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NOTICE OF ENTITLEMENT

We, **Vascular Graft Research Center Co., Ltd.**, the applicant/Nominated Person in respect of Application No. 14470/92 state the following:-

The Nominated Person is entitled to the grant of the patent because the Nominated Person derives title to the invention from the inventor by assignment.

The Nominated Person is entitled to claim priority from the application listed in the declaration under Article 8 of the PCT because the Nominated Person made the application listed in the declaration under Article 8 of the PCT, and because that application was the first application made in a Convention country in respect of the invention.

DATED this SIXTEENTH day of DECEMBER 1992

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a member of the firm of
DAVIES COLLISON
CAVE for and on behalf
of the applicant(s)

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COMPOSITE ARTIFICIAL BLOOD VESSEL

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(57) Claim

1. An artificial blood vessel comprising a tube of a porous synthetic polymer on a surface of which a protein or a peptide having cell adhesion and growth functions is covalently bonded through hydroxyl groups, carboxyl groups, epoxy groups or amino groups which are introduced onto the surface of the porous synthetic polymer.

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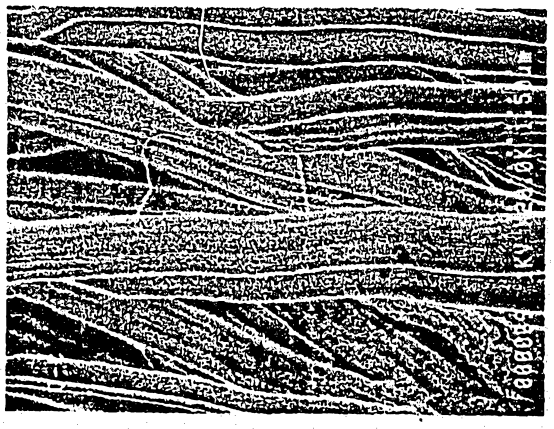

(57) Abstract

An artificial blood vessel comprising a tube made from a porous synthetic polymer and, covalently bonded to the surface of the tube via a hydroxyl, carboxyl, epoxy or amino group, a protein or peptide having an activity of cell adhesion and growth. It is possible to form a stable composite of a substance having a tissue inducing activity uniformly on the surface of the porous polymer material through covalent bonds without deforming the material, to form the tunica intima rapidly without inducing primary thrombus, and to maintain the formed tunica stably for long.

(57) 要約

多孔質合成高分子よりなるチューブの表面に、水酸基、カルボキシル基、エポキシ基又はアミノ基を介して細胞接着及び成長作用を有するタンパク質又はペプチドを共有結合させた人工血管。多孔質合成高分子材料の形状を変化させることなく、表面に均一に生体組織誘導作用を有する物質を共有結合によって安定に複合化でき、初期血栓を誘発することなく、速やかに内膜が形成され、しかも形成された内膜を長期間にわたって安定に維持することができる。

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SPECIFICATION

COMPOSITE ARTIFICIAL BLOOD VESSEL

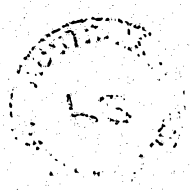
FIELD OF THE INVENTION

The present invention relates to an artificial blood vessel used for treatment of diseases in aortas, coronary arteries, peripheral blood vessels and the like.

PRIOR ART

Hitherto, a tube of a woven or knitted fabric of polyester fibers or expanded polytetrafluoroethylene (hereinafter referred to as "EPTFE") is used as an artificial blood vessel. The EPTFE tube is practically used in a smaller diameter range than the polyester tube, since polytetrafluoroethylene itself is excellent in an anti-thrombotic property and a porous structure comprising fibers and nodes which is obtained by drawing is excellent in biocompatibility.

However, EPTFE is not necessarily satisfactory in the anti-thrombotic property. In the case of an artificial blood vessel having an inner diameter of 6 mm or less, in particular 4 mm or less, a sufficient patency ratio is not achieved. To solve this problem, the following methods have been studied: (1) improvement of an anti-thrombotic property of a material itself, (2) imparting an anti-thrombotic property to an artificial blood vessel by forming an intima through induction of a tissue in an early stage after graf-



ting the artificial blood vessel and (3) seeding vascular endothelial cells having a good anti-thrombotic property on an inner wall of the artificial vessel.

Concretely, as the method (1), a development of an anti-thrombotic polymer material having a microphase separation structure or an anti-thrombotic agent-immobilized material is discussed (cf. Noishiki et al, Trans. A. S. A. I. O., 23, 253 (1977), etc.) Though such anti-thrombogenic materials can prevent the formation of thrombuses immediately after grafting, after a long term from the grafting, the thrombi are formed so that the vessel is occuluded.

As the method (2), is proposed an artificial blood vessel on which a cell-adhering protein such as collagen and fibronectin is coated and immobilized by crosslinking (cf. C. H. Lundgren et al, Trans. A. S. A. I. O., 32 346 (1986), etc.). Since the thrombus tends to adhere due to the coating of the protein in such artificial blood vessel, the patency ratio greatly decreases. Then, an artificial blood vessel to which an anti-thrombotic agent such as heparin is further composited is proposed, but sufficient patency is not achieved. If the patency is maintained, the artificial blood vessel does not have sufficient properties after a long time, since the patency ratio decreases due to thickening or peeling-off of the formed intima.

As the method (3), a method for seeding the vascular endothelial cells on the inner wall of the artificial

vessel is studied (cf. Takagi et al, JINKOZOKI (Japanese Journal of Artificial Organs), 17, 679 (1988), Japanese Patent Kokai Publication No. 170466/1989, etc.) However, it lacks immediate use since it takes a long time for collecting and culturing the vascular endothelial cells. In addition, stability and functions of the seeded endothelium are not complete and no good patency ratio is achieved.

As explained above, the prior arts cannot provide an artificial blood vessel which maintains a good patency ratio for a long time from the initial stage of the grafting.

SUMMARY OF THE INVENTION

As a result of the study on an artificial blood vessel in which an intima can be formed thereon quickly after grafting, the formed intima will be present stably and have a good patency ratio for a long time and an initial patency is good, it has been found that an artificial blood vessel, to which a protein or a peptide having cell adhesion and growth functions is covalently bonded through hydroxyl groups, carboxyl groups, epoxy groups or amino groups which are introduced on a surface of a porous synthetic polymer material by a physical or chemical treatment, is excellent in initial patency after grafting, an intima is quickly formed thereon, and the formed intima will be present stably for a long time without thickening or peeling-off of the formed intima so that a good patency continues. Thereby, the present invention has been completed.



Accordingly, the present invention provides an artificial blood vessel comprising a tube of a porous synthetic polymer on a surface of which a protein or a peptide having cell adhesion and growth functions is covalently bonded through hydroxyl groups, carboxyl groups, epoxy groups or amino groups which are introduced onto the surface of the porous synthetic polymer.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a scanning electron microphotograph of an inner surface of the artificial blood vessel of Example 1 comprising EPTFE to which gelatinated atelocollagen is chemically bonded,

Fig. 2 is a scanning electron microphotograph of an inner surface of the conventional artificial blood vessel of Comparative Example 2 comprising EPTFE to which gelatinated atelocollagen is coated and crosslinked using glutaraldehyde, and

Fig. 3 is a scanning electron microphotograph of an inner surface of the untreated EPTFE artificial blood vessel of Comparative Example 1.

DETAILED DESCRIPTION OF THE INVENTION

As the porous synthetic polymer material used in the artificial blood vessel of the present invention, a polyester woven or knitted fabric tube and a EPTFE tube which give satisfactory results are preferred since they have sufficient strength as the artificial blood vessel, are not decomposed or deteriorated in an organism and have no

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toxicity. Among them, the EPTFE tube is particularly preferred, which has a microporous structure comprising fibers and nodes, a high porosity and good compatibility with the tissue.

To form the intima in an early stage and maintain the formed intima stably, it is necessary to introduce the tissue or blood capillaries in the porous material. To this end, the porosity of the porous synthetic polymer material is, preferably at least 50 %, more preferably at least 70 %.

For the same reason, a pore diameter is preferably at least 20 μm. In particular, in the case of EPTFE, the pore diameter is preferably from 20 to 200 μm.

A ratio of an area of the synthetic polymer material which occupies the inner surface contacting a blood stream in the artificial blood vessel is, preferably from 15 to 80 %, more preferably from 25 to 55 %. This area ratio can be determined using a scanning electron microscope. When the area ratio of the synthetic polymer material is too high, a contact area between the immobilized protein or peptide and the blood is too large and a possibility of the thrombus formation becomes high so that the initial patency ratio decreases. When the area ratio of the synthetic polymer material is too low, the acceleration effect of the immobilized protein or peptide on the formation of the intima is, may be ~~not sufficiently~~ insufficiently achieved.

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To introduce the functional groups on the surface of the synthetic polymer material, there may be used a chemical treatment, or a physical treatment such as radiation exposure with a γ-ray or an electron beam and treatment with corona discharge or glow discharge. A suitable method is selected according to the polymer material. For example, in the case of polyethylene terephthalate (PET) which is one of the polyesters, ester linkages are hydrolyzed with an acid or an alkali to form the carboxyl groups, which are then converted to the ester groups, the hydroxyl groups, the amino groups or the epoxy groups by known reactions. It is possible to carry out graft polymerization by the irradiation of UV light or the corona discharging.

In the case of EPTFE, after defluorination with an alkali metal compound, the carboxyl groups, the hydroxyl groups, the amino groups, the epoxy or the like are introduced in EPTFE by reacting a compound having such functional groups.

Examples of the alkali metal compound are methyllithium, n-butyllithium, tert.-butyllithium, sodium-naphthalenide, naphthalene-benzophenone, vinyllithium and the like. They are used in the form of a solution. Among them, sodium-naphthalenide and sodium-benzophenone will form a dark brown layer on the EPTFE surface after treatment, and cannot treat EPTFE to the porous inner part uniformly. So, methyllithium, n-butyllithium and tert.-butyllithium are



preferred to produce the artificial blood vessel of the present invention. Since each of methyllithium, n-butyllithium and tert.-butyllithium has a weak force to withdraw the fluorine atom, it is necessary to add a chelating agent such as hexamethylphosphoric triamide or N,N,N,N-tetramethylethylenediamine.

Examples of the compound having the hydroxyl group, the carboxyl group, the epoxy group or the amino group are glycerol (meth)acrylate, 2-hydroxyethyl (meth)acrylate, 2-hydroxypropyl (meth)acrylate, polyethyleneglycol (meth)acrylate, glycidyl (meth)acrylate, (meth)acrylic acid, allylamine, 2-aminoethyl (meth)acrylate, acrylamide and the like. Alternatively, an acid anhydride such as maleic anhydride is added and then hydrolyzed.

More specifically, for example, the EPTFE tube is dipped in a solution of methyllithium in diethyl ether in a nitrogen atmosphere and hexamethylphosphoric triamide is added to the solution. Then, the solution containing the tube is kept standing for 30 minutes at 0°C to withdraw the fluorine atoms from EPTFE. After removing the solution, a solution of acrylic acid in tetrahydrofuran is added and reacted at 60°C for 10 hours. After the reaction, excessive acrylic acid or its polymer is washed off to obtain the acrylic acid-grafted polymer.

To introduce the functional groups in EPTFE, radiation exposure with the γ-ray or the electron beam, or the

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corona discharge may be used. But, as is well known, by the radiation exposure treatment, EPTFE is decomposed to its deep crystalline part so that a molecular weight of PTFE is lowered and its strength is considerably decreased and such EPTFE tube can hardly be used as the artificial blood vessel practically (cf. G. Morel et al, J. Appl. Polym. Sci., 24, 771 (1979)). With the glow discharge, it is difficult to treat the deep porous part of EPTFE so that only the outer and inner surfaces of the tube can be treated. Thereby, it is difficult to immobilize the protein on the pore surfaces in tube wall, and the formation of the tissue in the wall or the introduction of the blood capillaries cannot be accelerated.

On the other hand, treatment with the alkali metal compound can make the EPTFE tube to its porous inner part uniformly and also to the deep crystalline part of EPTFE without decomposing PTFE with a depth of about several hundred angstroms from the surface. Therefore, the strength is not decreased and the protein or peptide can be immobilized in all the parts of the porous wall. Consequently, it is desirable to treat the porous polymer material with the alkali metal compound for the production of the composite artificial blood vessel of the present invention.

A method for immobilizing the protein or peptide may be selected according to the material. Preferably, the method is selected so that the tissue induction function is

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not lost by the immobilization and the protein or peptide is immobilized by a bond which will remain without cleavage till the intima is formed. For example, with the hydroxyl group, the carboxyl group or the amino group, the covalent bond is formed by dehydration condensation, and with the epoxy group, the covalent bond is formed by an addition reaction. With the hydroxyl group, the protein or peptide is directly bonded by the dehydration condensation in the presence of carbodiimide as a catalyst, or an eliminating group such as a trifluoromethanesulfonyl group is introduced to improve the reactivity and then such group is reacted with the amino group of the protein. With the carboxyl group, the protein or peptide is directly bonded using a dehydration condensation catalyst such as carbodiimide, or N-hydroxysuccinimide is reacted to introduce an active ester group and improve the reactivity and then the active ester group is reacted with an amino acid of the protein.

The surface which is treated by coating and cross-linking in the prior arts has unevenness of several μm or larger and it is difficult to cover the entire surface of the porous synthetic polymer material. When the protein or peptide is immobilized according to the present invention, the surface unevenness is less than 5 μm and the pore cavities are completely covered.

As the protein to be composited, cell adhesion proteins, endothelial cell growth factors, blood vessel

growth factors and the like are exemplified. Among them, collagen, gelatin, albumin, laminin and fibronectin are preferred.

Since the protein or peptide is not covalently bonded to the porous polymer in the conventional coating-crosslinking type composite artificial blood vessel, there are many drawbacks that the coated protein or peptide is easily peeled off, uniformity in the pores is not sufficient, some parts may not be covered or some parts are coated too thick, or aggregates of the protein are deposited. Thereby, the initial patency ratio is low, the formed intima has poor stability and no good patency ratio is maintained for a long time.

The composite artificial blood vessel of the present invention comprises a porous synthetic polymer material, in particular EPTFE on which surface the protein or peptide having the cell adhesion and cell propagation functions is chemically bonded, and such protein or peptide strongly covers the whole surface of the porous wall of the polymer material covalertly at a thickness in a submicron order. The surface unevenness of the composite material is less than 50 nm, and it is possible to impart the tissue induction function to the surface without materially changing the shape of the porous synthetic polymer material. Since the surface of the porous polymer material is thinly composited, its shape is not changed by compositing. There-

fore, an area on which the protein or peptide is immobilized or an amount of the protein or peptide can be controlled by the porous structure of the synthetic polymer material.

As explained above, since the protein or peptide is strongly immobilized on the polymer material by the covalent bond, it is not peeled off during the handling in operation. Since the surface unevenness is less than 50 nm, the unevenness formed by the compositing does not induce the formation of thrombus by the blood rheology so that the patency ratio does not decrease after the grafting. Since, in the composite artificial blood vessel of the present invention, the tissue induction material is thinly composited only on the surface of the porous synthetic polymer material, no protein or peptide is present in the voids of the porous material. Therefore, the coated area and the composited amount of the protein or peptide can be controlled by changing the porous structure of the synthetic polymer material. As the result, since the contact area of the protein and the blood can be changed, the composite structure is controlled with balancing the thrombus formation due to compositing and the acceleration effect on the formation of the intima.

In the composite artificial blood vessel of the present invention, since the whole surface of the porous wall is composited with the tissue induction material, the tissue component is quickly introduced from outside the of

the artificial blood vessel wall and the growth of the intima quickly proceeds from an anastomotic site. In addition, since protein or peptide is strongly and uniformly immobilized by the covalent bond, the formed intima will not be peeled off and will be present stably for a long time.

PREFERRED EMBODIMENTS OF THE INVENTION

Example 1

In a mixture of a solution of methyllithium in ether (1.4 M) (20 ml) and hexamethylphosphoric triamide (2 ml), an EPTFE tube having an average fibril length of 30 μm, a porosity of 72 %, an area of the inner surface occupied by the resin of 45 %, an inner diameter of 1.5 mm, an outer diameter of 2.5 mm and a length of 10 mm was dipped at 0°C for 30 minutes in a nitrogen atmosphere, and the solution mixture was removed. Then, a solution of acrylic acid (1 g) in tetrahydrofuran (20 ml) was added and reacted at 60°C for 10 hours.

Thereafter, unreacted acrylic acid and polymerized acrylic acid were washed off to obtain an acrylic acid-grafted EPTFE tube. A grafted amount of acrylic acid was 45 μg per 1 cm of the tube.

An aqueous solution of 0.3 % gelatinated atelocollagen (hereinafter referred to as "GAC") which was prepared by thermally denaruring 0.3 % solubilized atelocollagen (Nitta Gelation Cellmatrix I-P) and 0.3 % 1-ethyl-3-(3-dimethylaminopropyl)carbodiimde was treated with 1N hydro-

chloric acid to adjust pH at 1.5. To this solution, the above prepared acrylic acid-grafted EPTFE tube was dipped for 24 hours and washed with water to obtain the GAC-bonded EPTFE tube.

The bonded amount of GAC was measured by the ninhydrin method to find that 55 μg of GAC was bonded per 1 cm of the tube.

The inner surface of the tube was observed by a scanning electron microscope. The microstructure of the fibrils and nodes of EPTFE was completely maintained, and the unevenness of the composited surface was less than 10 nm. No large unevenness which is seen in the coating complexing method was observed. The composited GAC was not peeled off by the handling such as flexing.

This artificial blood vessel had a tensile strength of 3.2 kg and a suture strength of 142 g, which were not significantly decreased from the values of 3.3 kg and 150 g of the untreated EPTFE, and the strength of this artificial blood vessel was practically acceptable. The suture strength is a load with which the tube is torn when a wire having a diameter of 0.2 mm is threaded at a position 3 cm apart from the tube end and pulled.

Each of six treated tubes was inplanted in an abdominal aorta of a rat. After three weeks, the inside of the porous wall of the artificial blood vessel was filled with the tissue components such as fibroblasts. A rate of

the endothelical cell-covered area of the inner wall was 100 % and a patency ratio was 100 %. The patency ratio did not decrease even after one year, the 100 % patency ratio was maintained, the formed intima was stable and no deposition such as thrombus was observed.

Example 2

In a mixture of a solution of methyllithium in ether (1.4 M) (20 ml) and hexamethylphosphoric triamide (2 ml), an EPTFE tube having an average fibril length of 30 μm, a porosity of 72 %, an area of the inner surface occupied by the resin of 45 %, an inner diameter of 2.0 mm, an outer diameter of 3.0 mm and a length of 20 mm was dipped at 0°C for 30 minutes in a nitrogen atmosphere, and the solution mixture was removed. Then, a solution of acrylic acid (1 g) in tetrahydrofuran (20 ml) was added and reacted at 60°C for 10 hours.

Thereafter, unreacted acrylic acid and polymerized acrylic acid were washed off to obtain an acrylic acid-grafted EPTFE tube. A grafted amount of acrylic acid was 45 μg per 1 cm of the tube.

An aqueous solution of 0.3 % gelatinated atelocollagen (GAC) which was prepared by thermally denaturing 0.3 % solubilized atelocollagen (Nitta Gelation Cellmatrix I-P) and 0.3 % 1-ethyl-3-(3-dimethylaminopropyl)carbodiimde was treated with 1N hydrochloric acid to adjust pH at 1.5. To this solution, the above prepared acrylic acid-grafted



EPTFE tube was dipped for 24 hours and washed with water to obtain the GAC-bonded EPTFE tube.

The bonded amount of GAC was measured by the ninhydrin method to find that 55 μg of GAC was bonded per 1 cm of the tube.

The inner surface of the tube was observed by a scanning electron microscope. The microstructure of the fibers and nodes of EPTFE was completely maintained, and the unevenness of the composited surface was less than 10 nm. ~~No~~ A large unevenness which is seen in the coating complexing method was not observed. The composited GAC was not peeled off by the handling such as flexing.

Each of six treated tubes was inplanted in a carotid artery of a rabbit. After four weeks, the inside of the porous wall of the artificial blood vessel was filled with the tissue components such as fibroblasts. A rate of the endothelial cell-covered area of the inner wall was 95 % and a patency ratio was 100 %. The patency ratio did not decrease even after one year, the 100 % patency ratio was maintained, the formed intima was stable and no deposition such as thrombus was observed.

Example 3

In the same manner as in Example 2, 2-hydroxyethyl acrylate was grafted on the same EPTFE tube as used in Example 2. The grafted amount was 65 μg per 1 cm of the tube.

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fluoroethanesulfonic acid (1 ml) and triethylamine (1 ml) in diethyl ether (20 ml) and reacted for 4 hours to introduce the 2,2,2-trifluoroethanesulfonic acid groups on the hydroxyl groups.

Then, fibronectin (derived from bovine plasma, manufactured by Nippon Ham) (5 mg) was dissolved in a sodium carbonate buffer (pH = 7) (20 ml). In this solution, the above prepared tube was dipped for 24 hours and washed with water to obtain the fibronectin-bonded EPTFE tube.

The bonded amount of fibronectin was measured by the ninhydrin method to find that 40 μg of fibronectin was bonded per 1 cm of the tube.

The inner surface of the tube was observed by a scanning electron microscope. The microstructure of the fibers and nodes of EPTFE was completely maintained, and the unevenness of the composited surface was less than 10 nm. ~~No~~ A large unevenness which is seen in the coating complexing method was not observed. The composited fibronectin was not peeled off by the handling such as flexing.

Each of six treated tubes was inplanted in a carotid artery of a rabbit. After four weeks, the inside of the porous wall of the artificial blood vessel was filled with the tissue components such as fibroblasts. A rate of the endothelial cell-covered area of the inner wall was 85 % and a patency ratio was 83 % (5/6). The patency ratio did not

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decrease even after one year, the 83 % patency ratio was maintained, the formed intima was stable and no deposition such as thrombus was observed.

Example 4

A polyester knit tube having an average pore size of 60 μm, a porosity of 60 %, an area of the inner surface occupied by the resin of 50 %, an inner diameter of 2.0 mm, an outer diameter of 3.0 mm and a length of 20 mm was subjected to hydrolysis with 6N hydrochloric acid to form carboxyl groups.

An aqueous solution of 0.3 % gelatinated atelocollagen (GAC) which was prepared by thermally denaturing 0.3 % solubilized atelocollagen (Nitta Gelation Cellmatrix I-P) and 0.3 % 1-ethyl-3-(3-dimethylaminopropyl)carbodiimde was treated with 1N hydrochloric acid to adjust pH at 1.5. To this solution, the above prepared tube was dipped for 24 hours and washed with water to obtain the GAC-bonded EPTFE tube.

The bonded amount of GAC was measured by the ninhydrin method to find that 25 μg of GAC was bonded per 1 cm of the tube.

The inner surface of the tube was observed by a scanning electron microscope. The shape of the polyester fibers was completely maintained, and the unevenness of the composited surface was less than 10 nm. No large unevenness which is seen in the coating complexing method was not

observed. The composited GAC was not peeled off by the handling such as flexing.

Each of six treated tubes was inplanted in a carotid artery of a rabbit. After four weeks, the inside of the porous wall of the artificial blood vessel was filled with the tissue components such as fibroblasts. A rate of the endothelial cell-covered area of the inner wall was 95 % and a patency ratio was 83 % (5/6). The patency ratio did not decrease even after one year, the 83 % patency ratio was maintained, the intima was stable and no deposition such as thrombus was observed.

Comparative Example 1

Each of the six EPTFE tubes which were the same as that used in Example 1 was inplanted in a abdominal aorta of a rat. After three weeks, a patency ratio was 100 %. But, the tissue components such as fibroblast was hardly introduced inside the porous wall of the artificial blood vessel, and a rate of the endothelial cell-covered area of the inner wall was 50 %. After one year, the patency ratio decreased to 67 %, and with the tubes having the patency, partial stenosis was observed.

Comparative Example 2

In the inside of the the same EPTFE tube as used in Example 1, the same 0.3 % GAC solution as that used in Example 1 was injected in vacuo and crosslinked with glutaraldehyde, followed by drying. A composited amount of GAC was 0.2 mg/cm.

The inner surface of the tube was observed by a scanning electron microscope. The composited GAC formed films between the fibrils of EPTFE partially, and unevenness of 0.5 to 1 μm was formed on the surface. Some parts of the inner wall were not covered by GAC. When this composite artificial blood vessel was flexed at a radius of curvature of 5 mm repeatedly, GAC was easily peeled off.

Each of six treated tubes was inplanted in an abdominal aorta of a rat. After three weeks, a patency ratio decreased to 67 %, and the endothelial cell-covered area of the inner wall was 85 % in the tubes having the patency. After one year, the patency ratio was further decreased to 33 %, and with the tubes having the patency, the formed intima were peeled off and partial stenosis was observed.

Comparative Example 3

Each of the six EPTFE tubes which were the same as that used in Example 2 was inplanted in a carotid artery of a rabbit. After four weeks, a patency ratio was 100 %. However, substantially no tissue component such as fibroblast was introduced inside the porous wall of the artificial blood vessel, and a rate of the endothelial cell-covered area of the inner wall was 50 %. After one year, the patency ratio decreased to 33 %, and with the tubes having the patency, partial stenosis was observed.

Comparative Example 4

In the inside of the the same EPTFE tube as used in Example 2, the same 0.3 % GAC solution as that used in Example 1 was injected in vacuo and crosslinked with glutaraldehyde, followed by drying. A composited amount of GAC was 0.3 mg/cm.

The inner surface of the tube was observed by a scanning electron microscope. The composited GAC formed films between the fibrils of EPTFE partially, and unevenness of 0.5 to 1 μm was formed on the surface. Some parts of the inner wall were not covered by GAC. When this composite artificial blood vessel was flexed at a radius of curvature of 5 mm repeatedly, GAC was easily peeled off.

Each of six treated tubes was inplanted in a carotid artery of a rabbit. After four weeks, a patency ratio decreased to 17 %.

Fig. 1 is a scanning electron microphotograph of an inner surface of the artificial blood vessel of Example 1 comprising EPTFE to which gelatinated atelocollagen is chemically bonded.

Fig. 2 is a scanning electron microphotograph of an inner surface of the artificial blood vessel of Comparative Example 2 comprising EPTFE to which gelatinated atelocollagen is coated and crosslinked using glutaraldehyde.

Fig. 3 is a scanning electron microphotograph of an inner surface of the untreated EPTFE artificial blood vessel of Comparative Example 1.

In the artificial blood vessel of the present invention, the gelatinated atelocollagen was thinly and uniformly composited and the shape of EPTFE was completely maintained, while the conventional one had the unevenness of several μm.

EFFECTS OF THE INVENTION

As explained above, the surface of the composite artificial blood vessel of the present invention is uniformly composited stably with the material having the tissue induction function by the covalent bond without deforming the shape of the porous synthetic polymer material. Therefore, by the synergistic effect with the porous structure, in particular, the porous structure of EPTFE, initial thrombus is hardly induced, and the intima is quickly formed and remains stably for a long time.

Accordingly, the composite artificial blood vessel of the present invention is useful as a substitute blood vessel of a small diameter blood vessel such as coronary artery, peripheral artery and the like with which no conventional material can achieve good patency.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:-

1. An artificial blood vessel comprising a tube of a porous synthetic polymer on a surface of which a protein or a peptide having cell adhesion and growth functions is covalently bonded through hydroxyl groups, carboxyl groups, epoxy groups or amino groups which are introduced onto the surface of the porous synthetic polymer.

2. An artificial blood vessel according to claim 1, wherein a ratio of a tube inner area which is occupied by the synthetic polymer material is from 15 to 80%.

3. An artificial blood vessel according to claim 1 or claim 2, wherein said porous synthetic polymer is expanded polytetrafluoroethylene.

4. An artificial blood vessel according to any one of claims 1 to 3, wherein said protein having the cell adhesion function is at least one protein selected from the group consisting of collagen, gelatin, laminin and fibronectin.

5. An artificial blood vessel according to any one of claims 1 to 4, wherein said porous synthetic polymer is expanded polytetrafluoroethylene, and said hydroxyl groups, said carboxyl groups, said epoxy groups or said amino groups are formed by defluorination of the surface of said polytetrafluoroethylene with an alkali metal compound and introduction of a compound of said functional group.

6. An artificial blood vessel according to claim 5, wherein said protein is gelatinized atelocollagen.

7. An artificial blood vessel according to claim 5 or claim 6, wherein said alkali metal compound to be used for defluorination is methyllithium, n-butyllithium or tert.-butyllithium, and is used in combination with hexamethylphosphoric triamide for the defluorination of the surface of polytetrafluoroethylene.

8. An artificial blood vessel according to claim 1 substantially as hereinbefore

described with reference to any one of the drawings or examples other than the comparative examples.

DATED this 15th day of November, 1993.

VASCULAR GRAFT RESEARCH CENTER CO., LTD.
By Its Patent Attorneys
DAVIES COLLISON CAVE

AUSTRALIAN PATENT OFFICE

ABSTRACT

An artificial blood vessel comprising a tube of a porous synthetic polymer on a surface of which a protein or a peptide having cell adhesion and growth functions is covalently bonded through hydroxyl groups, carboxyl groups, epoxy groups or amino groups. Its surface is uniformly composited stably with the material having the tissue induction function by the covalent bond without deforming a shape of the porous synthetic polymer material. Therefore, no initial thrombus is induced, and the intima is quickly formed and remains stably for a long time.

AUSTRALIAN PATENT OFFICE LS

Fig. 1

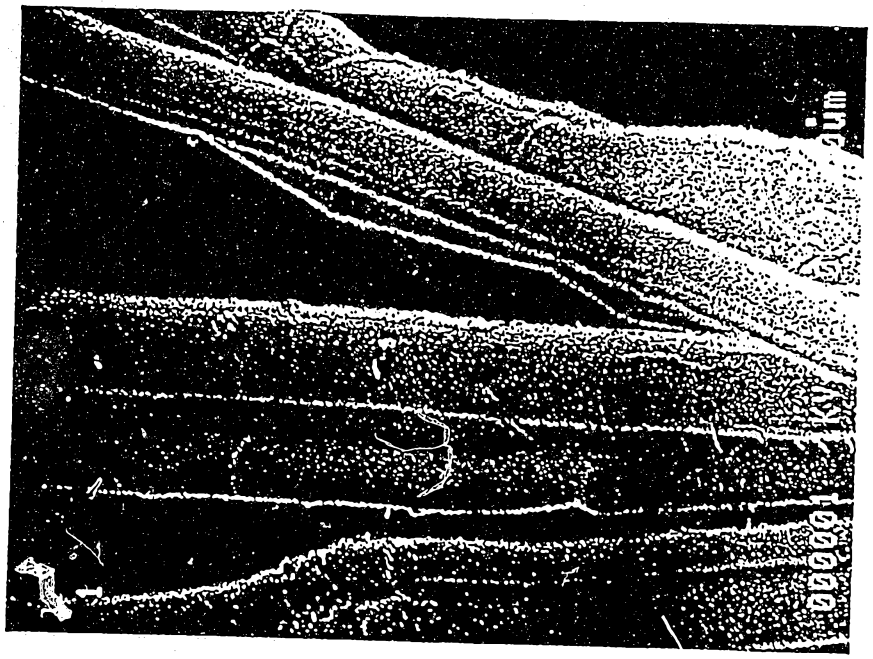


Fig. 2

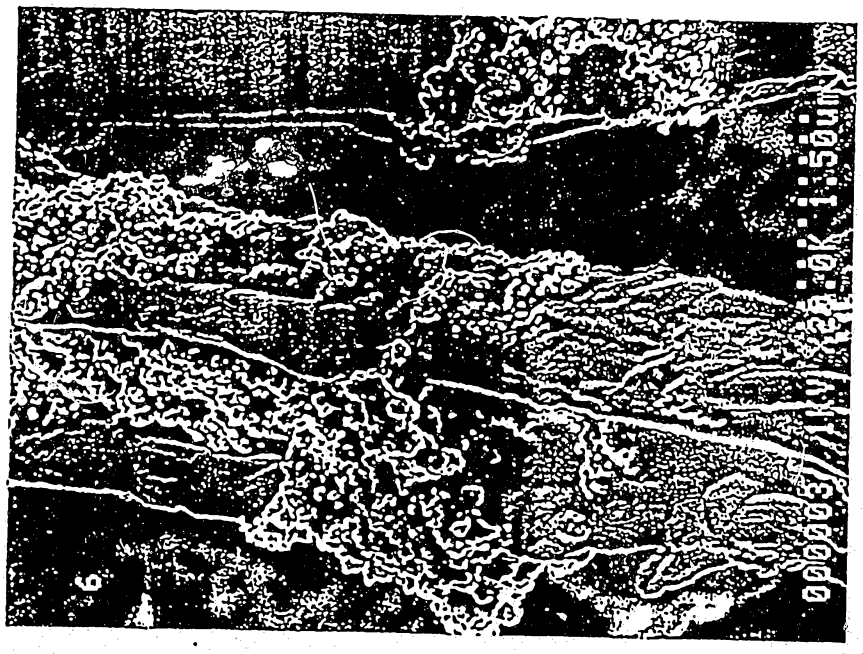
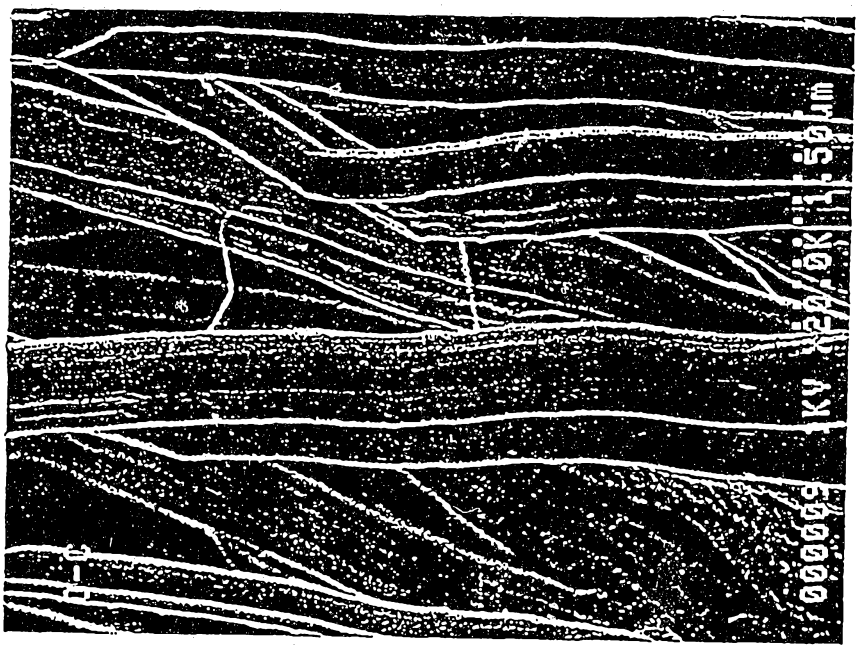


Fig. 3



INTERNATIONAL SEARCH REPORT

International Application No PCT/JP92/00373

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl[5] A61L27/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	A61L27/00, A61F2/06

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Jitsuyo Shinan Koho 1926 - 1992
Kokai Jitsuyo Shinan Koho 1971 - 1992

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A	JP, A, 1-170467 (Toray Industries, Inc.), July 5, 1989 (05. 07. 89), (Family: none)	1-7
A	JP, A, 63-46169 (Kogyo Gijutsuin-cho), February 27, 1988 (27. 02. 88), & EP, A, 241838	1-7
A	JP, A, 62-258666 (Intermedicat GmbH.), November 11, 1987 (11. 11. 87), & FI, A, 871065 & DK, A, 127987 & EP, A, 237037 & BR, A, 8701135 & DE, C, 3608158 & US, A, 4784659 & JP, B2, 1-51263	1-7
A	JP, A, 62-152469 (Sumitomo Electric Industries, Ltd.), July 7, 1987 (07. 07. 87), & EP, A, 230635 & US, A, 4822361	1-7

* Special categories of cited documents: [10]

"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

"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

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
June 19, 1992 (19. 06. 92)	July 7, 1992 (07. 07. 92)
International Searching Authority	**Signature of Authorized Officer**
Japanese Patent Office	

	Industries, Ltd.), July 7, 1987 (07. 07. 87), & EP, A, 230635 & US, A, 4822361	1-7
A	JP, A, 61-135651 (Vascutek Ltd.), June 23, 1986 (23. 06. 86), & GB, A, 8430265 & DK, A, 551185 & EP, A, 183365 & AU, A, 5059385 & AU, B, 569645 & ES, A, 548138 & US, A, 4747848 & CA, A, 1249490	1-7

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers , 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. ☐ Claim numbers , because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

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 of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ 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 claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	JP, A, 60-34450 (Terumo Corp.), February 22, 1985 (22. 02. 85), & JP, B2, 62-55426	1-7

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers , because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers , 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. ☐ Claim numbers , because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

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 of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ 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 claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

国際特許分類（IPC）

Int. Cl⁵

A61L27/00

II. 国際調査を行った分野

調査を行った最小限資料

分類体系	分類記号
IPC	A61L27/00, A61F2/06

最小限資料以外の資料で調査を行ったもの

日本国実用新案公報　1926－1992年
日本国公開実用新案公報　1971－1992年

III. 関連する技術に関する文献

引用文献のカテゴリー※	引用文献名　及び一部の箇所が関連するときは、その関連する箇所の表示	請求の範囲の番号
A	JP, A, 1－170467（東レ株式会社）, 5. 7月. 1989（05. 07. 89）,（ファミリーなし）	1－7
A	JP, A, 63－46169（工業技術院長）, 27. 2月. 1988（27. 02. 88）, &EP, A, 241838	1－7
A	JP, A, 62－258666（インテルメデイカット・ゲーエムベーハー）, 11. 11月. 1987（11. 11. 87）, &FI, A, 871065&DK, A, 127987 &EP, A, 237037&BR, A, 8701135 &DE, C, 3608158&US, A, 4784659 &JP, B2, 1－51263	1－7
A	JP, A, 62－152469（住友電気工業株式会社）,	1－7

※引用文献のカテゴリー
「A」特に関連のある文献ではなく、一般的技術水準を示すもの
「E」先行文献ではあるが、国際出願日以後に公表されたもの
「L」優先権主張に疑義を提起する文献又は他の文献の発行日若しくは他の特別な理由を確立するために引用する文献（理由を付す）
「O」口頭による開示、使用、展示等に言及する文献
「P」国際出願日前で、かつ優先権の主張の基礎となる出願の日の後に公表された文献
「T」国際出願日又は優先日の後に公表された文献であって出願と矛盾するものではなく、発明の原理又は理論の理解のために引用するもの
「X」特に関連のある文献であって、当該文献のみで発明の新規性又は進歩性がないと考えられるもの
「Y」特に関連のある文献であって、当該文献と他の1以上の文献との、当業者にとって自明である組合せによって進歩性がないと考えられるもの
「&」同一パテントファミリーの文献

IV. 認　証

国際調査を完了した日	国際調査報告の発送日
19. 06. 92	07.07.92

国際調査機関	権限のある職員
日本国特許庁（ISA/JP）	特許庁審査官　橋本伝一　4C 7038

第2ページから続く情報

	（Ⅲ欄の続き） 7. 7月. 1987（07. 07. 87）， &EP, A, 230635&US, A, 4822361	
A	JP, A, 62－152468（住友電気工業株式会社）， 7. 7月. 1987（07. 07. 87）， &EP, A, 230635&US, A, 4822361	1－7
A	JP, A, 61－135651（パスカテック リミテッド）， 23. 6月. 1986（23. 06. 86）， &GB, A, 8430265&DK, A, 551185	1－7

V. ☐ 一部の請求の範囲について国際調査を行わないときの意見

次の請求の範囲については特許協力条約に基づく国際出願等に関する法律第8条第3項の規定によりこの国際調査報告を作成しない。その理由は、次のとおりである。

1. ☐ 請求の範囲＿＿＿＿＿は、国際調査をすることを要しない事項を内容とするものである。

2. ☐ 請求の範囲＿＿＿＿＿は、有効な国際調査をすることができる程度にまで所定の要件を満たしていない国際出願の部分に係るものである。

3. ☐ 請求の範囲＿＿＿＿＿は、従属請求の範囲でありかつPCT規則6.4(a)第2文の規定に従って起草されていない。

VI ☐ 発明の単一性の要件を満たしていないときの意見

次に述べるようにこの国際出願には二以上の発明が含まれている。

1. ☐ 追加して納付すべき手数料が指定した期間内に納付されたので、この国際調査報告は、国際出願のすべての調査可能な請求の範囲について作成した。
2. ☐ 追加して納付すべき手数料が指定した期間内に一部分しか納付されなかったので、この国際調査報告は、手数料の納付があった発明に係る次の請求の範囲について作成した。
   請求の範囲＿＿＿＿＿＿＿＿
3. ☐ 追加して納付すべき手数料が指定した期間内に納付されなかったので、この国際調査報告は、請求の範囲に最初に記載された発明に係る次の請求の範囲について作成した。
   請求の範囲＿＿＿＿＿＿＿＿
4. ☐ 追加して納付すべき手数料を要求するまでもなく、すべての調査可能な請求の範囲について調査することができたので、追加して納付すべき手数料の納付を命じなかった。

追加手数料異議の申立てに関する注意

☐ 追加して納付すべき手数料の納付と同時に、追加手数料異議の申立てがされた。
☐ 追加して納付すべき手数料の納付に際し、追加手数料異議の申立てがされなかった。

Ⅲ. 関連する技術に関する文献（第2ページからの続き）		
引用文献のカテゴリー*	引用文献名及び一部の箇所が関連するときは、その関連する箇所の表示	請求の範囲の番号
	&EP, A, 183365&AU, A, 5059385 &AU, B, 569645&ES, A, 548138 &US, A, 4747848&CA, A, 1249490	
A	JP, A, 60-34450（テルモ株式会社）, 22. 2月. 1985（22. 02. 85）, &JP, B2, 62-55426	1-7