexamethasone, angiopeptin, somatostatin analogue, angiotensin converting enzyme inhibitors (Captopril, Cilazapril and Lisinopril), calcium channel blockers (Nifedipine), colchicine, fibroblast growth factor (FGF) antagonists, fish oil, heparin, histamine antagonists, lovastatin, methotrexate, monoclonal antibodies (to PDGF receptors, etc.), nitroprusside, phosphodiesterase inhibitors, prostacyclin analogues, prostaglandin inhibitor, seramin (PDGF antagonist), serotonin blockers, steroids, thioprotease inhibitors, and triazolopyrimidine.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023]   Fig. 1 is a schematic representation of the CH-PEO membrane covered stent with or without external porous layer, in accordance with one embodiment of the invention;

[0024]   Fig. 2 illustrates an *in vitro* assay showing inhibition of platelet adhesion of CH-PEG membrane incubated in the presence of sodium nitroprusside.

[0025]   Figs. 3A and 3B illustrate a macroscopic observation of the CH-PEO membrane covered stent showing the ability of the membrane to sustain a mechanical deformation following a compression (Fig. 3A) and the return to the original conformation (Fig. 3B) of a metallic stent;

[0026]   Figs. 4A to 4C illustrate scanning electron microscopic images of the membrane covered stent in which Fig. 4A represent an external view of the stent showing the adhesion of the membrane to the metallic struts, Fig. 4B is a view of the inside of the membrane showing the stent-struts underlaying and Fig. 4C is a 450X enlarged view the box in Fig. 4A;.

[0027]   Fig. 5A illustrates the swelling behavior in PBS at pH 7.4 of the CH-PEO membrane (▲), and of the CH-PEO porous membrane (■) in accordance with one embodiment of the present invention;

[0028]   Fig. 5B illustrates in an inlet of Fig. 5A the swelling behavior in PBS at pH 7.4 of the CH-PEO membrane (♦) in comparison with the chitosan (CH) membrane of the prior art (●);

[0029]   Fig. 6 illustrates ^{51}Cr-platelet adhesion (n≥5) in an *ex vivo* porcine model on CH-PEO membrane of the present invention, damaged (Media) and intact arteries (Endo), * $p < 0.05$ vs. Media;

[0030]   Fig. 7 illustrates ^{111}In-leukocyte adhesion (n≥5) in an *ex vivo* porcine model on CH-PEO membrane of the present invention, damaged (Media) and intact arteries (Endo), * $p < 0.05$ vs. Media;

[0031]   Fig. 8 illustrates ^{111}In-Platelet adhesion (n≥4) after a 90 min incubation in platelet rich plasma (PRP) on chitosan (CH) and CH-PEO or heparin complexed-CH-PEO (CH-PEO-Hep) membranes, where damaged arteries (Media) were used as control, * $p < 0.05$ vs. Media, + $p < 0.05$ vs. CH-PEO; and

[0032]   Fig. 9 illustrates the Arginine release behavior in PBS of the CH-PEO membrane of the present invention, either following direct loading (■) or

following precomplexation with anionic low molecular weight hyaluronic acid (LMW HA) (o).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0033] In accordance with the present invention, there is now provided a method of making chitosan-PEO based biodegradable membrane covered devices, such as stents. The membrane covered devices described here are intended to be used in a wide range of application such as the treatment of aneurysm, the treatment of vascular graft diseases, malignant obstructive disease and tissue engineering applications. The properties of the membrane were investigated in order to gain insight on its ability to be used in endovascular applications. Looking for vascular tissue engineering applications, an external porous membrane that could be used as a biodegradable scaffold was added. The haemocompatibility of the devices was characterized both in an *ex vivo* porcine model and *in vitro* and optimized through complexation with heparin. Finally, the ability of the membrane to act as a drug reservoir was investigated using the peptide precursor of nitric oxide L-Arginine and the NO donor sodium nitroprusside (SNP).

Materials

[0034] Chitosan derived from crab shell with a deacetylation degree > 85 % and PEO with an average molecular weight of 1,000,000 daltons were obtained from Sigma Co. Heparin was from Leo Pharma (Tornhill, CA)(1000 unit/mL). ^{3}H labeled and unlabeled L-Arginine were purchased from Sigma (L-Arginine hydrochloride; and L-Arginine-2,3-^{3}H in aqueous ethanol solution). LMW HA was obtained as previously described. Hyaluronan (HMW 500,000) was from Hyal Pharmaceutical Corp, Mississauga, Ca. All chemicals were chemical grade. SNP was from Sigma Chemicals.

Chitosan-polyethylene oxide (CH-PEO) polymers preparation

[0035] CH-PEO film was synthesized as a film or used for the membrane covered stent. Chitosan was dissolved in 0.1 M aqueous acetic acid (2% w/w) overnight and filtered to get ride of any insoluble material. PEO dissolved in

glacial acetic acid was then added to a ratio of CH/PEO: 70/30 by weight. The mixture was then stirred for 4 h and then degassed. A jelly-like solution was then obtained that was either poured in glass mold for characterization or used to prepare the membrane covered stent as described hereinafter. The gel was allowed to dry at room temperature (to obtain the membrane of the present invention upon rehydration) or was freeze dried (to obtain the porous membrane of the present invention upon rehydration). The dry material was then washed with aqueous 1 N NaOH to remove residual acid and finally thoroughly wash with ultrapure water.

Membrane covered stent preparation

[0036] Self-expandable stents made from NiTi alloy were mounted on a glass rod as schematized in Fig 1. The glass rod could be rotated on its longitudinal axis at controlled speed (rpm). A known amount (depending on the experiments, from 0.1g to few grams) of CH-PEO gel was then cast on the stent maintained in rotation. Controlling the rotation speed and as a function of the viscosity of the gel, a uniform layer of CH-PEO covered the stent in a controlled fashion. While maintained in rotation, the gel-covered stent was allowed to dry at room temperature. The dried membrane covering the stent was then neutralized as described above before removing the stent from the glass rod. The membrane-coated stent was then allowed to dry and was stored for characterization or further modifications.

surface complexation with anionic polymers

[0037] Heparin is being used herein as a model for anionic polymers. Heparin solution was prepared (1 mg/mL in water) and used to complex with CH-PEO film. The CH-PEO film was immersed in the heparin solution for two hours at room temperature (RT) to allow the electrostatic interaction between heparin and CH to occur and then thoroughly rinsed with water. The heparin complexed-CH-PEO-membrane (CH-PEO-Hep) was then dried and further used for the haemocompatibility assay.

Multilayered membrane covered-stent

[0038]   Additional layers of CH-PEO were deposited onto a previously prepared CH-PEO membrane layer to form a membrane having increased thickness/mechanical properties. This was achieved by repeating the same procedure as described above in reference to Fig. 1. External porous layers were also added onto membrane-covered-stents. A CH-PEO covered stent was prepared and kept on the glass rod (holder). The device was placed into a glass mold that was gently filled with CH-PEO mixture. The mold was then freeze at -20°C before being transferred to vacuum vessels and being lyophilized until dry. The membrane was then again neutralized with NaOH and rinsed with pure water. This procedure yielded an external macro-porous layer deposited onto a CH-PEO membrane layer itself deposited on a stent.

Characterization of the membrane

SEM observations

[0039]   Membrane covered-stents were observed using SEM. The devices were allowed to equilibrate in water and then freeze-dried. The membranes were processed (i.e. cut) prior to metallization with an ultrathin sputter coated gold layer. The samples were imaged using scanning electronic microscopy (SEM) at an accelerating voltage of 5 kV.

[0040]   The porosity of the membrane has been characterized by complex permitivity measurements using a vector network analyzer (Model PNA 8358, Agilent Technologies) and a dielectric probe (85070C, Agilent Technologies).

Swelling behavior

[0041]   The swelling behavior of chitosan-based membrane was investigated by gravimetric method. Discs of polymers were prepared and allowed to swell in 0.9 M NaCl solution. The swollen polymers were collected at various times, the excess water being removed by blotting against filter paper, and weighted (± 0.1 mg). The swelling ratio was calculated knowing the hydrated W_h and dry W_d weight: $(W_h-W_d)/W_h$.

Water permeation and burst strength

[0042]   The water permeation and burst strength of the membrane covered stent was determined in PBS as previously described (Berglund JD, et al., *Biomaterials*; **24**:1241-1254, 2003). The devices were hydrated for 30 min and completely crushed 5 times to simulate extensive manipulations. They were then cannulated with surgical sutures on semi-rigid silastic tubes at both sides. Water permeability is defined here as the amount of saline solution leaked per unit area and time under a physiologic pressure of 120 mm Hg. The burst pressure was characterized by pressurizing the device with an increasing hydrostatic liquid pressure (20 mmHg incremental steps) until membrane failure.

Thrombogenicity

[0043]   The thromboresistance of the CH-PEO membranes have been investigated using an extracorporeal procedure in *ex vivo* perfusion chambers using radiolabeled ^{51}Cr-platelets and ^{111}In Leukocytes. The radiolabeling, animal surgery, and extracorporeal procedures were achieved as previously described (Thierry B, et al., *Biomaterials*; **23**: 2997-3005, 2002). Six (6) pigs were used in the experiments. All procedures followed the American Heart Association guideline for animal research and were approved by the Montreal Heart Institute (MHI) ethic committee. Briefly, an extracorporeal shunt consisted in a two parallel silicon tubing channel circuit connecting the femoral artery to a perfusion chamber and returning to the femoral vein. Radiolabeled platelets and leukocytes were re-injected into the animals one hour before the beginning of the experiments. CH-PEO mixture was cast on glass slide, dried, rinsed with NaOH and water and finally allowed to dry and stored. Intact endothelium and injured arterial segments were used as respectively thromboresistant and thrombogenic control (Thierry B, et al., *Biomaterials*; **23**: 2997-3005, 2002). To simulate damaged arteries, intima denuded artery were prepared as followed. Normal porcine aortas were first dissected and then cut into rings, longitudinally opened and cut into segments. The aortic media was then exposed by lifting and peeling off the intima and the adventia together with a thin portion of the subjacent media. The segments were then cut to appropriate size using a

cutting device. Such aortic media segments were shown in previous experiments to be very thrombogenic and closely simulate angioplasty damaged arteries.

[0044]   The samples were hydrated in saline at least 30 min prior to the experiments and then placed in the perfusion chamber. The perfusion procedure was initiated by a 1 min saline wash. The blood was then allowed to circulate into the extracorporeal circuit for 15 min at a wall shear rate of 424 sec^{-1}. The circuit was then flushed with saline for 30 sec and the samples recovered and fixed in 1.5% glutaraldehyde in gamma-counter vials. The amount of radioactivity was then measured and calculated for background, decay and overlapping of the radionuclides. The total amount of platelets and leukocytes adsorbed on the surface was calculated knowing the activity of blood samples used as reference and using hematology achieved prior to each experiment.

Platelet adhesion

[0045]   Further insights on the haemocompatibility of the chitosan-based membranes were obtained using an *in vitro* platelet adhesion assays. Samples were prepared as 0.6 cm^2 discs using a cutting device. Chitosan (CH), CH-PEO and CH-PEO-Hep were prepared and kept at room temperature (RT). One (1) hour before the beginning of the experiments, samples were allowed to rehydrate in saline solution. Damaged arteries used as control were prepared as described above. Fresh blood was drawn from healthy volunteers (120 mL), (who were medication free) and used for radiolabeling of platelets. The labeled platelets were resuspended in the platelet poor plasma (PPP) to 2.5 x 10^6 platelets/mL. The samples were placed into the bottom of a 96 well polystyrene plate (Corning Inc.). 250 μl of freshly prepared ^{111}In-platelet solution was then added to each well taking special care that both sides of the samples were in contact with platelet solution. The platelet adhesion was allowed to proceed for 90 min with gentle shaking. After incubation, the samples were recovered and washed 4 times with saline. The samples were then fixed in 1.5 %

glutaraldehyde solution and the amount of platelet was determined using a gamma counter.

SNP loaded CH-PEO membrane.

[0046] Freshly dried CH-Peg films were rehydrated in an aqueous solution of the NO-donor sodium nitroprusside (SNP, Sigma) (83 mg/ml (10%) and 16.6 mg/mL (2%)) for 1 h and then washed with water and let to dry at room temperature. During the entire preparation procedure, the samples were kept in the dark to protect SNP from decomposition.

[0047] Platelet adhesion on SNP loaded membrane was investigated in an *in vitro* assays as described previously. To assess the effects of potency of SNP towards platelet adhesion, CH-PEG membranes were incubated in platelet rich plasma (PRP) with a physiological dose of SNP (50μMol/L).

L-Arginine release

[0048] L-Arginine (Arg), the precursor of nitric oxide (NO) has been used in the present invention as a model drugs. Arginine has been shown to reduce clinical restenosis when locally delivered through a NO mediated actions (Suzuki T, et al., *Am J Cardiol*; 89:363-367, 2002). The cationic drug has been either loaded directly in the membrane or precomplexed with anionic low molecular weight hyaluronic acid (LMW HA)(as a plasticizer polysaccharide). The complexation of the cationic drug with the plasticizer polysaccharide was expected to delay its release and to minimize drug lost during the washing procedures of the membrane.

[0049] CH-PEO mixture was prepared as described above. A stock solution of unlabeled Arg/[^{3}H] Arg was prepared in water (10^{-3} M with 2 μL [^{3}H] Arg/mL). In the direct loading experiments, L-Arginine was added to 2 g of the CH-PEO mixture to a final concentration of wt. 0.2 %. In the precomplexation method, LMW HA solubilized in water (1 mg/mL) was mixed with 100 μL of the Arg solution (COOH/Arg molar ratio: 5:1) and allowed to complex for 1 h. 260 μL of this mixture was then added to 2 g of the CH-PEO solution. The CH-PEO/Arg mixture with or without LMW HA was left under agitation for 2 h and then poured

in polystyrene wells. The membranes were allowed to dry and then neutralized for 2 min with 750 μL NaOH 1 M and rinsed twice with 1 mL water (2 min each). The rinsing solutions were kept for quantification of Arg released during the washing steps.

[0050]   The release behaviors of the Arg-loaded membranes were determined in PBS. Membrane cast in the wells were incubated with 1 mL PBS under gentle agitation at room temperature. At various time, the PBS solution was removed from the well. The Radioactivity of the solution was determined using liquid scintillation spectrometry (Beckman LS 6500 Liquid Scintillation counter) and correlated after correction for background to the amount of Arg using standard.

Device preparation

[0051]   The method described here to obtain the chitosan membrane-covered stent is reliable and reproducible. Once allowed to dry overnight and then neutralized with NaOH and rinsed with water, the device could be easily recovered from the holder without any damaged. Macroscopic observations showed that a thin membrane uniformly covered the whole stents as shown in Figs. 3A and 3B. Experiments have shown that the thickness of the membrane could be easily controlled by changing the viscosity of the chitosan mixture and to a lesser extent by controlling the speed of rotation of the glass holder. The thickness of a one-layer CH-PEO membrane was in the range of 100 μm. One of the challenges in the design of such a device was to optimize the mechanical properties of the membrane so that it could sustained the mechanical deformation associated with stent deployment. As shown in Figs. 3A and 3B, the hydrated membrane was able to sustain complete crush without any visible damages. Minimally invasive delivery of the device could be achieved easily. Hydrated membrane-covered stents were hand crimped on catheters and maintained cramped with small plastic clamps until dry. Device-loaded catheters were kept at room temperature until further used. The dilatation of the devices was achieved in glass tubing by rehydration and removal of the plastic clamps. The devices completely expanded inside the glass tubes. To investigate the

effect of the delivery procedure on the membrane, a stent was dilated and then quickly freeze-dried using liquid nitrogen. The lyophilized sample was then gold coated for SEM observations. Using low magnification, it was noted that the membrane conserved its integrity and remained firmly attached to the metallic structure (Figs. 4A to 4C). The luminal surface of the membrane was relatively smooth despite "waves".

Membrane characterization

[0052]   The swelling behavior of CH-PEO membrane in PBS (pH 7.4) is shown in Figs. 5A and 5B and compared to that of porous CH-PEO membrane obtained by freeze-drying (Fig. 5A) or to the CH membrane of the prior art (Fig. 5B). Rapid swelling of CH-PEO was observed and equilibrium was achieved within 1 min. As expected, porous CH-PEO presented a significantly higher swelling ratio than the CH-PEO membrane. The blend of Chitosan with PEO increased the swelling ratio in comparison to chitosan alone prepared in the same condition than the CH-PEO membrane (1.55 vs. 1.22 at 10 min).

[0053]   The water permeation at 120 mm Hg was determined to be less than 1 mL/cm^2 min^{-1}. No significant linkage of the membrane was observed when the device was pressurized at 120 mm Hg for 30 min. The burst pressure resistance of the 4 tested membrane-covered stents was determined to be higher than 500 mm Hg but the apparatus used in this work did not allow us to pressurize the membrane high enough to its failure. An external membrane could be added to the device as described herein with a mean porosity of about 95%.

Thromboresistance

[0054]   The thromboresistance of the CH-PEO membrane was investigated in an *ex vivo* porcine assays using radiolabeled platelets and leukocytes. Under the physiological flow conditions used in the study, the CH-PEO presented low amounts of platelet adhesion in comparison to damaged arteries (P < 0.05). Similar results were obtained with leukocytes (P < 0.005). Both platelet and leukocyte adhesion onto CH-PEG was in the same range as that on arteries with an intact endothelial layer (Figs. 6 and 7).

[0055]   The effect of PEO and heparin complexation on the haemocompatibility was investigated in the *in vitro* assays. The incorporation of PEO into the chitosan membrane tends to reduce the amount of platelet adhesion (52.5% reduction, P> 0.05, Student-t-test). Complexation of the CH-PEO membrane with heparin improved the resistance to platelet adhesion by 50.1% (p ≤0.05, Student t-test) (Fig. 8).

[0056]   Both 10% and 2% loading significantly decrease platelet adhesion in the *in vitro* assays (8.1±5.9 10^3 PLQ/cm^2 for 10% and 13.6±9.1 for 2% 10^3 PLQ/cm^2 Vs. 37.1±8.7 10^3 PLQ/cm^2). This corresponds to respectively a 78% and 63% inhibition of platelet adhesion (p<0.005). CH-PEG membrane incubated in the presence of SNP also presented a significantly reduced platelet adhesion (15.8±8.8 10^3 PLQ/cm^2), hence confirming the ability of SNP to inhibit platelet activation (Fig. 2).

Arginine release

[0057]   Both direct and indirect loading of the drug in the CH-PEO membrane were characterized by a high loss during the washing steps (> 80%). The precomplexation of the cationic drug with the anionic LMW HA resulted only in a minor increase in the retention of the drug during the washing steps (18 % Vs. 13.6 %). The release behavior of the Arginine loaded membrane in PBS is presented in Fig. 9. Both direct and indirect loading resulted in a high burst release in PBS with most of the drug remaining in the membrane being released by the first hour (91% and 86%).

Discussion

[0058]   The present invention describes the development of chitosan-based biodegradable membrane covered endovascular devices such as stents. The main finding was that metallic stent could be covered by a chitosan-based membrane displaying suitable properties for endoluminal implantation. To fulfill any clinical applications, such membrane should (1) be able to sustain the deformation during the endoluminal expansion of the stent; (2) not elicit extensive biological response; (3) be thromboresistant; (4) allowed cellular

ingrowths both at the luminal and external surfaces and (5) be able to resists physiological blood pressure. Minimally invasive implantation of stents/membrane-covered stents is widely used in the treatment of obstructive/degenerative pathologies such as coronary and peripheral artery diseases, aneurysm, ruptures and fistulas. They are also investigated in non-vascular malignant diseases in urology or gastroenterology for instance. The most straightforward applications of membrane-covered stents are currently the endoluminal exclusion of aneurysm and the revascularization of vein graft.

[0059] The excellent mechanical properties of the chitosan-PEO blend allowed the hydrated device to be completely crushed on a catheter without noticeable damages to the membrane upon redeployment. In turn, the membrane covered stent could be loaded onto a delivery catheter and expanded through the self-expanding properties of the supporting metallic stents. Assays have investigated the use of reticulated membranes, using glyoxal or glutaraldehyde, or chitosan alone but these membranes failed to sustain the elastic deformation required during expansion. The blend of chitosan with high molecular weight PEO have been shown to drastically improve the elasticity and strength of chitosan (Kolhe P, and Kannan RM. *Biomacromolecules*; 4:173-180, 2003; and Budtova T, et al., *J Appl Polym Sci*; 84: 1114-1122, 2002). The latter has been explained by the intermolecular interactions between chitosan and PEO, especially close to the stoichiometric monomer ratio.

[0060] The second key-point in the design of such an endovascular device was the overall biocompatibility and haemocompatibility of the material. Both chitosan and PEO are well-known biocompatible biodegradable materials. In particular, chitosan is currently widely investigated as wound dressing material, drug delivery vehicle and tissue engineering scaffold (Rao SB, and Sharma CP., *J Biomed Mater Res*; 34:21-28, 1997; and Madihally SV, and Matthew HW., *Biomaterials*; 20: 1133-1142, 1999) . Chitosan supports cell attachment and growth and has been successfully investigated in vascular tissue engineering applications (Chupa JM, et al., *Biomaterials*; 21:2315-2322, 2000). The poor endothelialization of synthetic graft materials has been called as a major cause

of failure. In contrast, chitosan supports the attachment and growth of endothelial cells which may in turn enhanced the mid-term biocompatibility of the device. Acute blood compatibility of chitosan have however been reported to be an issue with reported ability of the polymer to activate both complement and blood coagulation systems. A recent exhaustive investigation of the blood compatibility of chitosan has however showed that despite large adsorption of plasma proteins, the polymer was a weak activators of the alternative pathway of the complement and intrinsic pathway of coagulation (Benesch J, and Tengvall P., *Biomaterials*; **23**:2561-2568, 2002). CH-PEO membranes were tested in an *ex vivo* extracorporeal porcine model using radiolabeled platelets and leukocytes. Amount of adsorbed platelets and leukocytes were low and in the same range than those measured on intact aortic segments used a negative control. Both values were significantly lower than the damaged artery model used as positive control. Endothelial denuded arteries have been previously used to simulate angioplasty-damaged blood vessels. To get further insight of the haemocompatibility of the CH-PEO membrane, an *in vitro* assay was used to compare the platelet adhesion on CH-PEO membrane with chitosan alone and heparin complexed CH-PEO membrane. The blending of chitosan with PEO tends to reduce the adhesion of platelets. This may be related to the hydrophilicity of PEO that may decrease non-specific adhesion. A further improvement was observed upon surface complexation with heparin. Complexation of chitosan with anionic polysaccharide such as heparin has been widely used to improve its thromboresistance. Conversely, electrostatic complexation with glucosaminoglycans (GAGs) such as hyaluronic acid or heparin has been reported to modulate its biological activity (Chupa JM, et al., *Biomaterials*; **21**:2315-2322, 2000). Complex of chitosan and heparin have also recently been reported to enhance wound healing (Kweon DK, et al., *Biomaterials*; **24**:1595-1601, 2003).

[0061] Along with its mechanical properties and biocompatibility, the choice of chitosan as a base materials relied on its ability to achieve local delivery of biologically active components (Singla AK, and Chawla M. *J Pharm Pharmacol*; **53** :1047-1067, 2001). In particular chitosan has been investigated in therapies

directed against cancer or hyperproliferative vascular disease using drugs such as doxorubicin or paclitaxel (Mitra S, et al., *J Control Release*; **74**:317-323, 2001; and Nsereko S, and Amiji M., *Biomaterials*; **23**:2723-2731, 2002;). The loading and delivery of the NO-precursor L-Arginine was investigated in the present application. Arginine is a low molecular weight cationic drug that has been proved to be effective in the reduction of neointimal hyperplasia (Suzuki T, et al., *Am J Cardiol*; **89**:363-367, 2002; and Kalinowski M, et al., *Radiology*; **219**:716-723, 2001). The release behavior presented in Fig. 9 shows an initial burst release with small amount of the drug being released after 1 h. Importantly, a significant amount (86.4 %) of the drug was lost during the neutralization and washing procedures. The possibility to control the release behavior by precomplexation of the cationic drug with the plasticizer low molecular weight hyaluronic acid (LMW HA) was thus investigated. Such precomplexation method has been used to improve the release behavior of doxorubicin in chitosan nanoparticles. Only a modest improvement was obtained as the initial loss during the washing procedure remained high (82 %) and most of the drug was released during the initial burst (86 % at 30 min Vs. 91 % for the direct method). Much longer release profile could however be achieved using anionic drug or higher molecular weight molecules. The NO donor sodium nitroprusside was also loaded into the CH-PEO membrane. A dose-dependant reduction in the amount of adhered platelets was obtained in the *in vitro* assays. The latter further confirmed the ability of the membrane to act efficiently as a drug delivery system.

[0062]   While the membrane-covered device was initially designed for the treatment of occlusive diseases and vein graft stenoses, its potential use in the setting of endovascular aneurysm closure is also considered as a promising application in the present invention. The possibility to exclude the aneurysm by the chitosan-based membrane as well as the potential for local delivery of biologically active components such as growth factors are appealing. The CH-PEO membrane acts as a temporary barrier while being used as a template for arterial reconstruction. Organization of the blood thrombi into connective tissue may be expected to solve the issue of endoleak that plague endovascular

aneurysm exclusion. Such organization is enhanced by the presence of the chitosan based membrane and further accelerated by incorporation of appropriate growth factors. Sealing of the membrane to prevent growth of the aneurysm is however required. The water permeation assays used here showed that the CH-PEO membrane covered stent showed an excellent blockade of saline solution and less than 1 mL/cm^2 min^{-1} was measured. The low volume of solution prevented more precise determination but these values are better or comparable to that reported for commercially available graft such as the cross-linked collagen Dacron impregnated Hemashield$^®$ (<10 mL/cm^2 min^{-1}). The burst resistance of the membrane should also be high enough to prevent failure upon the aneurismal transmural pressure. Burst pressure resistance of a monolayered CH-PEO membrane was determined to higher than 500 mm Hg. This value is expected to sustain transmural pressure occurring in aneurysm. Additional CH-PEO layer could be useful to increase the burst resistance. In addition, thrombus formation and tissue ingrowths within the membrane in contact with fluids of the excluded area of the aneurysm could act as additional sealing and reinforcing material. In an attempt to apply the concept of tissue engineering in the field of aneurysm closure, the possibility to add an external porous layer that could be truly used as a scaffold for vascular cells was also investigated. Tissue engineering of vascular graft has been the object of much interest due to the unsolved clinical issue of small vascular graft. The advantage of using biological materials such as collagen as construct is counterbalanced by the inadequate mechanical properties of these polymers. Hybrid graft using dacron sleeves combined with collagen-based construct have been proposed to assure enough burst resistance for *in vivo* implantation (Berglund JD, et al., *Biomaterials*; **24**:1241-1254, 2003; and Xue L, and Greisler HP., *J Vasc Surg*; **37**:472-480, 2003;). The presence of synthetic sleeves may however be damageable for the long term success of the graft. On the other hand, graft made completely from biodegradable polymers required lengthy *in vitro* tissue formation to display appropriate mechanical properties for implantation. A hybrid device conjugating the mechanical scaffold of the metallic device and the biodegradable chitosan-based porous matrix open new opportunities in this field. Metallic stents do not elicit intensive biological

reactions such as those commonly observed with polymeric synthetic materials. The metallic component of the hybrid construct can provide the necessary mechanical strength, at least at the acute phase of the tissue organization, while minimizing the amount of non-biodegradable material within the artery in comparison to synthetic polymeric sleeves. In addition, cell ingrowths naturally occurring in chitosan matrix could be easily enhanced by incorporation of growth factors in the chitosan-based membrane (Lee JY, et al., *J Control Release*; **78** :187-197, 2002).

Conclusion

[0063]   This study showed that a biodegradable membrane covered stent could be designed based on the excellent mechanical properties of chitosan-PEO blend. The device could be loaded onto a catheter and expanded without noticeable damage to the membrane, thus authorizing endoluminal delivery. The biological properties of chitosan and its biocompatibility associated with the potential to achieve uniform local delivery of biologically active components to the vascular wall suggest that such device could be used in the treatment of various pathologies such as vascular obstructive diseases, aneurysms and malignant diseases. While the *in vitro* experiments presented here are promising, further *in vivo* investigations are warranted to prove its clinical potential.

[0064]   While the invention has been described in connection with specific embodiments thereof such as stents, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims. More particularly, a simple filling wire could be used for example for filling an aneurismal sac, instead of using the membrane coated stent (as per one embodiment of the invention) for covering or blocking the opening or the neck of the aneurismal sac.

WHAT IS CLAIMED IS:

1.   A biodegradable membrane covered endovascular device, said device being coated with a solution comprising chitosan and a chitosan plasticizer or cross-linker, said solution being dried on the device, forming a flexible polymeric membrane on the device, upon rehydration.

2.   The device of claim 1, wherein the solution is freeze-dried on the device, forming a flexible porous polymeric membrane on the device upon rehydration.

3.   The device of claim 1 or 2, wherein the chitosan plasticizer or cross-linker is selected from the group consisting of polyethylene oxide (PEO), cellulose, keratin, collagen, anionic polysaccharide, glutaraldehyde, and poly(α-hydroxy acid).

4.   The device of any one of claims 1 to 3, wherein the solution further comprises an active ingredient.

5.   The device of claim 4, wherein said active ingredient is selected from the group consisting of growth factor, anti-proliferative drug, anti-inflammatory drug, nitric oxide donor compound, radionuclide, nucleic acid, peptide, and protein.

6.   The device of claim 5, wherein said anti-proliferative drug is selected from the group consisting of paclitaxel, sirolimus, tacrolimus, everolimus, dexamethasone, angiopeptin, angiotensin converting enzyme inhibitor, calcium channel blocker, colchicine, fibroblast growth factor (FGF) antagonists, fish oil, heparin, histamine antagonist, lovastatin, methotrexate, monoclonal antibody specific to PDGF receptor, nitroprusside, phosphodiesterase inhibitor, prostacyclin analogue, prostaglandin inhibitor, seramin, serotonin blockers, steroids, thioprotease inhibitor, triazolopyrimidine.

7.   The device of any one of claims 1 to 6, wherein said device is a stent, a stent graft or a coil.

8.   The device of claim 3, wherein said solution is a 70:30 weight to weight chitosan:polyethylene oxide solution.

9.   The device of any one of claims 1 to 8, wherein said device has a metallic scaffold made of stainless steel, NiTi alloy, titanium alloy, Conichrome, Phynox, MP35N, cobalt-based alloy, tantalum titanium-zirconium-niobium alloy, titanium-aluminum-vanadium alloy, platinum, tungsten, tantalum, or a combination thereof.

10.   The device of any one of claims 1 to 9, wherein the polymeric membrane comprises chitosan, or chitosan blends or composites complex with a biocompatible synthetic or natural polymer.

11.   A method for producing a biodegradable membrane-covered device, said method comprising the steps of:

   a)   preparing a solution containing chitosan and a chitosan plasticizer or cross-linker;

   b)   contacting a device to be covered with the solution of step a); and

   c)   allowing the solution to dry onto said device.

12.   The method of claim 11, wherein the solution in step c) is freeze-dried onto said device to produce a porous biodegradable membrane-covered device.

13.   The method of claim 11 or 12, further comprising after step c) the steps of:

   d)   rinsing the device of step c) with an alkaline solution;

   e)   rinsing the device of step d) with a physiological buffer;

   f)   allowing the device of step e) to dry.

14.   The method of claim 11, 12 or 13, wherein said chitosan plasticizer or cross-linker is selected from the group consisting of polyethylene oxide (PEO), cellulose, keratin, collagen, anionic polysaccharide, glutaraldehyde, and poly(α-hydroxy acid).

15.   The method of any one of claims 11 to 14, wherein the solution is prepared by dissolving in solutions chitosan in an acidic medium and the chitosan plasticizer or cross-linker in another acidic medium and mixing the solutions of chitosan and chitosan plasticizer or cross-linker together to form the solution used in step a).

16.   The method of claim 14, wherein said solution is a 70:30 weight to weight chitosan:polyethylene oxide solution.

17.   The method of any one of claims 11 to 16, wherein the solution of step a) further comprises an active ingredient.

18.   The method of claim 17, wherein said active ingredient is selected from the group consisting of growth factor, anti-proliferative drug, anti-inflammatory drug, nitric oxide donor compound, radionuclide, nucleic acid, peptide, and protein.

19.   The method of claim 18, wherein said anti-proliferative drug is selected from the group consisting of paclitaxel, sirolimus, tacrolimus, everolimus, dexamethasone, angiopeptin, angiotensin converting enzyme inhibitor, calcium channel blocker, colchicine, fibroblast growth factor (FGF) antagonists, fish oil, heparin, histamine antagonist, lovastatin, methotrexate, monoclonal antibody specific to PDGF receptor, nitroprusside, phosphodiesterase inhibitor, prostacyclin analogue, prostaglandin inhibitor, seramin, serotonin blockers, steroids, thioprotease inhibitor, and triazolopyrimidine.

20.   The method of any one of claims 11 to 19, wherein said device is a stent, stent graft or a coil.

21.   A method for treating an aneurysm comprising the steps of implanting a device as defined in any one of claims 1 to 10 in a blood vessel of a patient suffering from an aneurysm, such that the device is implanted over the opening of the aneurismal sac in the blood vessel or within the aneurismal sac, the membrane covered endovascular device allowing cells to adhere to the device, thereby closing or filling up the aneurismal sac.

22.   Use of a device as defined in any one of claims 1 to 10, for treating a disease located at the surface of a tubular organ.

23.   The use of claim 22, wherein the tubular organ is a blood vessel or the esophagus.

24.   The use of claim 22, wherein the disease is a tumor.

25.   The use of claim 22, wherein the disease is a vasculature-related disease.

26.   The use of claim 25, wherein the vasculature-related disease is stenosis or restenosis.

27.   The use of claim 22, wherein the organ is a vein graft used in a by-pass procedure.

CH-based mixture

Mixture cast on the rotating stent

Drying, neutralizing and rinsing

Membrane-covered stent

The holder is placed in a mold filled with CH-based mixture
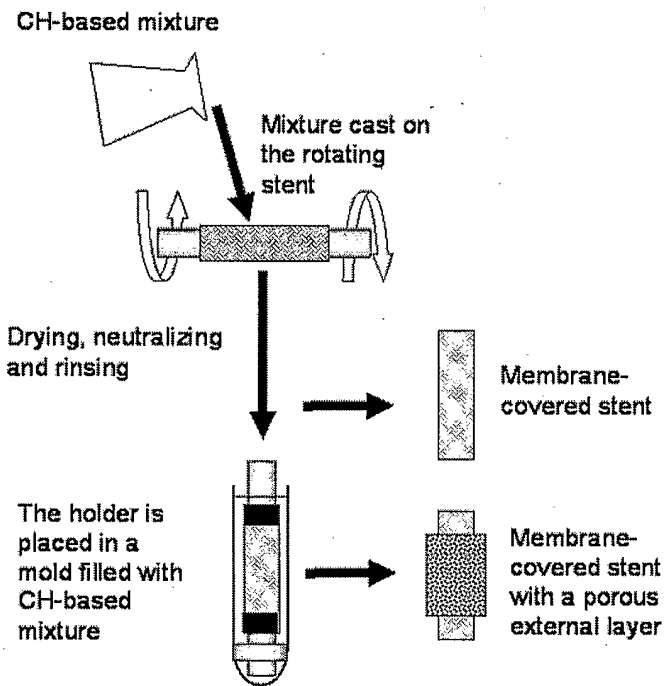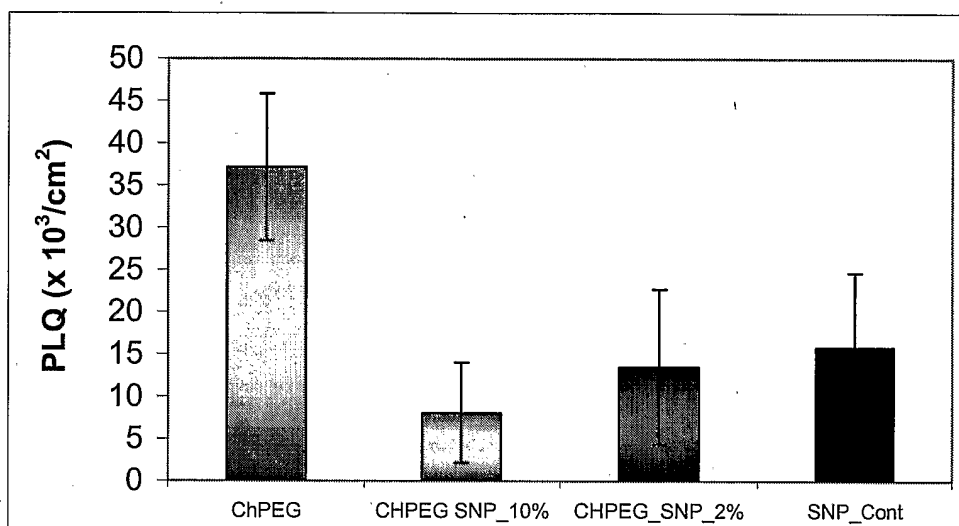
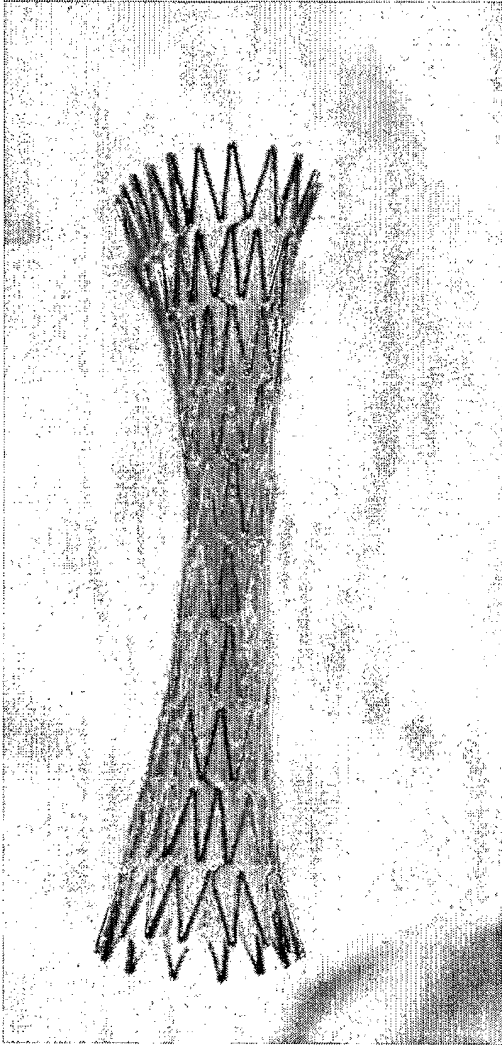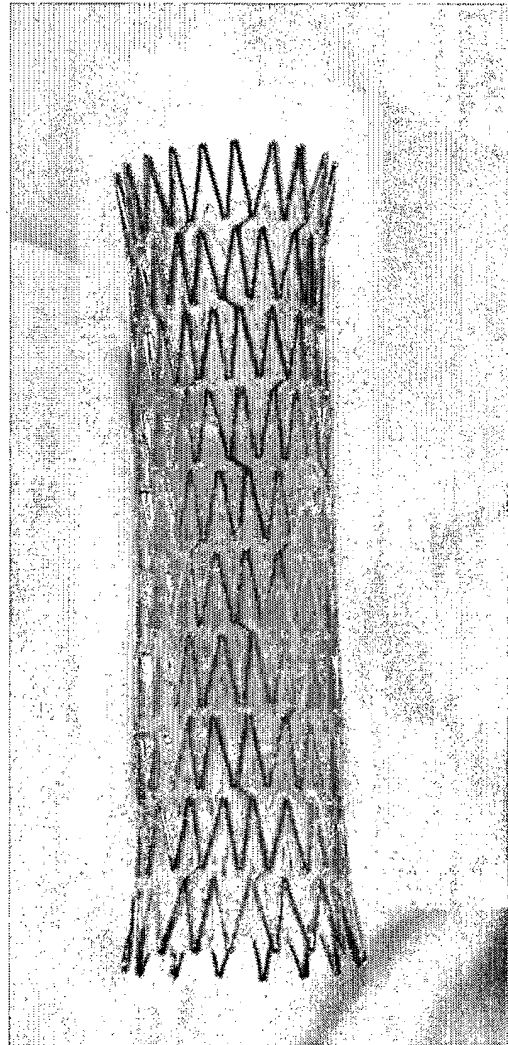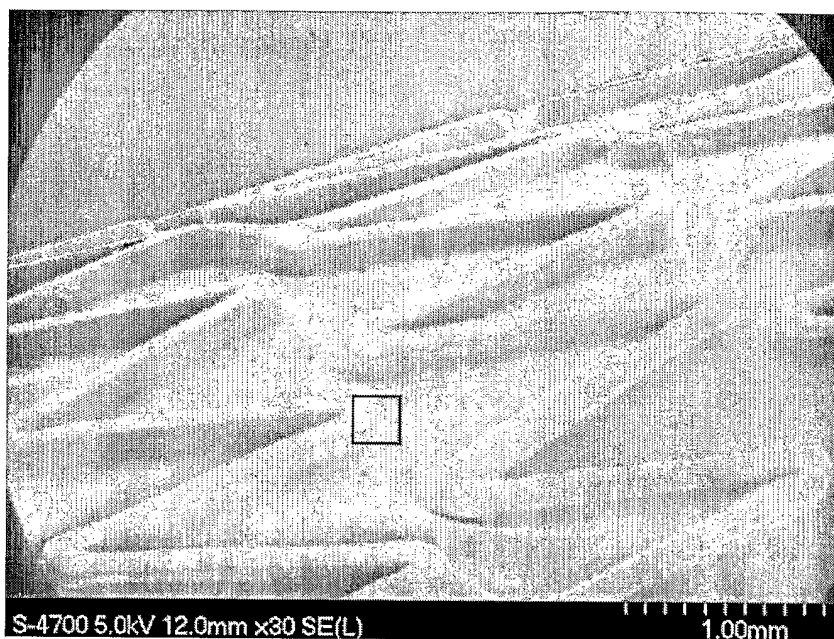Membrane-covered stent with a porous external layer
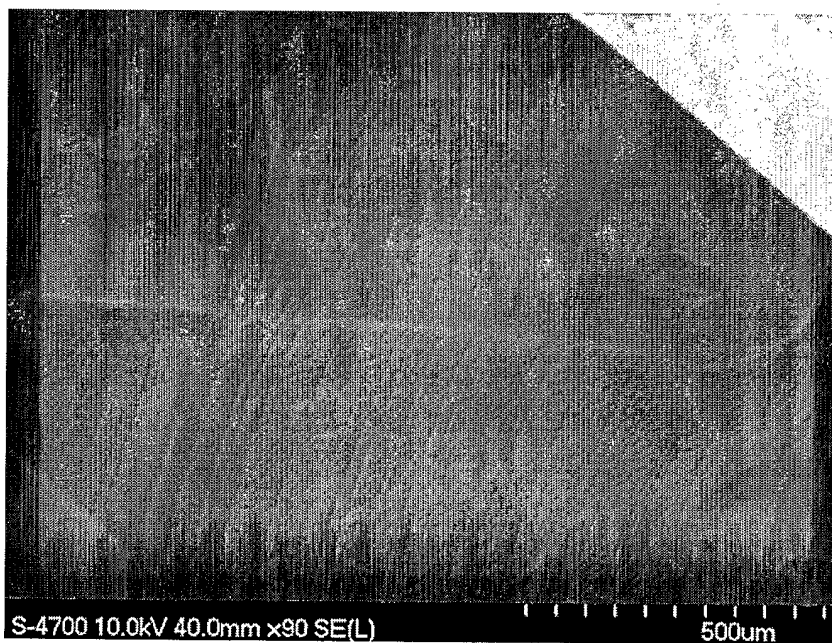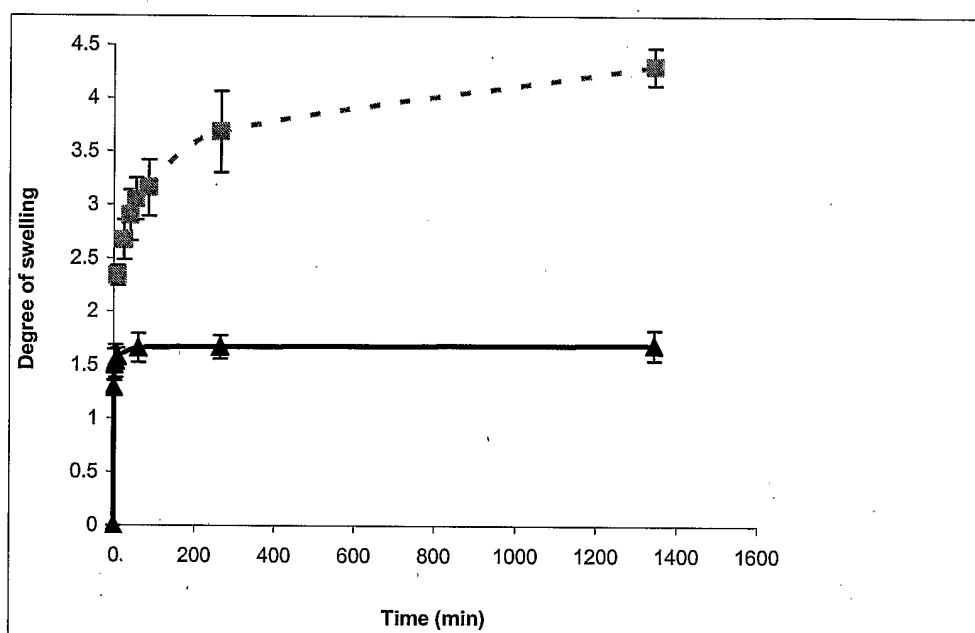
Fig. 1

Fig. 2

Fig. 3A   Fig. 3B

Fig. 4A

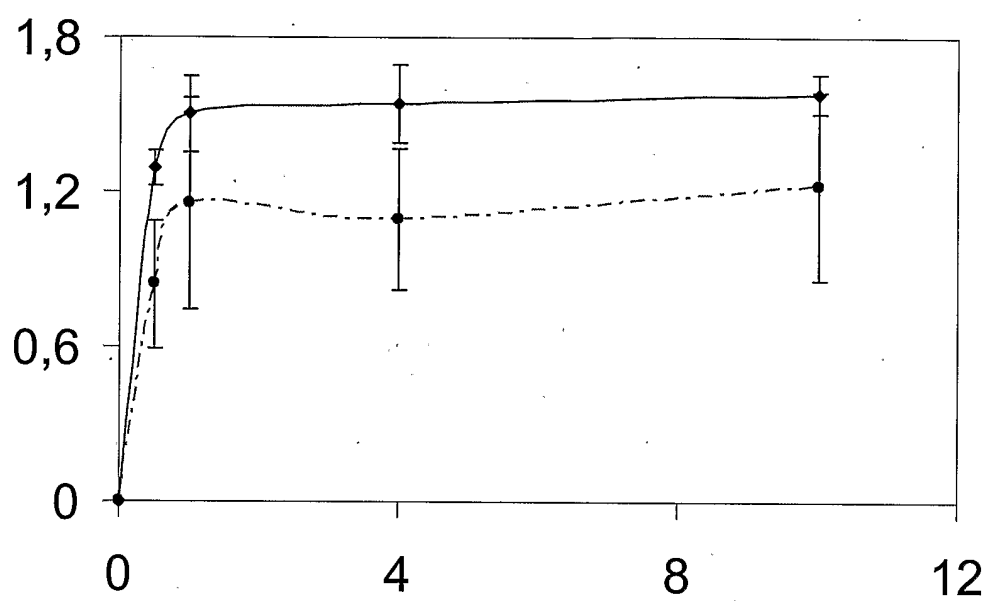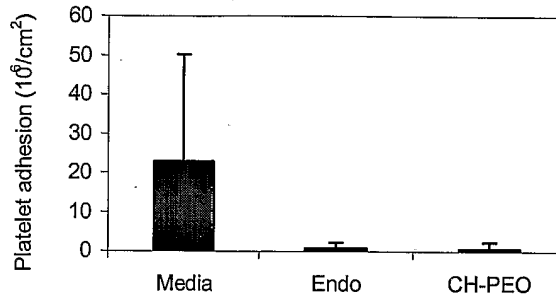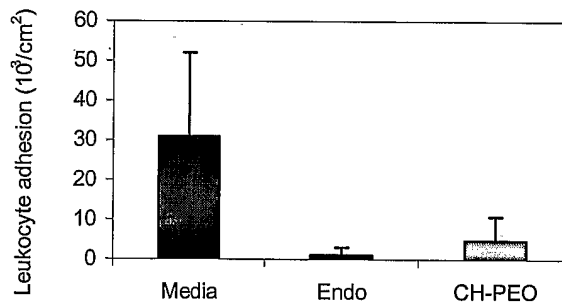Fig. 4B

Fig. 4C

Fig. 5A

Fig. 5B

Fig. 6

Fig. 7
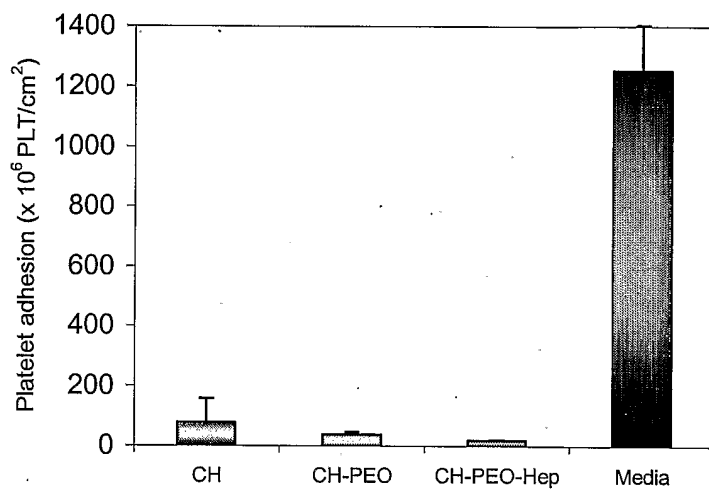
Fig. 8
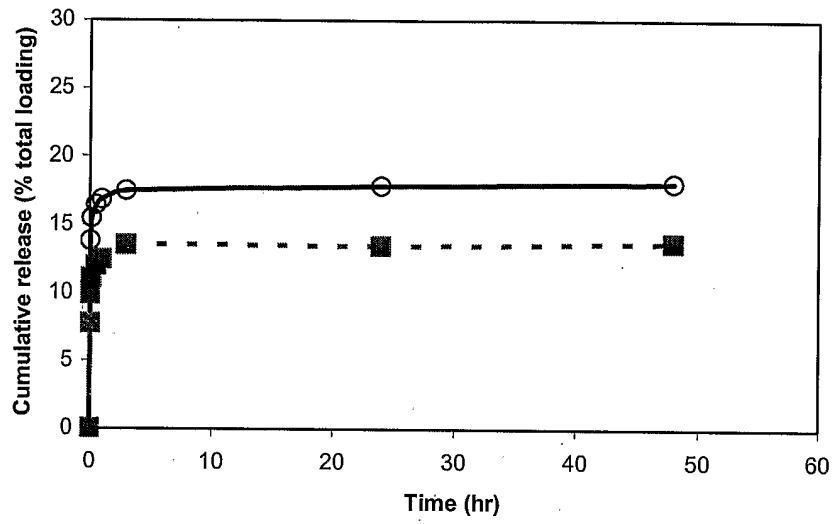
Fig. 9

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   A61L31/10   A61L29/08   A61F2/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   A61L   A61F

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

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

EPO-Internal, WPI Data, PAJ, INSPEC, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 2004/012676 A (GP MEDICAL) 12 February 2004 (2004-02-12) paragraph '0020! paragraph '0045!; examples 1,3,7 paragraph '0170!; claims 1,3,6,9-14	1-27
X	WO 03/020771 A (PRINZ MARTIN ; MUCOBIOMER BIOTECHNOLOGISCHE F (AT)) 13 March 2003 (2003-03-13) page 2, paragraph 4 - page 3, paragraph 1 claims 1,14; example 1	1-10, 21-27
Y	US 5 904 927 A (AMIJI MANSOOR M) 18 May 1999 (1999-05-18) claims 1,3-6,9-12	1-10, 21-27
	-/--	

[X] Further documents are listed in the continuation of box C.   [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 October 2004	04/11/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Ganschow, S

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INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BRIEGER DAVID ET AL: "Local drug delivery systems and prevention of restenosis" CARDIOVASCULAR RESEARCH, XX, XX, vol. 35, no. 3, 1997, pages 405-413, XP002202664 ISSN: 0008-6363 the whole document	1-10, 21-27
X	WO 00/71136 A (CHITOGENICS INC) 30 November 2000 (2000-11-30) page 1, line 5 – line 11 page 5, line 36 – page 6, line 3 page 7, line 9 – line 24 page 8, line 18 – line 25; claims 1,5,7-9	1-10
A	US 2003/065377 A1 (DAVILA LUIS A ET AL) 3 April 2003 (2003-04-03) the whole document	21
A	WO 03/037219 A (LOETZBEYER THOMAS ; PAHMEIER ANDREA (DE); ALVITO BIOTECHNOLOGIE GMBH () 8 May 2003 (2003-05-08) claims 1,3,7,11,13	1-27
A	US 5 885 609 A (AMIJI MANSOOR M) 23 March 1999 (1999-03-23) the whole document	1-27

2

Box II   Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:   —
   because they relate to subject matter not required to be searched by this Authority, namely:

   > Although claims 21-27 are directed to a method of treatment of the
   > human/animal body, the search has been carried out and based on the alleged
   > effects of the coated stent.

2. ☐ Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such
   an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III   Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
   searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
   of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
   covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

                                ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2004)

INTERNATIONAL SEARCH REPORT

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US 5885609	A		23-03-1999	NONE		