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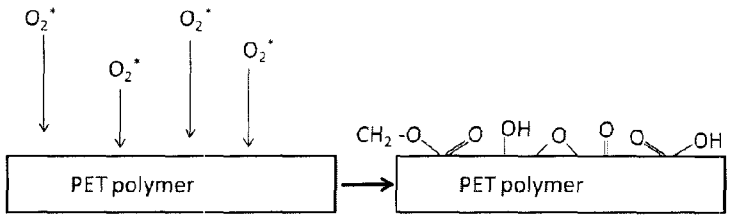

Figure 1.

(57) Abstract: Method of treatment of a vascular graft addresses the need for improved methods of increasing hemocompatibility of vascular grafts made from polyethylene terephthalate polymers against the state of the art. The present invention does not involve deposition or grafting of any anti-thrombogenic coating on the inner walls of vascular grafts. Hence, it provides a method for improved hemocompatibility of vascular grafts by treatment of inner walls by excited oxygen molecules. The efficiency of the method which is the subject of this invention is confirmed by behavior of blood platelets on modified vascular grafts. Against the state of the art the methods of the invention prevents transformation of platelets from normal state in healthy blood to highly activated states, which would further lead to undesired thrombus reactions. Thus the present invention improves hemocompatibility of artificial polyethylene terephthalate surfaces, as transformation of platelet shape from its normal state- round or discoid and dendritic to spread dendritic, spread and fully spread shape is highly reduced.

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Method for treatment of a vascular graft

FIELD OF TECHNOLOGY

The present invention relates to methods for manufacturing of grafts. In particular the present invention relates to optimization of vascular grafts made from polyethylene terephthalate polymers by treatment of inner walls.

TECHNICAL PROBLEM

Technical problem addressed by this invention is a need for improved methods of increasing hemocompatibility of vascular grafts made from polyethylene terephthalate polymers, current methods in some cases showing hemocompatibility problems.

STATE OF THE ART

Atherosclerotic cardiovascular disease is still the largest cause of mortality in Western society. Arterial damage produces localized reductions in the calibre of arteries (stenosis) which ultimately stops the flow of blood through the affected vessels. The disease is treated surgically by bypassing the segment of affected vessels to restore blood flow (Chandy, T.; Das, G. S.; Wilson, R. F.; Rao, G. H. R.; *Biomaterials* **21**, 699 (2000)). Wherever possible, an autograft is the best choice for a replacement vessel. However many patients, especially those with pre-existing vascular diseases and patients who have already undertaken autograft procedures, do not have blood vessels healthy enough to adequately serve as replacements. In such cases, the most common form of

treatment is by using synthetic polymeric materials, such as Dacron (PET - polyethylene terephthalate) or ePTFE (extended polytetra fluoroethylene), because they exhibit a unique through-pore microporous wall structure and because they also have highly flexible mechanical properties. These synthetic vascular grafts have been used successfully to replace large-diameter blood vessel, however the long-term patency for small-diameter vascular grafts is still unsatisfactory; this is primarily due to thrombus formations (Wissink, M.J.B.; Beernink, R.; Poot, A.A.; Engbers, G.H.M.; Beugeling, T.; van Aken, W.G.; Feijen, J.; Improved endothelialization of vascular grafts by local release of growth factor from heparinized collagen matrices. *Journal of Controlled Release* **64**, 103 (2000)). Significant efforts have been made in recent years to develop novel vascular grafts, and some novel grafts have indeed been produced, however the long term patency is still disappointing. At every 10 % of patients post-surgical complications are observed, mostly due to inflammatory reactions, infections and aneurysm. In such cases the artificial blood vessel has to be removed and the new autologous material for vascular graft should be implemented, which increases the expenses of treatment for at least two times.

When polymer materials come into contact with blood they can cause various undesirable host responses such as thrombosis, inflammatory reactions, infections and various other responses. Such responses are minimised if the materials are hemocompatible. In fact surface-induced thrombosis is one of the main problems associated with blood-contacting biomaterials. Polyethylene terephthalate (PET- Dacron grafts) and polytetrafluoroethylene (PTFE) are the two most common prosthetic materials available today for vascular grafts. Though PTFE and Dacron vascular grafts possess many of the properties of ideal vascular prostheses, successful results for small-calibre vascular grafts have not yet been reported.

Normal blood is a metastable state stabilized between two opposing driving forces coagulation and anticoagulation. Almost any external perturbation in the form of an artificial surface tends to trigger the coagulation system, i.e. blood clotting may occur (Kasemo, B.; Biological surface science. *Surface Science* 500, 656; 2002). Once biomaterials are exposed to blood the adsorption of blood proteins immediately follows. The type, the amount and the conformational state of the adsorbed proteins determine whether platelets will adhere and become activated or not. The adsorption of fibrinogen (FNG), which is present in blood plasma, has been shown to be closely related to surface-induced thrombosis. Fibrinogen is the most abundant protein in blood plasma

and has been closely linked to adhesion and activation of platelets. As thrombus formation begins with protein adsorption, the main efforts to improve hemocompatibility of materials have been directed towards controlling (mainly preventing) protein adsorption. Therefore, a modification of the material surface with protein-repulsive molecules has become a widely used approach for improving hemocompatibility of materials (Tzoneva-Velinova et al.; Journal of Material Science 19, 3203, 2008). The modification of these grafts materials with a suitable protein coating may improve cell adhesion and proliferation. Commercially available collagen-, gelatin-, heparin- and albumin- coated vascular grafts have been developed to prevent the procedure of clotting of the vascular graft before implantation, some of them are disclosed in patents: WO 9608149, EP 1501565, FR 2793693, DE 19724869, WO2005053765, US 4326532, US 20060228391, US 4987181 in US 20040234575. Heparin impregnation is also usually performed to prevent coagulation as disclosed in patents US 4613517 and US 4521564.

Another method for surface modification of a vascular grafts is by gaseous plasma in which various gases have been employed in order to obtain desirable surface features and enhance and optimize adhesion of coatings and/or biological materials. Some of these methods are disclosed in patents: US60033582 and US7803393. Gasous-plasma treatment is used together with various protein coatings to to tailor surface properties and improve biocompatibility/hemocompatibility.

DESCRIPTION OF NEW INVENTION

Method of treatment of a vascular graft addresses the need for improved methods of increasing hemocompatibility of vascular grafts made from polyethylene terephthalate polymers against the state of the art. The present invention does not involve deposition or grafting of any anti-thrombogenic coating on the inner walls of vascular grafts. Hence, it provides a method for improved hemocompatibility of vascular grafts by treatment of inner walls by excited oxygen molecules. The efficiency of the method which is the subject of this invention is confirmed by behavior of blood platelets on modified vascular grafts. Against the state of the art the methods of the invention prevents transformation of platelets from normal state in healthy blood to highly activated states, which would further lead to undesired thrombus reactions. Thus the present

invention improves hemocompatibility of artificial polyethylene terephthalate surfaces, as transformation of platelet shape from its normal state- round or discoid and dendritic to spread dendritic, spread and fully spread shape is highly reduced.

Vascular grafts are cardiovascular implants which replace injured or diseased blood vessels. The methods of invention deals with vascular grafts made from polyethylene terephthalate in any forms; tubes made from foils, or prosthetic grafts made by weaving or knitting. The term "polyethylene terephthalate" will be used identically as its abbreviation "PET". Expression PET polymers relates to at least one PET polymer. Vascular grafts are of any length but usually of length between 0.5 and 50 cm. They are of any diameter but typically of diameter between 1 and 20 mm. The wall thickness is typically between 0.05 and 1 mm. These figures are given for information purposes in order to better present embodiment of this invention, and do not, in themselves, limit scope of this invention.

The disease or condition to be treated with vascular graft according to this invention is selected from group comprised of atherosclerotic cardiovascular disease, inflammatory reactions, infections, aneurysm.

Electronically excited oxygen molecules are molecules of oxygen gas containing two atoms bonded by covalent forces for which one of the electrons is found in an energetically less favorable orbital. Against normal state of oxygen molecules which have an electron configuration in energetically most favorable state the electronically excited molecules have one electron in an energetically higher orbital. Electronically excited molecules are often described with symbols. A normal oxygen molecule is often known as an oxygen molecule in the ground state and has been assigned the state ($X\ ^3\Sigma_g^-$). Electronically excited oxygen molecules are found either in the first excited state which has been assigned the state ($a\ ^3\Delta_g^-$), or in the second excited state which has been assigned the state ($b\ ^1\Sigma_g^+$). Electronically excited molecules do not de-excite by electrical dipole radiation and are often referred as metastable oxygen molecule excited states. The radiative life time of electronically excited molecules in the first excited state is about 45 minutes, and the radiative life time of the electronically excited molecules in the second excited state is about 10 seconds. Radiative life time means an average time an electronically excited molecule remains in the

excited state until it is transformed to a molecule in the ground state by simultaneous emission of a light quantum (a photon).

Broadly, this new invention provides a method for improved hemocompatibility of vascular grafts by treatment of inner walls by excited molecules, preferably oxygen. The efficiency of the method which is the subject of this invention is confirmed by behavior of blood platelets on modified vascular grafts. Against the state of the art the methods of the invention prevents transformation of platelets from normal state in healthy blood to highly activated states, which would further lead to undesired thrombus reactions. Thus the present invention improves hemocompatibility of artificial polyethylene terephthalate surfaces, as transformation of platelet shape from its normal state- round or discoid and dendritic to spread dendritic, spread and fully spread shape is highly reduced.

In preferred embodiments a vascular graft made from PET is subjected to the electronically excited molecules, preferably oxygen, in a suitable processing chamber. The electronically excited molecules will slowly react chemically with PET materials leaving excessive hydroxyl, ether, carbonyl, ester and carboxyl groups on the surface. These groups will form hydrophilic surface with a negative surface charge which will in turn reduce adsorption of fibrinogen and lower adhesion of platelets and their activation. The enzyme thrombin acts on the release of small peptides from fibrinogen, which causes the polymerization of fibrinogen monomers into fibrin polymer. Thus the fibrin structures, if appearing on a surface of vascular grafts, may cause accumulation of blood proteins, and further activation of blood platelets which will with high probability result in aggregation of platelets and surface induced thrombosis.

In further preferred embodiments the electronically excited molecules are dosed to vascular grafts in such a way that the flux of the electronically excited molecules will be applied at a temperature range between 0 and 50°C or even at room temperature said temperature ranging from 15 and 25 °C.. This temperature range is favorable because it prevents condensation of water molecules on the surface and allows for good stability of hydroxyl, ether, carbonyl, ester and carboxyl functional groups on the surface. Higher temperatures, at more than 50°C, are avoided in preferred embodiments since the excessive hydroxyl, ether, carbonyl, ester and carboxyl groups on the

surface of PET polymers are thermally not stable and decay spontaneously at elevated temperatures, i.e. above about 50°C.

Preferably the treatment of vascular grafts made from PET polymers is performed under reduced pressures, i.e. pressures below atmospheric pressure. Reduced pressure is favorable since it allows for rapid desorption of water molecules that might be present on PET polymers and since it prevents a loss of electronically excited molecules by gas phase thermallization. The term thermallization stands for transformation of electronically excited molecules to molecules in the ground state through superellastic collisions with other molecules at three body collisions. A three body collision is referred as a collision of at least one electronically excited molecule with two other molecules that may or may not be electronically excited.

Brief description of the drawings

Other advantages of the invention will become apparent upon reading the following descriptions and accompanying drawings, said drawing forming part of this application, presenting as follows:

Figure 1. shows schematic of the formation of excessive hydroxyl, ether, carbonyl, ester and carboxyl functional groups on the surface of PET polymers upon exposure to electronically excited oxygen molecules

Figure 2. shows measured values of hydroxyl, ether, carbonyl, ester and carboxyl functional groups by ESCA (Electron Spectroscopy for Chemical Analyses) on the surface of untreated material and materials treated by electronically excited oxygen molecules

Figure 3. an image from SEM (Scanning Electron Microscopy) of a standard vascular graft after incubation with whole blood

Figure 4. an image from SEM (Scanning Electron Microscopy) of a standard vascular graft after treatment by the method of invention and incubation with whole blood

Figure 5. shows the surface density of selected forms of blood platelets: (R)- round or discoid; (D) dendritic or early pseudopodia; (SD) spread-dendritic or intermediate pseudopodial and (FS) fully spread on untreated vascular grafts and vascular grafts treated by the method of the invention

Figure 6. shows the adsorbed mass of fibrinogen on untreated PET surface and on PET surface treated by the method of the invention

Detailed description of the invention

The hemocompatibility of standard vascular grafts is not optimal. The blood platelets often adhere to PET material, and transform from inactive to active types. The active types release an enzyme called thrombokinase which favors transformation of blood proteins, in particular fibrinogen to fibrin.

Platelets are self sufficient as they contain all the necessary ingredients needed for adhesion, aggregation and formation of thrombi. In fresh blood platelets have spheroidal form, but have a tendency to extrude hair-like filaments from their membranes and can adhere to each other (Shi et al.; Biomaterials and Tissue Engineering, Springer-Verlag, Berlin; 2004). Their function is to arrest bleeding through the formation of platelet plugs and to stabilise platelet plugs by catalyzing coagulation reactions which lead to the formation of fibrin, as described above.

The basis for understanding platelet function is their structure. In an un-stimulated state platelets have a discoid shape, which is maintained by a cytoskeleton of microtubules. The external surface coat of platelets contains membrane-bound receptors (glycoproteins Ib and IIb/IIIa) that mediate contact reactions of adhesion (platelet – surface) and aggregation (platelet – platelet). The membrane also provides a phospholipid surface, which accelerates the coagulation cascade and forms a spongy, canal-like open network that represents an expanded reactive surface to which plasma factors are selectively adsorbed (Ratner et al.; Academic Press, San Diego; 2008).

A possible way to asses the activation degree of adherent platelets is to study their shape and the number of adherent platelets. According to Goodman (Goodman et al.; Platelet responses to

silicon-alloyed pyrolytic carbons. *Journal of Biomedical Materials Research Part A* 83A, 64; 2006), their shape can be categorised on a scale from lower to higher level of activation as: round or discoidic (R); dendritic or early pseudopodial (D); spread-dendritic or intermediate pseudopodial (SD); spreading or late pseudopodial (S) and fully spread (FS).

According to the methods of invention, the adsorption of blood proteins and transformation to active types is prevented by formation of a stable negative charge on PET polymers. The negative surface charge is formed because of presence of excessive hydroxyl, ether, carbonyl, ester and carboxyl functional groups on the surface. In the preferred embodiment the functional groups are formed on the surface of PET polymers by exposure to a flux of electronically excited molecules. Unlike oxygen molecules in the ground electronic state which are not capable of chemical interaction with PET polymers at room temperature, the electronically excited molecules interact with PET polymers predominantly by breaking bonds between hydrogen and carbon in polymer materials and thus enrich the surface of PET polymers with hydroxyl, ether, carbonyl, ester and carboxyl functional groups. The schematic of the interaction between an electronically excited molecule and PET polymers is presented in Figure 1. The enrichment of surface with functional groups is determined by the ESCA method. ESCA is the abbreviation for "Electron Spectroscopy for Chemical Analyses". The atomic concentration of these groups is presented in Figure 2. Increase in hydroxyl, ether, carbonyl, ester and carboxyl groups is observed after treatment by the method of the present invention.

Presence of excessive hydroxyl, ether, carbonyl, ester and carboxyl functional groups on the surface of vascular grafts improves hemocompatibility of vascular grafts made from PET polymers. Namely, the amount and orientation of blood proteins, which always adsorb on a polymer surface to form a thin film, influence on the biological response. A change in the surface charge will result in the amount and reorientation of blood proteins adsorbed on the surface, which further influence on adhesion and activation of platelets. The long term equilibrium is always established in such a manner that the blood proteins orient on the surface in energetically most favor state. This state is often called the equilibrium state. When an equilibrium state is reached the surface becomes passivized by blood proteins so further accumulation is unlikely to occur. The major task is to obtain an appropriate amount and orientation of blood proteins, which adsorb on the inner

walls of vascular grafts made from PET polymers, to prevent activation of blood platelets on the surface of vascular grafts. According to the methods of invention, an optimal amount and orientation of blood proteins is obtained by functionalization of the inner walls of vascular grafts with excessive hydroxyl, ether, carbonyl, ester and carboxyl functional groups. The presence of these functional groups in a preferred concentration will cause a change in the surface charge and thus favorable amount and orientation of blood proteins adsorbing on the vascular graft surface from blood plasma. Such a film of blood proteins is stable since it is found in energetically most favorable state.

Electronically excited oxygen molecules could be produced in large quantities by two different procedures: i) heating of gas containing molecules in the ground state to very high temperatures (typically several 1000 K), and ii) formation from neutral oxygen atoms in the ground state and association of said oxygen atoms in the ground state collisions in the gas phase. Since PET materials will not stand heating to high temperatures the first method should be applied in such a way that gas is heated at high temperature and then cooled down to room temperature by adiabatic expansion to a chamber pumped continuously in order to achieve pressure much lower than in the hot chamber. The second method could be applied at lower temperatures. In a preferred embodiment oxygen gas is leaked to a dissociation chamber. The pressure in the dissociation chamber should be in the range of rough vacuum, typically between 100 and 10000 Pa. In the dissociation chamber, the molecules found essentially in the ground state are dissociated to neutral oxygen atoms also essentially in the ground state by irradiation with free electrons. The kinetic energy of electrons used for dissociation of oxygen molecules in the ground state should be in a narrow bracket between 1 and 6 eV. If the electron energy is lower the molecules would not be likely to dissociate. If the electron kinetic energy is higher than about 6 eV, the dissociation process might lead to heating of gas which is an effect that should be minimized, otherwise the gas would be heated and a need for cooling down would become an important consideration. Once the atoms are dissociated the gas is lead from the dissociation chamber to an association chamber. In the association chamber the atoms associate to molecules in electronically excited states. The gas in the association chamber is essentially kept at almost the same pressure as the gas in the dissociation chamber. Since it is essential to have a flow of gas from the dissociation to the association chamber a small pressure gradient is needed anyway, but

this pressure gradient is essentially so small that the ratio of pressures in the dissociation and association chambers rarely exceeds a factor of 1.05. Association of neutral oxygen atoms in the ground state to electronically excited oxygen molecules is slightly exothermic process so in a preferred embodiment the association chamber is cooled by forced air. Despite cooling the gas in the association chamber is still found at elevated temperature (about 400 K) so it should be cooled down by expansion to the treatment chamber. The pressure difference between the association and treatment chambers should allow for cooling the gas of electronically excited molecules down to the room temperature. In practice, this means that the ratio of pressures in the association and treatment chambers is a factor of about 1.5. This value depends on the temperature of gas in the association chamber – a higher temperature in the association chamber will dictate a higher pressure difference between the association and the treatment chambers.

Once the electronically excited molecules are prepared they are let to interact with vascular grafts made from PET polymers. In cases when the ratio between the vascular graft diameter and its length is rather large (say above a factor of 0.3 cm) the electronically excited molecules are left to diffuse freely throughout the treatment chambers. Many electronically excited molecules will enter the vascular graft and will interact chemically with the inner walls of the vascular graft. The chemical reactions are presented schematically in Figure 1. Since the vascular grafts during treatment with electronically excited molecules are kept essentially at room temperature, where the interaction probability and thus a loss of electronically excited molecules due to surface chemical reactions is low, many electronically excited molecules will reach the entire surface inside the vascular graft so the surface functionalization will be uniform throughout the entire length of the vascular graft.

If the ratio between the vascular graft diameter and its length is rather small (say below a factor of 0.3 cm) the electronically excited molecules will not reach all surfaces inside the vascular graft so free diffusion is not enough to assure for uniform surface modification. In the case of small ratio between the vascular graft diameter and its length the electronically excited molecules should be forced to flow through a vascular graft. In a preferred embodiment forcing is realized by establishing a pressure gradient across the length of a vascular graft. Such a pressure gradient is commonly established by pumping gas containing electronically excited molecules through the vascular graft.

Presence of water molecules adsorbed on surfaces of vascular grafts may prevent chemical interaction between electronically excited molecules and PET polymers and thus prevent successful functionalization of the inner walls of vascular grafts with excessive hydroxyl, ether, carbonyl, ester and carboxyl functional groups. In the preferred embodiment the vascular grafts are dried before treatment using any of available drying techniques. Still, some water molecules may be present either on vascular grafts or in the treatment chamber. In order to minimize the influence of adsorbed water molecules on functionalization of vascular grafts with excessive hydroxyl, ether, carbonyl, ester and carboxyl functional groups by electronically excited molecules the following precautions are adopted: gas leaked into the dissociation chamber is of high purity and/or is dried by any available technique prior to leakage into the dissociation chamber; the whole system is made from materials that contain water molecules only in minimum quantities; rubber materials are particularly avoided; the system is pumped by powerful vacuum pumps; the entire system is heated to elevated temperature and pumped during continuous leakage of dry gas prior to treatments and then cooled down to room temperature; after the treatment the entire system is filled with dry gas rather than air from laboratory room.

In the preferred embodiment the treatment of vascular grafts with electronically excited molecules is essentially performed at room temperature. This temperature assures for favorable reactivity between electronically excited molecules and vascular grafts made from PET materials, allows for desorption of water molecules that might be formed on the PET polymer surface during chemical reactions and prevents structural changes of PET polymers that might otherwise occur due to thermal effects.

Subject of this invention is also vascular graft product made of PET polymer or plurality thereof which has been treated by method as decribed in this application.

Said vascular graft products are used when the blood flow through the natural vessel is restricted. In such cases the part of the natural blood vessel has to be replaced by an artificial one. In therapeutic treatment said vascular graft products may be used for replacement or re-inforcing of already existing blood vessel..

Example 1

In the example disclosed here a vascular graft made from PET polymer has been treated in a treatment chamber by electronically excited molecules. The electronically excited molecules were produced by dissociation and subsequent association of oxygen molecules following the procedure described in details in the text above. The vascular grafts have been dried under vacuum conditions for prolonged time at essentially room temperature. A dried graft has been mounted into the treatment chamber containing electronically excited molecules at the density of about $2x10^{22}$ m^{-3}. The vascular graft made from PET polymer was left in the treatment chamber for 100 seconds and the electronically excited molecules were left to diffuse freely. The electronically excited molecules reacted with vascular graft made from PET polymer following reactions presented schematically in Figure 1. Excessive hydroxyl, ether, carbonyl, ester and carboxyl functional groups were formed on the surface of the vascular graft made from PET polymer and their concentration has been determined immediately after treatment by ESCA method. The results of the characterization are presented in Figure 2. The vascular grafts made from PET polymers have been incubated with whole blood taken by vein puncture from a healthy donor. The activation of blood platelets has been analyzed by scanning electron microscopy (SEM). Figure 3 represents an SEM image of untreated vascular grafts after incubation with whole blood. And on Figure 4 SEM image of vascular grafts treated by the methods of the invention after incubation with whole blood is presented. A huge difference is observed: high concentration of highly activated blood platelets is observed on the surface of an untreated vascular graft, while much lower number of platelets and mostly in normal- round state are seen on the surface of vascular graft treated by methods of the invention. An important effect governing the hemocompatibility of vascular grafts is transformation of blood platelets from inactive form (round shape) present in real blood of a healthy donor not in contact with any foreign materials to activated forms. Several forms have been indicated and the surface concentration has been determined using a common counting technique. The results are summarized in Figure 5. The results show that the vascular graft treated by methods of the invention prevent transformation of platelets to the most active-fully spread (FS) form, while the transformation to other forms such as dendritic (D), spread dendritic (SD) and spread (S) forms are strongly reduced as compared to the untreated vascular graft. Huge difference in the amount of adsorbed blood plasma protein fibrinogen is observed between untreated and treated PET vascular grafts by the method of the invention. The amount of

fibrinogen was measured by quartz crystal microbalance (QCM) and much lower amount of fibrinogen was shown to adhere on treated PET surface, as can be seen from Figure 6. As lower adhesion of fibrinogen to artificial surfaces has been linked with lower activation of platelets and surface induced thrombosis, these results further support improved hemocompatibility of PET vascular grafts after treatment by the method of the present invention.

CLAIMS

1. Method for treatment of a vascular graft made from PET polymer or plurality thereof, said method comprised of the following steps:
   - Drying said vascular graft;
   - Mounting of the said vascular graft into a vacuum chamber with essentially negligible amounts of water molecules;
   - Essentially evacuating said treatment chamber to achieve pressure below atmospheric pressure, preferably in a range between about 1 Pa to about 1000 Pa;
   - Applying electronically excited molecules into said treatment chamber, preferably electronically exited oxygen molecules;
   - Maintaining pressure in a range of 1 – 1000 Pa in said treatment chamber;
   - Reacting said electronically excited oxygen molecules with said vascular grafts;
   - Venting said treatment chamber with essentially dry air.

2. Method according to claim 1, wherein the treatment procedure takes place at temperatures between 0 and 50 degrees Centigrade, preferably at temperatures between 15 and 25 degrees Centigrade.

3. Method according to claim 1, wherein water vapor partial pressure in said treatment chamber is below 10 Pa, preferably below 1 Pa.

4. Method according to claim 1, wherein the density of said electronically excited molecules, preferably electronically excited oxygen molecules is between about $1x10^{19}$ m^{-3} and about $1x10^{24}$ m^{-3}.

5. Method according to claim 1, wherein the treatment time is between 1 s and 10.000 s, preferably between 10 s and 1000 s.

6. Method according to claim 1, wherein the said electronically excited molecules, preferably electronically excited oxygen molecules are produced by any known technique including thermal excitation and formation by association of free oxygen atoms.

7. Method according to claim 1, wherein the said electronically excited molecules, preferably electronically excited oxygen molecules are at their temperature between -20 degrees Centigrade and 100 degrees Centigrade, preferably at temperatures between 15 and 25 degrees Centigrade.

8. Method according to claim 1, wherein the said electronically excited molecules, preferably electronically excited oxygen molecules are cooled down to the temperature according to claim 7 by essentially adiabatic expansion.

9. Vascular graft made from PET polymer or plurality thereof treated by a method of any of preceding claims.

10. Use of vascular graft of claim 9 in therapeutic treatment of disease or condition.

11. Use of vascular graft according to claim 10 sherein said therapeutic treatment is replacement or reinforcement of blood vessel.

12. Use of vascular graft according to any of claims 10 or 11 whereas the disease or condition is selected from the group comprised of atherosclerotic cardiovascular disease, inflammatory reactions, infections, aneurysm.

13. A product comprising vascular graft of claim 9.

AMENDED CLAIMS
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CLAIMS

1. Method for treatment of a vascular graft made from PET polymer, said method comprised of the following steps:
   - Drying said vascular graft;
   - Mounting of the said vascular graft into a vacuum chamber with negligible amounts of water molecules;
   - Evacuating said treatment chamber to achieve pressure below atmospheric pressure, preferably in a range between 1 Pa to 1000 Pa;
   - Applying electronically excited molecules into said treatment chamber;
   - Maintaining pressure in a range of 1 – 1000 Pa in said treatment chamber;
   - Reacting said electronically excited oxygen molecules with said vascular grafts;
   - Venting said treatment chamber with dry air.

2. Method according to claim 1, wherein the treatment procedure takes place at temperatures between 0 and 50 degrees Centigrade, preferably at temperatures between 15 and 25 degrees Centigrade.

3. Method according to claim 1, wherein water vapor partial pressure in said treatment chamber is below 10 Pa, preferably below 1 Pa.

4. Method according to claim 1, wherein the density of said electronically excited molecules is between $1x10^{19}$ m^{-3} and $1x10^{24}$ m^{-3}.

5. Method according to claim 1, wherein the treatment time is between 1 s and 10.000 s, preferably between 10 s and 1000 s.

6. Method according to claim 1, wherein the said electronically excited molecules are produced by any known technique including thermal excitation and formation by association of free oxygen atoms.

7. Method according to claim 1, wherein the said electronically excited molecules are at their temperature between -20 degrees Centigrade and 100 degrees Centigrade, preferably at temperatures between 15 and 25 degrees Centigrade.

8. Method according to claim 1, wherein the said electronically excited molecules are cooled down to the temperature according to claim 7 by adiabatic expansion.

9. Vascular graft made from PET polymer treated by a method of any of preceding claims.

10. Use of vascular graft of claim 9 in therapeutic treatment of disease or condition.

11. Use of vascular graft according to claim 10 wherein said therapeutic treatment is replacement or reinforcement of blood vessel.

12. Use of vascular graft according to any of claims 10 or 11 whereas the disease or condition is selected from the group comprised of atherosclerotic cardiovascular disease, inflammatory reactions, infections, aneurysm.

13. A product comprising vascular graft of claim 9.

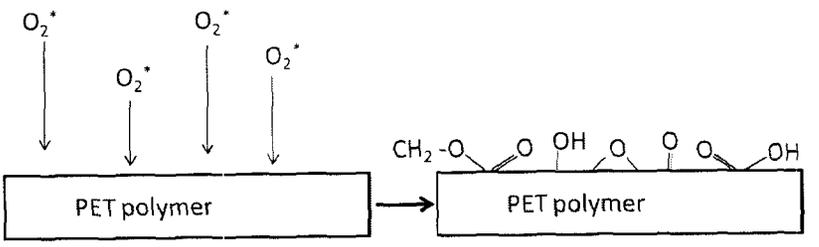

Figure 1.

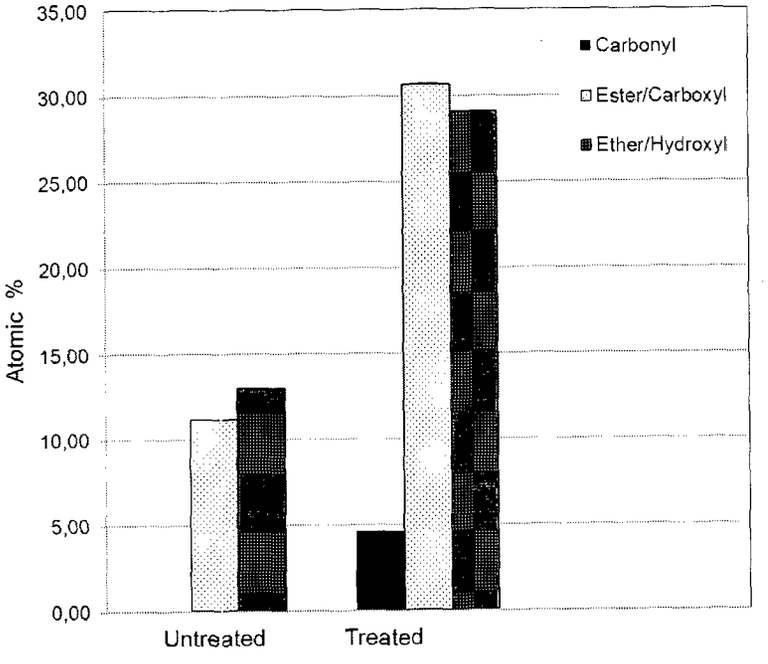

Figure 2.

Figure 3.

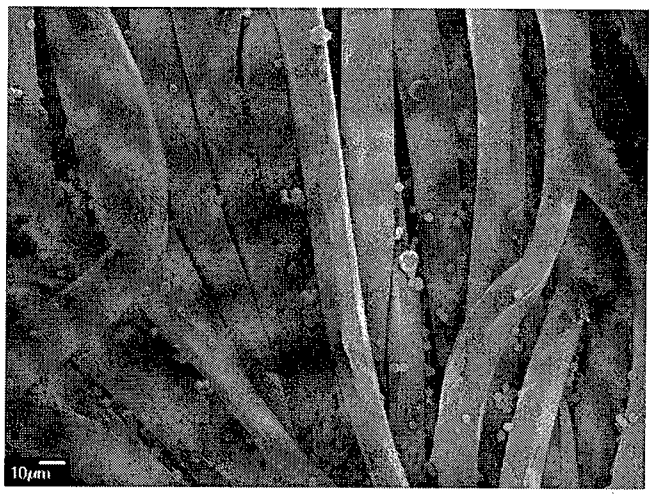

Figure 4.

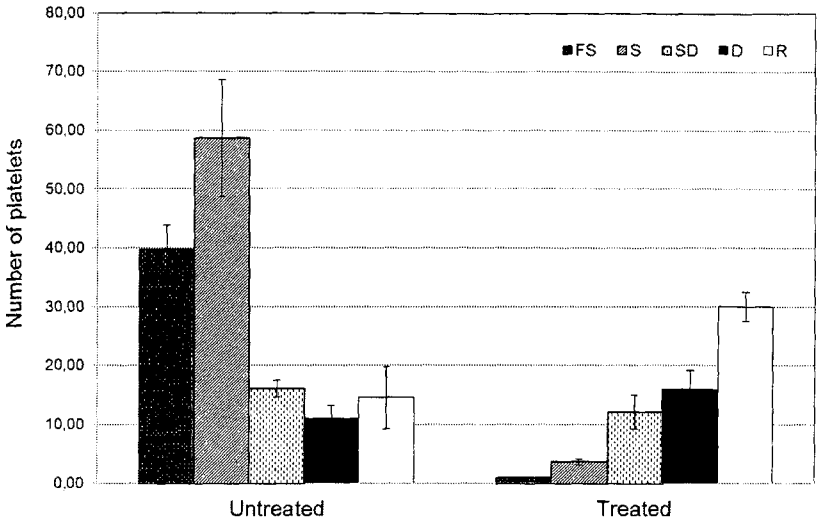

Figure 5.

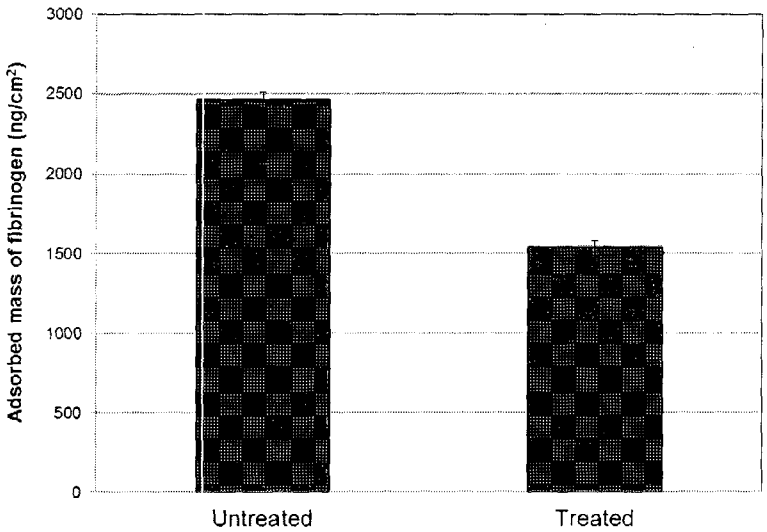

Figure 6.

INTERNATIONAL SEARCH REPORT

International application No
PCT/SI2013/000003

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L27/18 A61L33/00 A61F2/06
ADD.

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)
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 practicable, search terms used)
EPO-Internal, COMPENDEX, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/123465 A2 (INST STEFAN JOSEF [SI]; JUNKAR LTA [SI]; MOZETIC MIRAN [SI]; VESEL ALE) 28 October 2010 (2010-10-28) page 5, lines 16-21 page 7, lines 8-27 page 9, lines 17-26 page 11, line 26 - page 12, line 6	1-13
A	VESEL A ET AL: "Surface functionalization of organic materials by weakly ionized highly dissociated oxygen plasma", JOURNAL OF PHYSICS: CONFERENCE SERIES, INSTITUTE OF PHYSICS PUBLISHING, BRISTOL, GB, vol. 162, no. 1, 1 April 2009 (2009-04-01) , page 12015, XP020157453, ISSN: 1742-6596 abstract	1-13

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 October 2013	28/10/2013

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, Fax: (+31-70) 340-3016	Zalfen, Alina

INTERNATIONAL SEARCH REPORT

International application No
PCT/SI2013/000003

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	UROS CVELBAR ET AL: "Hemocompatible Poly(ethylene terephthalate) Polymer Modified via Reactive Plasma Treatment", JAPANESE JOURNAL OF APPLIED PHYSICS, vol. 50, no. 8, 1 August 2011 (2011-08-01) , page 08JF02, XP055084367, ISSN: 0021-4922, DOI: 10.1143/JJAP.50.08JF02 abstract ----	1-13
A	ALENKA VESEL ET AL: "Surface modification of polyester by oxygen- and nitrogen-plasma treatment", SURFACE AND INTERFACE ANALYSIS, vol. 40, no. 11, 10 October 2008 (2008-10-10), pages 1444-1453, XP055084372, ISSN: 0142-2421, DOI: 10.1002/sia.2923 abstract ----	1-13

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/SI2013/000003

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010123465 A2	28-10-2010	SI 23021 A	29-10-2010
		WO 2010123465 A2	28-10-2010