Development of Porous polyurethane Arterial-Venous Shunt by Thermal Control

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온도 조절을 통한 다공성 폴리우레탄 동정맥 누관의 개발

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Abstract

A technique for the preparation of porous polyurethane vascular prostheses was investigated. Small-diameter vessels are not in general clinical use due to their limited long-term biocompatibility and low patency rates in experimental trial. These limits are mainly due to the failure of mechanical function of the vascular grafts. This failure has been suggested to result partially from compliance mismatch. The long-term patency is considered to depend critically on the properties of the material and the fabrication process of the graft. So the control of pores is very important and main points to develop a available vascular grafts.

Two-kind polymer sheets was compared. One was the porous PU-sheet made at room temperature by the solvent/non-solvent exchange. And the other was the porous PU-sheet which was fabricated by thermal phase transition and solvent-/non-solvent exchange using the thermal controller. The polymer sheets had a uniform pore size and pore occupation. According to the result of the above experiments, polyurethane solution was injected into a mold designed for U-type tube. The average pore size and pore occupation were easily changed by changing polyurethane concentration, freezing temperature, and methods. This technique can give a proper pore size (10~45 ㎛) for tissue ingrowth, and suitable compliances for matching with arteries and veins. Besides, the fabrication of more complicated shaped vessels such as the U-type vascular grafts is easily controlled by using the fixed mold. this method might give a desired compliant graft for artificial implantation with the presently valid medical polymers.

Introduction

For the artificial vessels, many synthetic materials have been used in attempts to replace diseased small diameter blood vessels including polymers such as poly(ethylene terephthalate) (Dacron®), poly(tetrafluoroethylene) and polyurethanes[1,3-6]. Polyurethanes have been found to have excellent physical properties which have been put to good use in the artificial heart, pacemaker leads and catheters. One of these, Pellethane®, a segmented poly(etherurethane urea), is known to have excellent compatibility with blood[4-6,8]. However, there is conflicting evidence concerning the performance of polyurethanes such as Pellethane®. In animal experiments, vascular grafts made from polyurethanes and having diameter <6 ㎜ have generally failed to remain patent following long-term implantation, although Annis has reported excellent 2 yr patency rates in dogs[2].

A variety of methods have been developed to allow the reliable production of porous vessels based on spinning technology. For example, recently Nakayama and Matsuda presented microporous polymer tubes prepared by and excimer laser ablation technique[8-10]. In their paper, the irradiation of a KrF excimer laser (248 ㎚) was applied to several polymer films and tubes by passing a laser pulse through an optical microscope, resulting in ablative photodecomposition. And they reported that as an application of the ablation technique, polyurethanes films were micropored by the excimer laser ablation technique in conjunction with open-cell structured, small diameter grafts under development. Other techniques for the manufacture of vascular have also been used for the preparation of compliant grafts.

However, these methods always need some special apparatus and can not change the compliance of the vessels over a large range to match the compliances of the native veins and arteries[1]. Generally, the compliance of veins and arteries change from several percent to about 20% in the physiological pressure range (60-160mmHg). And, it is very important and practical to fabricate vessels with adequate compliance to match the arteries or veins to be replaced. Thus, it is necessary to develop a technique which can form vessels with various compliances. In addition to these, many methods for porous PU vessels are not applicable for U-type shunt because they are usually for the straight vessels and it is hard to control the properties of U-type vessels. In this study, a simple and convenient technique to form compliant vessels for U-type arterial-venous shunt was investigated. The porosity (pore size and pore occupation) and compliance could be easily controlled to match those of living tissues and to enhance ingrowth of endothelial cells[11].

Materials and Methods

Materials

Pellethane® 2363-80AE(PU pellets) was provided by DOW Chemicals Co., U.S.A. and N,N-Dimethylacetamide(DMAc) was purchased from Shinyo Ltd, Japan. HETO® thermostats (01DBT623 and CB13-45E) was provided by Heto-Holten A/S, Allered, Denmark for thermal control. The thermal bath was filled with ethanol/HETO-cold fluid, which was used for the temperature change from -120 ℃ to +5 ℃.

Preparation of polyurethane (PU) sheets

PU pellets were extracted with methanol for 3 days to remove low-molecular-weight components. After extraction, the pellets were dried under vacuum at 60 ℃ for 48 h to remove residual solvents.

The methanol-extracted PU pellets were dissolved in DMAc to make 10, 12, 14,16 % (wt/v) solution, respectively.

The PU sheets (6 × 12 ㎝) were made from the solutions by the two methods. First, each solution was cast on the glass plate and the plates was dipped into the absolute ethanol solution (25 ℃). The plate was moved into absolute methanol at room temperature. The methanol was changed every 12 h to permit solvent in the polymer sheet to dissolve out. The solvent exchange was carried out for 2days to form a uniformly porous sheets. Porous sheets were moved into the distilled water to remove the methanol solution and dried under vacuum for 2 days.

Secondly, each solution was cast on the lower glass plate and it was covered with the upper plate and the combined plate was moved into the HETO® thermostat. After keeping the polymer-solution-cast plate at the temperature change from -120 ℃ to +5 ℃ for 1 day, the upper plate was removed. The lower plate was moved into the cold methanol (< 0 ℃). The cold methanol was changed every 12 h to permit solvent in the frozen polymer sheet to dissolve out. And the next step followed the previous procedure.

Vessel fabrication

The methanol-extracted PU pellets were dissolved in DMAc to 14 % (wt/v) solution. And the solutions were injected into the mold. The experimental mold is shown in Figure 1. The vessels were fabricated by two methods, that is, the acute freezing method and the slow freezing method.

Briefly, polymer solution was injected into the mold, and the combined mold was moved into the refrigerator (- 45 ℃) in the acute freezing method and the HETO® thermostat in the slow freezing method. After keeping the polymer-solution-cast mold at the temperature change from 25 ℃ to -46 ℃ for 1 day, the inner mold was moved into the cold methanol. The cold methanol was changed every 12 h to permit solvent in the frozen polymer graft to dissolve out without the shape change of the grafts. The solvent exchange was carried out for 2days to form a uniformly porous vessel. Porous vessels were moved into the distilled water to removed the methanol solution and dried under vacuum for 3 days.

Scanning electron microscopy and image analysis

The surface morphology of modified PU grafts was examined with a Hitachi S-510 scanning electron microscopy (SEM) at an accelerating voltage 15kV. Samples were mounted and then sputter coated with gold using an ion coater.

Measurement of Stress and Strain

Uniaxial stress-strain analysis was performed using Instron 4201 (USA) testing machine according to ASTM D882-91 (Standard Test Methods for Tensile Properties of Thin Plastic Sheeting). The films were 0.20-0.26 ㎜ thick, 5 ㎜ wide and samples were cut using a razor blade to grip length, 30 ㎜ and total length, 50 ㎜. Respectively, 5 samples were tested at room temperature with a 5 ㎏ load cell. Compliance was determined with 1/modulus (modulus = stress/strain, stiffness) after evaluating the ultimate tensile strength (maximum load), maximum stress, maximum strain.

EC treatments

The assay of cell ingrowth *in vitro* was performed as followings: Thin porous PU film (less than 0.20 ㎜ thick) were prepared by the two methods, one at room temperature and the other at -45 ℃, slow freezing method. Endothelial cells(ECs) were then seeded onto the PU films. After incubation in a designed wells for 1 day, PU films, on which confluent monolayer of ECs was formed, were stripped carefully from the wells without damaging the cellular sheet. The EC monolayered PU films were then incubated. The confluent region and leading edge of cells that migrated from the monolayered upper sheet to the cell free lower surface through one micropore were examined with a scanning electron microscope.

Preliminary animal experiment for implantation

A male dog (weight, 40 ㎏) was used in this study according to the "Principles of Laboratory Animal Care" (formulated by the National Society for Medical Research) and the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication No. 86-23, revised 1985). After systemic anticoagulation with intravenous heparin, treated U-type vessels were implanted end-to-end in the limb of the dog. All implanted grafts were harvested after surgery.

Results

Surface characterization of Polyurethane sheets

Porous PU sheets of thickness between 0.2 and 0.26 ㎜ were fabricated solvent/non-solvent exchange at room temperature and at -45 ℃ using the slow freezing method. The initial cooling rate is approximately 1 ℃/min in thermostat.

All sheets were opaque, white and very flexible. SEM photographs of the upside, downside and cross-section are shown Figure 2-9. Pore characterizations are listed in Table 1, 2. At room temperature, the average pore size decreased with the decrease of the concentration of polymer solutions but it was not significant. The pore size was distributed from 4 ㎛ to 29 ㎛ but the pore occupation was less than 15 - 20 %. The uniform and continuous pore distribution was observed from the downside to upside of all samples. In case of the slow freezing method, the characteristics were different from those in the above case. Especially the polymer granules were observed in all cases. The average pore size was the greatest in 14 wt/v %, and the pore range was high in 12 wt/v %. Pore occupation was declined to decrease with the increase of the concentration.

Vessel fabrication

Vessels of wall thickness between 0.22 and 0.25mm, determined by the mold, were fabricated using the acute freezing method and the slow freezing method. The acute freezing method means that the polymer solution-filled mold is moved directly into a low temperature-fixed refrigerator, whereas the slow freezing method means that the polymer solution-filled mold is into the thermostat bath and frozen gradually by the controller. Typical SEM photographs of the cross-section, inside surface, and outside surface of the wall, are shown in Figure 10,11 and Table 3. From these photographs, a uniform distribution of pores on the surface is observed. Pores sizes are 3 ㎛ to 4.5 ㎛ and 30 ㎛ to 40 ㎛, respectively. This show that pore characteristics are a function of freezing temperature. The pore size was decreased with decreasing of freezing temperature, and was increased with decreasing of the cooling rate.

Mechanical properties

The compliances changes with porosity, pore size and distribution, and nonuniformity as well as the mechanical properties. Usually, larger porosity and pore size increase the compliance. Compliances of porous vessels can be controlled by changing polymer concentration and freezing temperature, and methods. Table 4~6 showed the compliance change with parameters as mentioned above. The maximum stress and maximum strain were increased with a increase of concentration of polymer solution. Table 3 and 4 showed that the values of the sheets made at room temperature were larger that those of sheets made at -45 ℃, but table 6 showed that the compliance was increased with decreasing the concentration of polymer solution and that the compliance of the sheet made at lower temperature was larger. It is because the porosity of the sheet fabricated at lower temperature was lager.

Cell ingrowth In Vitro

At first, an EC confluent monolayer was formed on a micropored PU film mounted on a designed kits and then stripped carefully from the kits. The PU film, which has a confluent EC monolayer sheet at one face and a cell-free one on the other, was immersed into the culture medium and cultured further. Scanning electron microscopic observation of the EC showed that a circular cell sheet was being expanded by migration and proliferation of ECs as the culturing proceeded. The cell population was gradually reduced in the vicinity of the leading edge.

Preliminary animal experiment for implantation

When a treated microporous PU graft was implanted in the limb of a dog, the ingrowth of ECs on the vessel effectively prevented blood leakage through the micropores. Macroscopically, all grafts exhibited smooth inner surfaces.

Discussion

A convenient and simple technique for porous vessels has been developed. The vessels with a large range of compliances having different pore size and distributions or the same pore size and distribution can be easily formed by this technique without special apparatus. These parameters are very important for the performance of artificial vessels. To match the compliance completely, a series of grafts with various compliances can be prepared.

At first, the porous polymer sheets fabricated by the two different methods were compared each other changing the concentration of the polymer solution. One was the fabrication at room temperature, the other was the preparation at -45 ℃, controlled slow cooling rate. SEM photographs have shown that the tow method were different and the temperature had significant influence on the pore sizes and pore distributions. In addition to these comparison, another method, the acute freezing method was compared. According to these experiments, the pore range, the pore average size and the pore distribution was dependent on the cooing rate. The slow freezing method make a uniform and available pores compared with the acute freezing method.

Calculated compliances have showed that vessels with larger pore size have a larger compliance. And these data also elucidated that the compliance also changes with the change in porosity, pore size and pore distribution. The control of micropores can solve the problem of the compliance mismatch. This technique provides a series of compliant grafts conveniently and quickly for clinical selection.

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Table 1. Pore Dependence on Polymer Concentration

Polymer conc. (wt/v %)	Pore range (㎛)	Average pore size (㎛)	Pore occupation (%)
10	5 - 24	14 - 16	5 - 15
12	4 - 29	14 - 19	5 - 10
14	8 - 27	12 - 16	15 - 20
16	5 - 27	10 -16	< 5

Table 2. Pore Dependence on Polymer Concentration

Polymer conc. (wt/v %)	Pore range (㎛)	Average pore size (㎛)	Pore occupation (%)
10	7 -24	14 -17	45 - 50
12	11 -93	20 - 30	20 - 30
14	10 -47	27 - 30	30 - 40
16	2.5 - 17	3 - 4	5 - 10

Table 3. Pore Distribution of U-type graft (14 wt/v %, -45 ℃)

Polymer conc. (wt/v %)	Pore range (㎛)	Average pore size (㎛)	Pore occupation (%)
acute freezing	1.5 - 6	3 - 4.5	10 -15
slow freezing	14 - 47	30 - 40	35 - 40

Table 4. Mechanical properties of porous sheets (25 ℃)

Polymer conc. (wt/v %)	Max. load	Max. stress	Max. Strain
10	0.8131 ± 0.2213	0.4084 ± 0.0636	422.68 ± 46.02
12	0.9238 ± 0.1354	0.4686 ± 0.4994	498.55 ± 40.07
14	1.0904 ± 0.1389	0.5837 ± 0.0670	531.54 ± 33.85
16	1.3616 ± 0.0761	0.8129 ± 0.0834	541.34 ± 28.35

Unit: Load; kg, Stress; kg/㎟, Strain; %

Table 5. Mechanical properties of porous sheets (-45 ℃)

Polymer conc. (wt/v %)	Max. load	Max. Stress	Max. strain
10	0.6259 ± 0.1592	0.3246 ± 0.1530	385.80 ± 35.95
12	0.8019 ± 0.2903	0.3927 ± 0.0225	405.24 ± 59.12
14	0.9871 ± 0.2389	0.4867 ± 0.1017	480.56 ± 97.31
16	1.3110 ± 0.1274	0.7491 ± 0.2275	507.37 ± 46.84

Unit: Load; kg, Stress; kg/㎟, Strain; %

Table 6. Mechanical properties of porous sheets

Polymer conc. wt/v %	25 ℃		-45 ℃	
	Mod	Comp	Mod	Comp
10	0.1597 ± 0.0179	6.3001 ± 0.6363	0.1457 ± 0.0098	6.8612 ± 0.6751
12	0.2398 ± 0.0207	4.1356 ± 0.3485	0.1925 ± 0.051	5.1948 ± 0.2489
14	0.2406 ± 0.0263	4.1992 ± 0.4384	0.2036 ± 0.0871	4.9127 ± 0.8745
16	0.3144 ± 0.0424	3.2392 ± 0.4559	0.2599 ± 0.0125	3.8740 ± 0.5563

Unit: Modulus; kg/㎟, Compliance; ㎟/kg

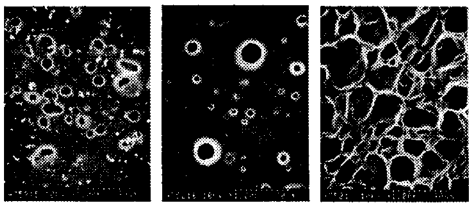

Figure 3. SEM photographs (original mag. X 1000) of upside, downside and cross-section of the sheet (10wt/v %, 25 ℃)