Development of Thermoplastic Polyurethane Vascular Prostheses

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ABSTRACT: In this study, the optimum electrospinning conditions for a thermoplastic polyurethane/*N*,*N*-dimethylformamide solution were sought. Under these conditions, polyurethane tubes for artificial blood vessels were produced via electrospinning, and various properties of the specimen were measured. The optimum electrospinning conditions were a concentration of 16 wt %, a voltage of 13.0 kV, and a tip-to-collector distance of 15 cm at 27°C and 80% relative humidity. The thermal behavior from a differential scanning calorimeter showed that this polyurethane web was thermally stable in the body temperature range. Enzyme hydrolysis of the web revealed that the polyurethane vascular prostheses would perform as nondegradable blood vessels in the body system. The pore size range of the electrospun polyurethane vascular prostheses was 0–3.5 μm. With a tensile stress of about 8–9 MPa and a tensile strain of about 80%, the tensile properties were expected to be superior to those of existing vascular prostheses made of polyester and expanded polytetrafluoroethylene. In the last stage, heparin and collagen were treated on the surfaces of artificial vessels to reduce the probability of thrombi forming during the initial stage of grafting, and so the treated specimens exhibited a lower possibility of thrombi forming than untreated ones in a blood coagulation test. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 3267–3274, 2008

Key words: biomaterials; elastomers; polyurethanes

INTRODUCTION

Synthetic biomaterials include silicone rubber, poly (vinyl chloride), nylon, polytetrafluoroethylene, poly (ethylene terephthalate) (PET), and polyurethanes. Thermoplastic polyurethanes represent a class of polymers that possess a range of very desirable properties: they are elastomeric, have excellent hydrolytic stability, and are resistant to microorganisms and abrasion. Many commercially available types of thermoplastic polyurethanes can be used to make good electrospinning solutions. An electrospun polyurethane web is expected to be applied not only to materials for the manufacture of ultralight protective clothing but also to biomaterials such as artificial vascular grafts after biofriendly, antithrombotic, and inert properties have been added.

There are three different types of pipe tubing biomaterials that can be made with polyurethane: extruded tubing, solvent-cast tubing, and porous and fibrous tubing. The first method synthesizes a tube on emitting and correcting polymers. The second forms a pipe tube via solvent casting within the tubed polyethylene or poly(vinyl chloride) produced by the first method. The last method synthesizes a tube used mainly as a vascular graft that may perform as efficiently as a real vessel when porous polyurethane is employed.[1]

Studies on the development of materials for vascular grafts using polyurethane began in the 1960s, and various types of vascular grafts, including foam and film types, have been developed since. In 1978, Annis attempted to develop electrospun polyurethane as a biomaterial for artificial vascular grafts with tetrahydrofuran as a solvent. Since then, more studies have been conducted with the aim of synthesizing pipe tubing material by coiling and adhering a minutely porous electrospinning material to a spinning mandrel with *N*,*N*-dimethylformamide and neighboring desirable properties with such property-changing phenomena as elasticity according to the orientation of the electrospun fibers.[2–5] Until now, however, research in artificial vascular grafts has not definitely established a materialistic examination of appropriate properties in terms of vascular materials. Moreover, studies have not made any significant

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development in presenting irresolvable electrospinning materials because initial attempts to do so were made many years ago.

The aim of this study, therefore, was to explore the appropriate conditions for forming porosity in artificial vascular grafts and to look at conditions for forming porous polyurethane thin films via electrospinning on the basis of ideas introduced in previous studies. Under such conditions, a method to form small-diameter artificial vascular grafts with an inside diameter of less than 3 mm was attempted. Through various property evaluation methods, the polyurethane material was examined to see if it possessed distinguished properties and the possibility of high biomedical suitability for applications such as artificial vascular grafts.

EXPERIMENTAL

Materials

Pellethane 2103-80AE, an ether-based thermoplastic polyurethane used for electrospinning, was obtained from Dow Chemical Co. (Seoul, Korea; weight-average molecular weight = 26,000). *N*,*N*-Dimethylformamide (first grade, Dae Jung Chemical Corp., Korea) was used as a solvent for electrospinning. The existing expanded polytetrafluoroethylene (ePTFE) vascular grafts (3-mm small inside diameter) and Dacron vascular grafts (10-mm large inside diameter) were used for a comparison of properties.

Methods

Electrospinning conditions

The optimal electrospinning conditions were examined to determine the concentration of the solution, applied voltage, tip-to-collector distance, flow rate of the automatic syringe pump, and so on. The electrospun web was collected on the face fabric with a thickness of 0.02 mm. The optimum electrospinning conditions of the polyurethane solution were a concentration of 16 wt %, a voltage of 13.0 kV, and a tip-to-collector distance of 15 cm at 27°C and 80% relative humidity.

Figure 1 shows the electrospinning scheme for making artificial vascular grafts. Small-diameter artificial vascular grafts created by electrospinning were formed where a metal bar with the same diameter as the desirable vessels was installed on a flat collector, and discharged fibers were coiled and coagulated on the spinning bar.

Antithrombosis treatment

For reducing thrombosis responses, electrospun polyurethane vascular grafts were treated with heparin and collagen (Sigma–Aldrich, Yong-in, Korea). Three specimens of heparin treatment, collagen treatment, and a 1 : 1 mixture were prepared to evaluate blood coagulation. The treatment was as follows: the specimens were soaked in heparin and collagen solutions and shaken in a thermostat water bath for 24 h. After that, the specimens were picked out carefully and dried for 24 h in room-temperature desiccators.

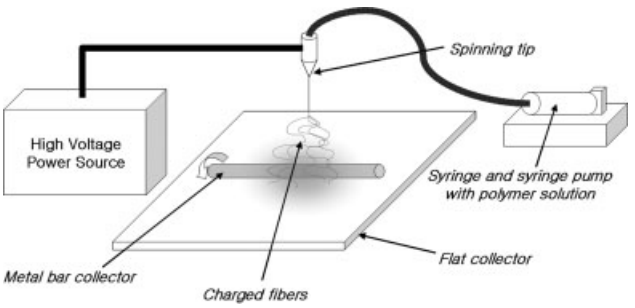

Figure 1 Electrospinning scheme for making vascular prostheses.

Measurements

Surface observations

Video microscopy. A video microscope (SV-32, SomeTech, Seoul, Korea) was used to examine the uniformity of the electrospun fibers and the formation of beads. The magnification was 600×.

Scanning electron microscopy (SEM). The specimens were coated with Au by an ion-sputtering device (JFC-1100E, JEOL, Tokyo, Japan). A scanning electron microscope (JSM5310LV, JEOL, Japan) was used to observe the electrospun spandex web and measure the fiber diameters. The acceleration voltage was 10 kV, and the SEM magnification was 3500×.

Biosuitability of the materials

Differential scanning calorimetry (DSC). The thermal behavior of the vascular prostheses was measured with a differential scanning calorimeter (DSC-Q100, TA Instrument, NY). The temperature was increased by 10°C/min from 0 to 50°C. The nitrogen purge of DSC was 50 mL/min.

Enzyme hydrolysis. An enzyme hydrolysis of the electrospun polyurethane web was carried out with proteinase K and lipase (Sigma–Aldrich). Proteinase K (5.3 units/mL) and lipase (50 units/mL) were added to phosphate-buffered saline (1*M*, pH 7.4) to make an enzyme concentrate. The specimen was soaked with 100 mL of the enzyme solution and degraded for 6 weeks in a water bath (37°C and 120 rpm).

Platelet adhesion test. The possibility of making thrombi when they were grafted onto the body was tested by platelet adhesion observation. Specimens

were immersed in human platelet plasma for 2 h at 37°C. After that, the specimens were rinsed with 1*M* phosphate-buffered saline (pH 7.4) and fixed with a 2% formaldehyde solution in 1*M* phosphate-buffered saline (pH 7.4) at room temperature. After being dehydrated with a series of ethanol–water solutions and dried in a desiccator at room temperature, specimens were observed with a scanning electron microscope (JSM5310LV, JEOL).[6]

Thrombus adhesion test. An *in vitro* test was designed to evaluate the thrombogenicity of the polyurethane and ePTFE vascular grafts. Standard extended polytetrafluoroethylene vascular grafts (n = 5), each with a diameter of 3 mm, were used for comparison with the newly developed polyurethane grafts (n = 5). In the gravimetric analysis, the weight of the adherent thrombi was recorded at 1-min intervals for 8 min.

Physical properties of the vascular graft materials

Tensile properties. Tensile properties were measured with an Instron 5543 universal testing machine (Instron, Bucks, UK). The specimens were cut and spread into rectangular forms (1 cm × 4 cm). Five samples in each group of specimens were measured to obtain the results. The crosshead speed was 10 mm/min.

Pore size. The porosity of the electrospun web was measured with a PMI (NY) porometer with a maximum pressure of 120 SPI and a maximum flow rate of 25,000 cc/min in conformity with ASTM F 316-03.

RESULTS AND DISCUSSION

Thermal properties of the electrospun polyurethane

Thermoplastic polyurethane, which was used as the material for making artificial vascular grafts in this study, is known to be thermally stable under normal temperatures and a range of body temperatures. Therefore, it is not expected to have any significant changes in its properties upon being exposed to body temperature for a long time when applied to the internal organs, and this meant that a differential scanning calorimeter had to be used to measure the rise and drop in temperature to confirm the thermal stability of the thermoplastic urethane.

Figure 2 shows that the thermoplastic polyurethane specimen used in this research was stable in the range of 0–50°C, so it hardly exhibited an endothermic reaction with a rise in the temperature of calorification or with a drop in temperature that would have a significant effect on the internal structure of the polymer. Consequently, it is expected that constant exposure to body temperature would

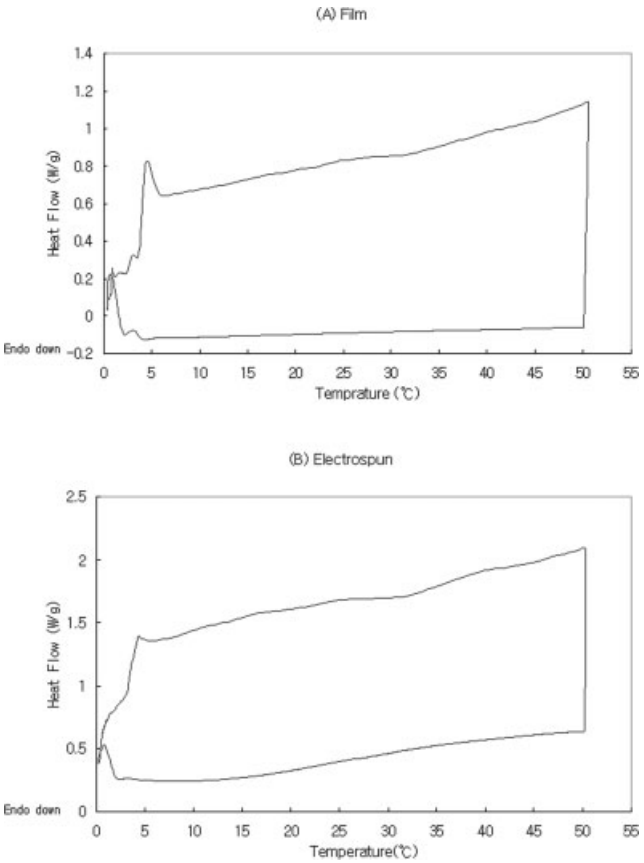

Figure 2 DSC diagram of (A) a polyurethane film and (B) an electrospun web (maximum sensitivity = 0.2 μW).

cause hardly any changes in the structure of the polymer.

Generally, polymers with a possibility of melting form a greater orientation via electrospinning. As their crystallization point rises, the thermal behavior, including the heat of fusion, tends to increase in comparison with the state of the bulk film. However, the specimen used in this research, polyurethane, was very stable thermally and was not affected by crystallization. Thus, the electrospun web did not exhibit any thermal behavior that was different from that of the bulk state.

Surface characteristics

The existing Dacron artificial vascular grafts used for comparison were tricot knit tissues threaded with Dacron yarns for cardiovascular purposes, with a large inner diameter of 1 cm and a thickness of 0.7 mm on the walls of the arteries (Fig. 3).

Artificial vascular grafts made of ePTFE had a small internal diameter with an inner diameter of 3 mm and a thickness of 0.5 mm on the walls. Such vascular grafts were folded with several ePTFE films with a minute porosity to minimize the possibility of

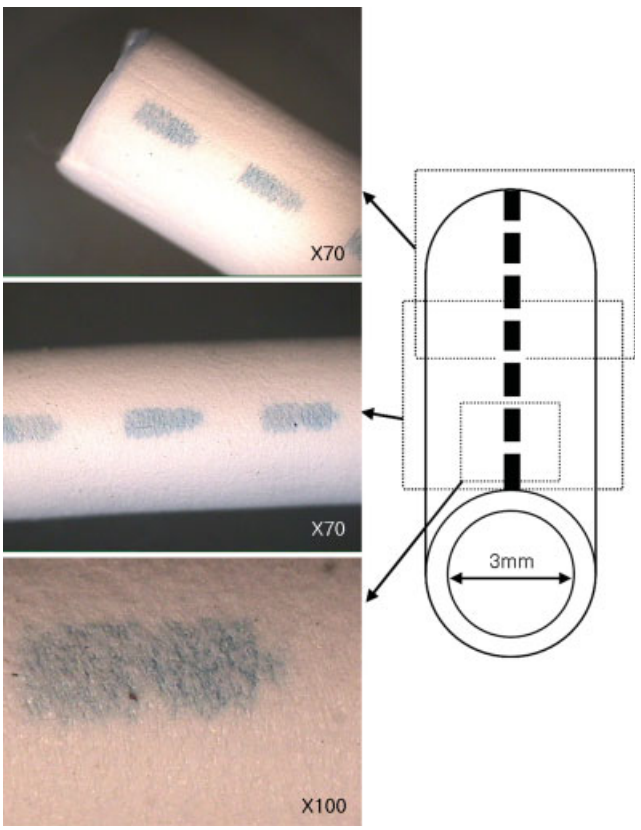

Figure 3 Video microscopy pictures of Dacron vascular prostheses. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

thrombi developing on the flexible and smooth surfaces of the walls of the arteries (Fig. 4).

The polyurethane used in our research was a material that had been tested for application to artificial organs with excellent strength. In addition, one of the advantages of using this material is that its lack of viscosity makes it is easy to inject into the body. The polyurethane material is discharged into thin fibers that form a web without any directional difference (Fig. 5).

Degradability from enzyme hydrolysis

The thermoplastic polyurethane used as the biomaterial in this study is known to be greatly elastic, resistant to germs and abrasion, and stable for hydrolysis.[7] When polymer prostheses are grafted onto the body, enzymes recognize the polymer surfaces chemically and attack the surfaces in various ways.[8] Thus, hydrolysis using enzymes was performed directly to determine whether the thermoplastic polyurethane was an appropriate material for developing irresolvable artificial vascular grafts.

The electrospun polyurethane web that was developed in our research showed weight loss due to enzyme breakdown in the range of three decimals, which was in the error tolerance range. Thus, this material was expected to exhibit irresolvable properties in the body.

Appearances by platelet adhesion

In the case of artificial vascular grafts, their function replaces the role of living vessels immediately upon grafting because blood streams along the interior walls of artificial vascular grafts. At this time, a platelet adheres onto and is destroyed on the inner walls; this process forms thrombi and creates a barrier to a successful graft. For this reason, studies on coating the interior walls of artificial vascular grafts with silicon and antibiotics or on reforming surfaces of polymers have continued to be conducted to prevent platelets from adhering to the walls.

Among the several methods used to test the forming properties of materials on platelets, the most frequently used in biomaterial engineering is platelet adhesion because an evaluation of it can be conveniently performed *in vitro*.[9]

A platelet under normal conditions is shaped like a disc with a diameter of 2–4 μm, and it adheres to the walls of arteries and activates and transforms

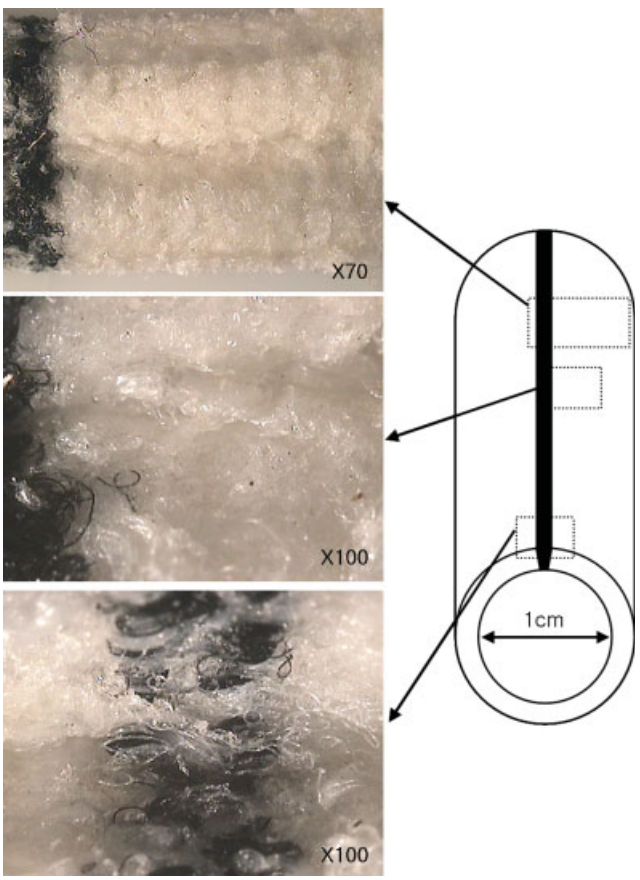

Figure 4 Video microscopy pictures of ePTFE vascular prostheses. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

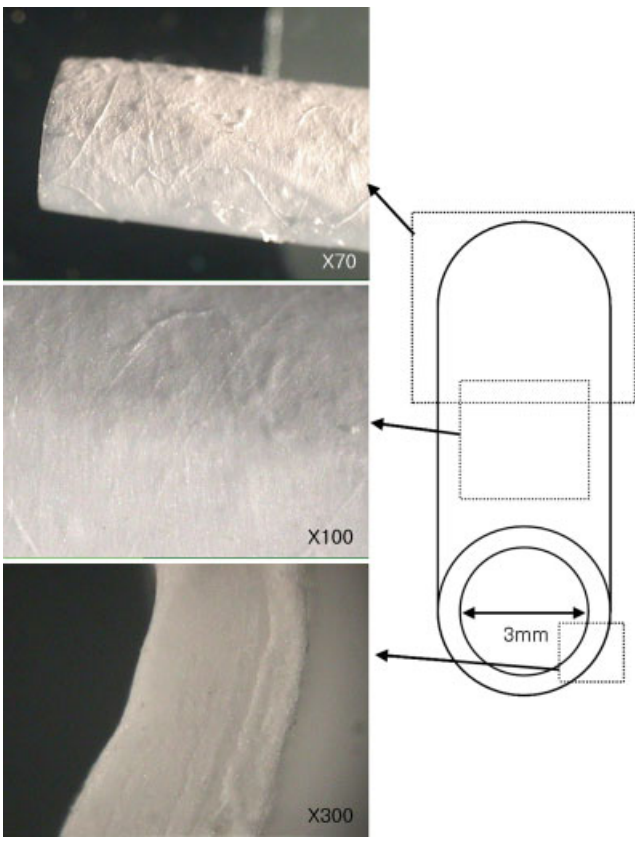

Figure 5 Video microscopy pictures of electrospun polyurethane vascular prostheses. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

when facing physical stimuli or stimulants that have penetrated from the outside. Because thrombi are formed by coagulating substances emitted from transforming platelets, it is possible to predict the probability of thrombus development via observations of the quantity of platelets grafted onto the material and their morphology.[10–12]

A platelet adhesion test was performed to learn whether the material in the electrospun web figure would be appropriate for blood in the way of artificial vascular grafts. The prevention of thrombus development in artificial vascular grafts and the probability of a successful graft were mainly determined by the existence and nonexistence of permeability due to surface conditions and opened pores. Thus, glass, a typical material without permeability or openings, was compared with a thin film having the same length as a polyurethane casting web to determine if an electrospun web figure with an opening would be appropriate for blood vessels. In Figure 6, the electrospun web specimen, with a diameter smaller than that of a platelet and a large number of openings, exhibited the lowest level of platelet adhesion and a transformation time of 120 min upon digestion of the platelet. Thus, the electrospun web was verified to be an appropriate material for artificial vascular grafts.

Tensile properties

Figure 7 compares the tensile properties of a large-diameter material used for artificial vascular grafts on the market and a small-diameter material. The tensile stress of the artificial vascular grafts made with a small-diameter thermoplastic polyurethane was greater than that of Dacron, which has been widely used in artificial vessel grafting. Indeed, its tensile stress was greater than 8 MPa, making it similar to the ePTFE material. In addition, the tensile strain of polyurethane exhibited a higher value than the other two materials. Thus, it showed outstanding efficiency in terms of physical properties, and this makes its use in artificial vascular grafts possible.

Pore size distributions and pore diameters

Table II exhibits the pore size distributions and mean pore sizes of artificial vascular grafts made of the existing large-inner-diameter Dacron, small-inner-diameter ePTFE, and small-inner-diameter electrospun polyurethane. The pore size of artificial vascular grafts has been claimed to be the determining factor in the interior development of cells and in the opening of artificial vessels. Indeed, the adhesion rate of interior cells will rise with a larger pore size diameter when a material with the same properties is used.[13] In addition, blood may leak out of the vascular grafts upon grafting if the pore diameter of a material used for artificial vascular grafts is too large. On the other hand, the adhesion rate of interior cells will drop if the pore size is too low or nonexistent. Thus, an appropriate pore size is vital to the grafting possibility of artificial vessels.

The pore size of a commercial, artificial vascular material with a large inner diameter was known to be 19–24 μm, which was distributed in the range of generally large-diameter artificial vascular materials (15–35 μm). The average pore size of the ePTFE vascular grafts was 0.39 μm, of which the distribution did not significantly deviate from the average. In contrast, the pore size of the electrospun artificial vascular grafts was 0.18–3.57 μm, of which the distribution was very broad. Its mean pore diameter was measured at 1.30 μm, which was smaller than that of the Dacron artificial vessels, but it exhibited a larger mean pore diameter and distribution than ePTFE. Thus, it was expected to provide greater advantages for the grafting of interior cells.

Thrombus adhesion onto untreated specimens

The thrombus development reactions of artificial vascular grafts can be classified into two categories:

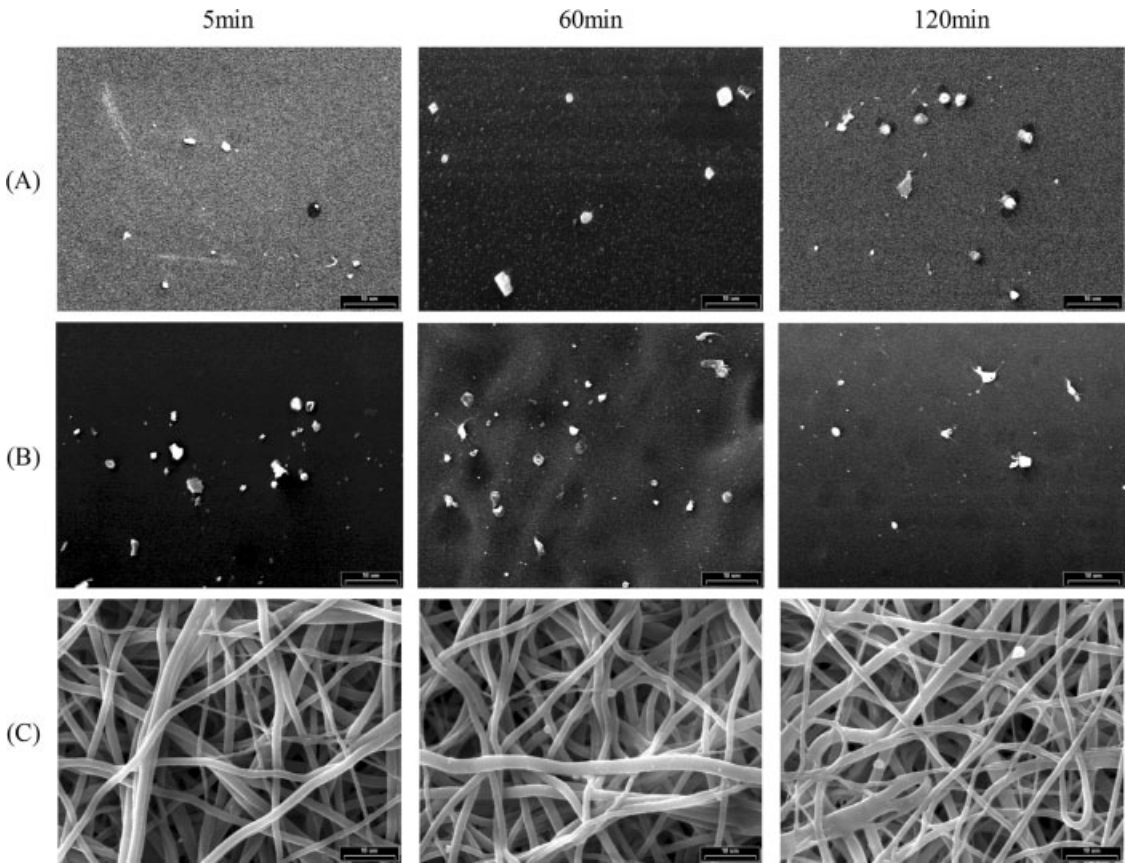

Figure 6 Results of platelet adhesion testing: (A) glass, (B) polyurethane film, and (C) polyurethane web (SEM; magnification, 2000×).

a temporary reaction caused by the adhesion of a platelet as blood reaches the surface *in vitro* at the time of the initial stage of grafting and a long-term reaction due to the adhesion of thrombus-inducing materials such as proteins, which are grafted for a long period of time. The former can be solved by an anticoagulant treatment on the grafting surfaces, whereas adhesion of the interior cells of living vessels on the surfaces of artificial vascular grafts may prevent thrombi from forming.

Thus, the blood coagulation of the electrospun polyurethane artificial vascular grafts developed in this study and of the existing ePTFE artificial vessels on the market was evaluated (Fig. 8). This was done by an *in vitro* experimental method used to determine the possibility of thrombi forming when an artificial material first comes into contact with blood immediately after grafting. As the weight became greater, it became possible to deduce a greater

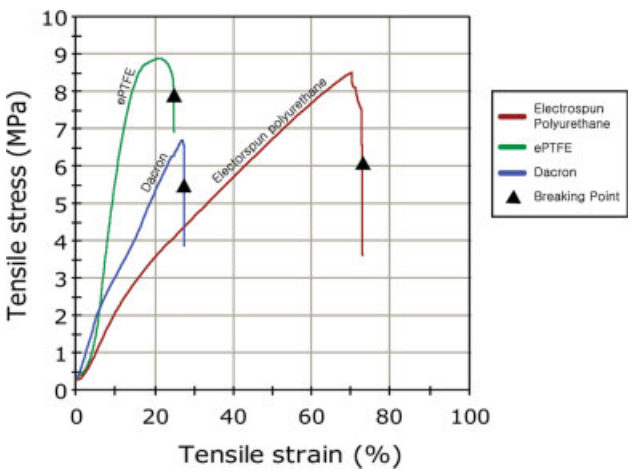

Figure 7 Tensile properties of vascular prostheses. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I
Weight Decrease (g) in the Electrospun Polyurethane Web by Enzyme Hydrolysis

Period of time (weeks)	Control (g)	Protease (g)	Lipase (g)
1	0.001	0.002	0.000
2	0.001	0.002	−0.001
3	0.001	0.002	−0.005
4	0.001	0.001	−0.004
5	−0.001	0.001	−0.001
6	0.001	0.001	−0.002

TABLE II
Pore Size Distributions and Mean Pore Diameters of the Vascular Prostheses

Specimen	Range of pore sizes (μm)	Mean pore diameter (μm)
PET	19.31–24.59	22.89
ePTFE	0.38–0.56	0.39
Polyurethane	0.18–3.57	1.30

possibility of thrombi forming during the initial stage of grafting. Electrospun polyurethane artificial vascular grafts exhibited a rapid increase in weight (>0.05 g after 4 min), whereas the weight of ePTFE increased after 6 min. In other words, the surfaces of the latter were smoother than those of the former, and the latter also had a low surface energy for its properties. Thus, the possibility of thrombi forming on the former immediately upon grafting could be evaluated to be lower than that of the latter.

Thrombus adhesion onto antithrombosis-treated specimens

According to the previously outlined results, the polyurethane material has a higher critical surface tension than Dacron or ePTFE, the existing material used for artificial vessels, and this means that it should have a higher possibility of blood coagulation due to the adhesion of coagulant agents in the blood. Moreover, it would be necessary to improve its properties in blood coagulation and thrombus formation through an improvement in its biocompatibility with blood. Consequently, the interior and exterior walls of artificial vascular grafts were treated with heparin, an anticoagulant, and collagen, a type of hydrophilic element, to improve the biocompatibility of the material. Three specimens of heparin treatment, collagen treatment, and a 1 : 1 mixture of the two were prepared to evaluate blood coagulation.

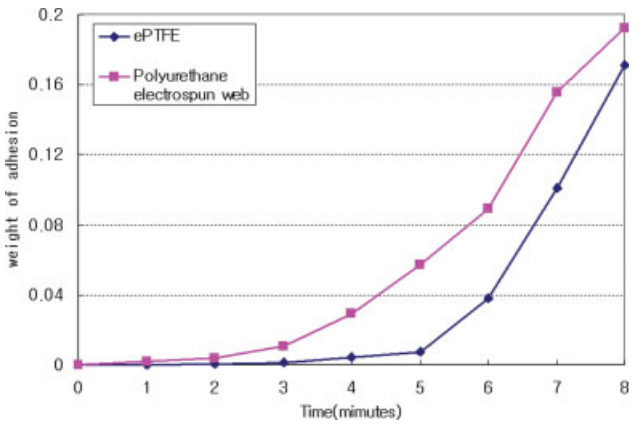

Figure 8 Weight increase by thrombus adhesion to untreated specimens. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

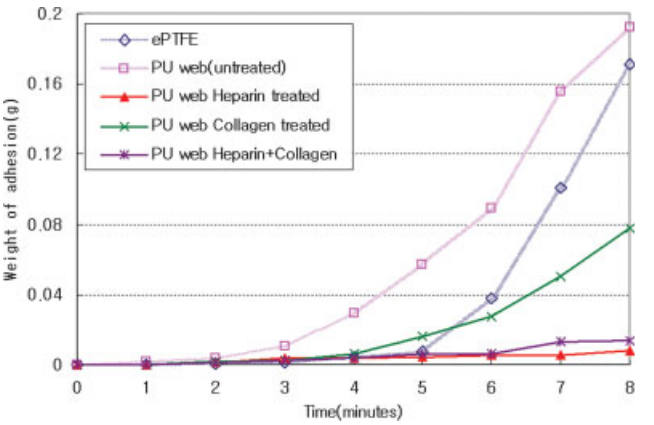

Figure 9 Weight increase by thrombus adhesion to heparin- and collagen-treated specimens (PU = polyurethane). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

As a result, the polyurethane artificial vascular grafts treated with the specimens showed a lower weight increase from the adhesion of blood coagulation than the unprocessed polyurethane and existing ePTFE grafts (Fig. 9). It was therefore expected that the reaction of thrombi during the initial stage of grafting would decrease and that the blood flow rate would increase because of the treatment with heparin and collagen. In particular, the specimen treated with collagen alone exhibited a lower weight increase than the specimens treated with heparin alone or with the mixture of heparin and collagen. Thus, heparin appears to be a more appropriate element than collagen for increasing the biocompatibility of the material with blood.

CONCLUSIONS

1. A method of forming a tube by piling up electrospun material in a cylindrical collector with a desirable diameter was attempted through the application of polyurethane electrospinning to a biomaterial. As a result, a possible material for artificial vascular grafts was yielded because of its exterior and form.
2. An electrospun polyurethane web for biomaterials exhibited thermal stability at temperatures ranging from 0 to 50°C and was also stable during the hydrolysis of enzymes. Thus, it could be used as an irresolvable material for artificial vascular grafts.
3. The number of adherent platelets on the electrospun polyurethane web, the diameter of which was smaller than that of the platelets, decreased in comparison with the state of the film. Thus, it would be possible to use an electrospun fiber web, with a diameter of nano/micrometers and a low risk of creating thrombi, as a material for artificial vascular grafts.

4. The tension stress of the material of the artificial vessels developed in this study exhibited higher values than those of the existing Dacron and ePTFE, and the values of all the specimens ranged from 8 to 9 MPa.
5. The pore sizes of the biomaterial of the electrospun polyurethane artificial vascular grafts, which were in the range of 0.18–3.57 μm (of which the average pore diameter was 1.30 μm), were smaller than those of the existing large-inner-diameter Dacron vessels and were larger than those of the small-inner-diameter ePTFE artificial vessels.
6. Electrospun polyurethane artificial vascular grafts were expected to have a higher probability of forming thrombi at the time of initial grafting than ePTFE ones because of the higher amount of adhesion of coagulation in the blood coagulation test. Thus, heparin and collagen were treated on the surfaces of artificial vessels to reduced the probability of such thrombi forming during the initial stage of grafting. As a result, the treated specimens exhibited a lower possibility of thrombi forming than the untreated ones. The specimen treated with heparin in particular showed significantly higher biocompatibility than the others.

Because of its porous characteristics, the tube material formed through the electrospinning of the polyurethane biomaterial was determined to have potential for use in the development of artificial vascular grafts. Thus, it is expected that electrospinning polyurethane artificial vessels with a small inner diameter could yield higher biocompatibility than that of other materials if their compatibility with blood increases and their weak points are improved through a treatment with an anticoagulant. However, it will be necessary to verify the grafting behavior of the material upon its application *in vivo* and to research this matter in greater detail in future studies.

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