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Preparation of Segmented Polyurethane Hollow Fiber with Tissue Compatible Properties for Hybrid Blood Vessel

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The purpose of the research is preparation of a new-type hybrid artificial blood vessel with small inner diameter less than 4mm. We paid attention to the mechanical property and blood compatibility of the material for preparing the artificial blood vessel. As a core material, segmented polyurethane (SPU) was used. The inner surface of the artificial blood vessel should have an excellent antithrombogenicity at early stage after implantation and it goes adhesiveness of human umbilical endothelial cells (HUVEC). Thus, the surface should be covered with a blood compatible phospholipid polymer, that is, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer. On the outer surface, the smooth muscle cells (SMC) can be adhered and connected well with living organisms. Since the core material should be avoided to make a mismatch of mechanical compliance, the same of the dynamic characteristic of living blood vessels; J-curve is needed. The porous structure of the core material is suitable for this purpose. The core material with a sponge structure was prepared from SPU by hollow fiber processing technique. Young's modulus in the low load region of J-curve when making it at 60°C in temperature of the outer coagulant solvent at SPU : dimethylsulfoxide (DMSO) : ethanol = 20:75:5 (wt %), became smaller than that of the SPU hollow fiber prepared from its N,N-dimethylformamide (DMF) solution. Breaking strength increased, and an ideal J-curve property was obtained. The appearance where SMC invades on the substrate coated with MPC polymer was observed.

Key words: phospholipid polymer, hollow fiber, segmented polyurethane, smooth muscle cell, human umbilical endothelial cell

1. INTRODUCTION

1.1 Overview of artificial blood vessels

Currently, cardiovascular disease is the secondary cause of death to malignant tumor and blood vessel disease including cerebrovascular disorder is the biggest killer. The best treatment for such disease is replacement by artificial blood vessels or auto grafts. But, now, auto grafts are almost used and less small-diameter artificial grafts are used in affected area such as coronary artery whose inner diameter is less than 4mm. The serious problem of autogenously graft is that the diameter of grafts decreases and they are occluded by thrombosis because of arteriosclerosis.

Accordingly, the development of small-diameter artificial grafts with good patency is urgently needed. Tissue-engineered blood vessels have problems that the rupture strength of them is very weak – about 0.1 MPa – and the strength decreases as the scaffold degrades. Shortly, they have risk of vascular rupture and blood leakage after transplantation. Recently, polyester small-diameter artificial grafts covered with polymer alloy of segmented polyurethane (SPU) and phospholipid polymer can have antithrombogenic property and maintain good patency over a period up to about eight months. They looked carefully at the situation in occluded grafts and found that all grafts are occluded at the anastomotic site [1-3]. Compliance mismatch between native artery and artificial graft is thought to be aftereffects of this occlusion.

Artery consists of intima, media and adventitia. Intima consists of human umbilical endothelial cell (HUVEC) and basal lamina. Media consists of internal elastic lamina, smooth muscle cells (SMC) and external elastic lamina and it controls blood flow. Internal elastic lamina consists of erastin fibers whose Young's modulus is 0.2-0.5MPa. External elastic lamina consists of collagen fibers whose Young's modulus is 50-100MPa. Adventitia consists of fibroblast, elastin and collagen and it supports strength of vessels. At low pressures, an artery can largely expand and contract elastically mainly due to elastin fibers, whereas collagen fibers remain unscratched. As the intraluminal pressure is increased, collagen fibers serve to protect against the pressure load and play an important role in preventing the rupture of the artery at high pressures. These histological and mechanical features of a native artery produce the specific J-curve in the stress-strain relationship [4].

Present small-diameter artificial graft has mainly the following problems; i) inner surface covered with mural thrombus after transplantation, ii) imperfectly-formed layer of endothelial cells, iii) compliance mismatch with native artery. As complete solution to these problems, we proposed tissue compatible hybrid blood vessel as follows.

For i) Coating of antithrombogenic MPC polymer

For ii) Controlled-release of endothelial growth factor

For iii) Design of materials which have J-curve property

1.2. Design concept of the hybrid blood vessel

In this research, the following two points were instituted as hybrid blood vessel similar to the living body blood vessel. (I)The core material , SPU hollow fiber (HF), whose dilation and contraction is done by SMC having equal dynamic strength and dynamic characteristic (J-curve) in that of native artery. For this

purpose, SPU HFs need three conditions, (a)outer surface whose pore size as big as 20 μm that SMC can invade, (b)breaking strength stronger than native artery, (c)J-curve whose Young's modulus is smaller than that of it. (II)We suggest a new-type inner surface, which promotes growth of HUVEC. First, the inner surface is covered with some gradient concentration by blood compatible water-soluble phospholipid polymer (PMBBU), composed of 2-methacryloyloxyethyl phosphorylcholine (MPC), *n*-butyl methacrylate (BMA), and 2-methacryloyloxyethyl butylurethane (MEBU) [5]. The MPC polymer could suppress blood coagulation well even when the polymer contacts with human whole blood without anticoagulant. Also, the MPC polymer with the MEBU moieties was good property to attach the SPU surface through hydrogen bonding between urethane unit in both the SPU and the MPC polymer. Then, the drug delivery system of vascular endothelial growth factor (VEGF) in the core material makes entire field of the inner surface covered with HUVEC. To that end, we need to control the good timing that water-soluble phospholipid polymer begins to slowly dissolve as the speed of the growth of HUVEC. So that means we strive to do the temporal and spatial control of cell response in the material interface. In this study, the appearance of PMBBU that was invaded by SMC was observed as the former stage of the experiment.

2. EXPERIMENT

2.1 Preparation of SPU HFs

SPU HFs were fabricated using a double injection nozzle with an annular spinneret by the dry-jet wet spinning process, as the same procedure reported by Ye, et al [6]. The spinneret diameters of inner and outer tube were 15 mm and 20 mm. The polymer solution was pumped at constant speed (10 mL/min) by a metering pump. After leaving the spinneret, the SPU solution dropped freely by gravity into an outer coagulant (distilled water) bath (Fig.1). Polymer was SPU, Tecoflex EG-60D, solvent was *N,N*-dimethylformamide (DMF) or dimethylsulfoxide (DMSO), and poor solvent was ethanol (EtOH). The change in the pore size on outer surface of the hollow fiber by the temperature change of the outer coagulant solvent was observed with the scanning electron microscope (SEM). Moreover, J-curve of the hollow fiber and the change in breaking strength were examined by the tensile test. Composition of polymer solution is shown in Table 1 and spinning conditions of HFs are shown in Table 2. The dynamic characteristic value aimed was assumed to be a value of bovine arteria femoralis.

2.2 Synthesis of PMBBU

The desired amounts of MPC, BMA and MEBU were placed in a glass ampoule, and the mixture was diluted with ethanol to 1 mol l^{-1} of monomer concentration. Into this solution, 2,2'-azobisisobutyronitrile (AIBN) was dissolved (1 mmol l^{-1}). Argon was bubbled into the solution to displace the oxygen, and then the ampoule was sealed. The polymerization was carried out at 60°C for 4h. After cooling, the contents were poured into a large amount of a mixture of diethyl ether and DMF (9/1 by volume) to remove unreacted monomer and precipitate the polymer formed. The precipitate was filtered off and dried *in vacuo*. The structure of the copolymer obtained was confirmed by ^{1}H NMR and IR.

The mole fraction of each component in the copolymer was determined from the results of phosphorus analysis

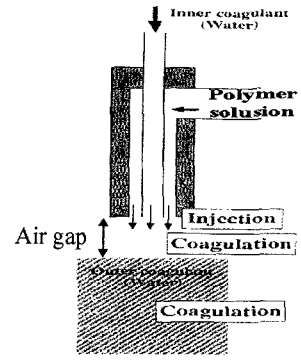


Fig.1. Dry-jet wet spinning process

Table 1. Composition of polymer solution

Abb.	Composition (wt%)			
	Polymer	Solvent		
	SPU	DMF	DMSO	Ethanol
SPU (DMF)	20	75	0	5
SPU (DMSO)	20	0	75	5

Table 2. Spinning conditions of HFs

Abb.	Polymer Solution Temp. (℃)	Inner Coagulant Temp. (℃)	Outer Coagulant Temp. (℃)	Air Gap (mm)
SPU (DMF)	23	23	15-95	25
SPU (DMSO)	60	23	23-60	25

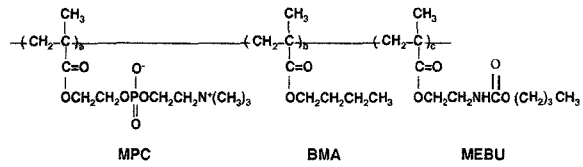


Fig.2. Chemical structure of PMBBU

Table 3. Synthesis of PMBBU

Mole fraction (MPC/BMA/MEBU)		Time	Yield	Mw [a]
	NMR			
In feed	In copolymer	(h)	(%)	
0.30/0.50/0.20	0.38/0.43/0.19	4	15	2×10^5

[Monomer]=1mol/L, [AIBN]=1mmol/L, Solvent: EtOH, Polymerization Temperature:60 °C

a) Mw represents weight-averaged molecular weight. Elution time by gel-permeation chromatography was calibrated with a poly(ethylene glycole) standards.

for both the MPC and MEBU. The chemical structure of PMBBU and results of the polymerization are shown in Fig.2 and Table 3.

2.3 Cell experiment

PMBBU was coated on half of the poly(ethylene terephthalate)(PET) board. SMCs were seeded on the PET board (3×10^5cells/ml). SMCs were cultured in SmGM-2 (Sankojyunyaku. Co. Japan). SmGM-2 was exchanged every 24 hours.

3. RESULTS AND DISCUSSION

3.1 Dynamic characteristic change of SPU HFs

3.1.1 Effect of Temp. of the outer coagulant solvent

Fig.3(a) and Fig.3(b) are SEM photographs of outer surface of SPU(DMF) HFs at 50°C and 90°C in temperature of the outer coagulant solvent. The relationship between pore size of outer surface of SPU(DMF) HFs and outer coagulant temperature is shown in Fig.3(c). The pore size increased along with the rise in heat of the outer coagulant solvent, and became large 20 μ m at 90°C or more. Fig.4(1) is the J-curve of bovine arteria femoralis. Fig.4(2) and Fig.4(3) are the stress-strain curves of SPU(DMF) HF prepared at 50°C and 90°C in temperature of the outer coagulant solvent at SPU:DMF:EtOH = 20:75:5 (wt%). Fig.4(3) was more adjacent to Fig.4(1) than Fig.4(2). Breaking strength of Fig.4(3) was also larger than that of Fig.4(2).

3.1.2 Effect of solvent

Fig.5 are SEM photographs of the cross section of SPU HFs. Generally, the morphologies of the cross

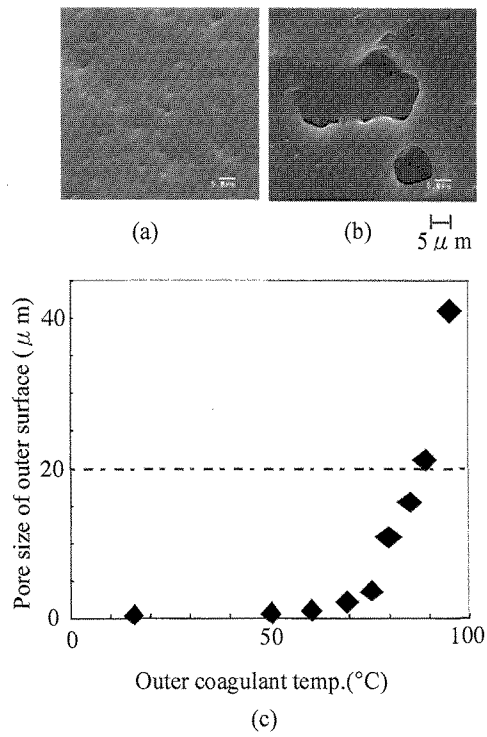


Fig.3. Pore size of outer surface of SPU(DMF) HFs
(a) Outer surface of SPU(DMF) HFs at 50℃
(b) Outer surface of SPU(DMF) HFs at 90℃
(c) Change of pore size by outer coagulant temp.

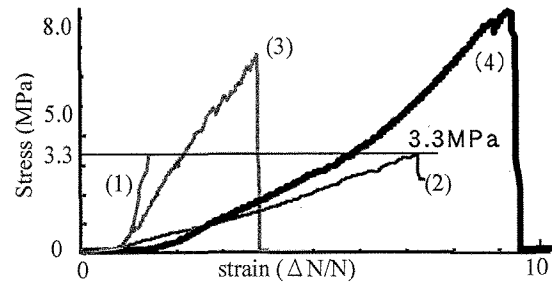


Fig.4. Stress-strain curve of SPU HFs

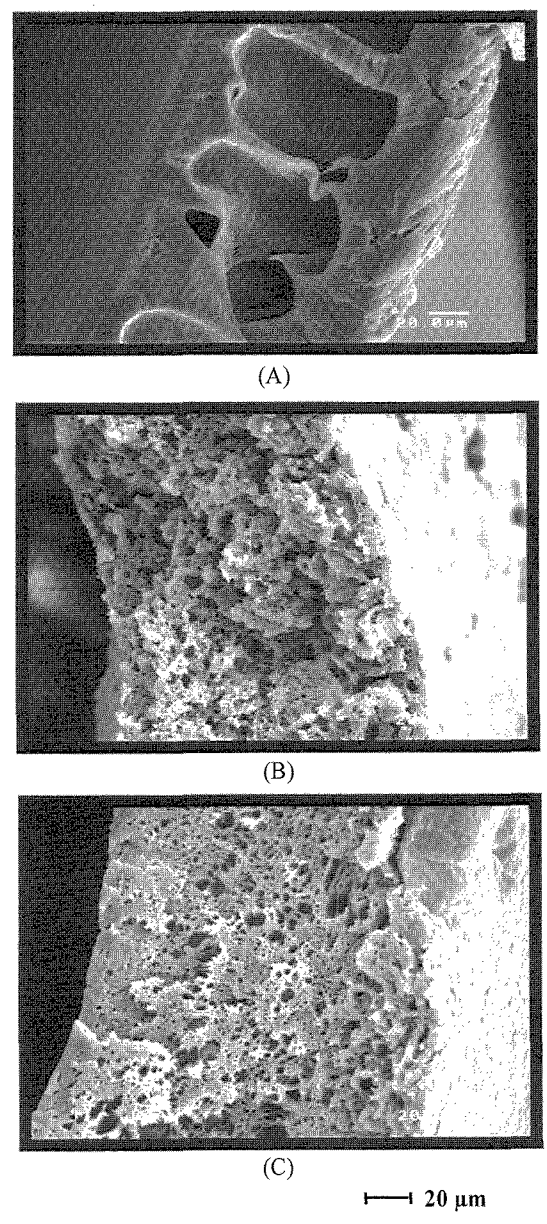
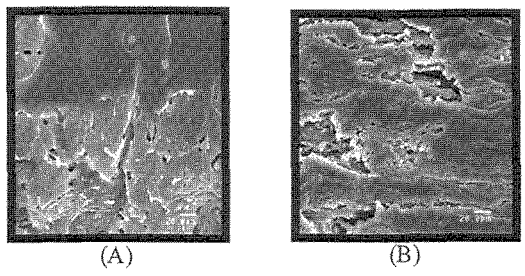


Fig.5. Cross section of SPU HFs
(A):SPU(DMF)
(B):SPU(DMSO) Outer Coagulant Temp.:23℃
(C):SPU(DMSO) Outer Coagulant Temp.:60℃



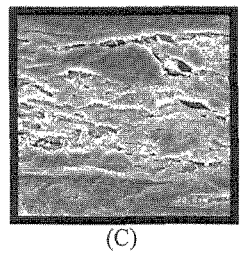


Fig.6. Outer surface of SPU HFs
(A):SPU(DMF)
Outer Coagulant Temp.:23°C
(B):SPU(DMSO)
Outer Coagulant Temp.:23°C
(C):SPU(DMSO)
Outer Coagulant Temp.:60°C

section of SPU(DMF) HFs are seen in Fig.5(A). They seem sponge structure which has a space between the dense structures of two layers. Fig.5(B) and Fig.5(C) are SEM photographs of the cross section of SPU(DMSO) HFs prepared at 23°C and 60°C in temperature of the outer coagulant solvent at SPU:DMSO:EtOH = 20:75:5 (wt%). The cross section structure of the SPU(DMSO) HFs were exact for the SPU(DMF) hollow fiber, and became tubular with which externals were steady. Fig.6 are SEM photographs of outer surface of SPU HFs. The pore size of outer surface of SPU(DMSO) HFs was large 20 μ m. Fig.4(4) is the stress-strain curve of SPU(DMSO) HF prepared at 60°C in temperature of the outer coagulant solvent at SPU:DMSO:EtOH = 20:75:5 (wt%). Young's modulus in the low load region of Fig.4(4) became smaller than that of SPU(DMF) HF. And breaking strength increased.

3.2 Cell experiment

SMC proliferation in borderline was seen in Fig.7. SMC broke the borderline with PMBBU six days later after sown, and would be covered completely in two weeks. SMC proliferation in interfacial neighborhood was seen in Fig.8. It seemed that SMCs cooperated each other to surround a part of PMBBU coating part and proliferated there.

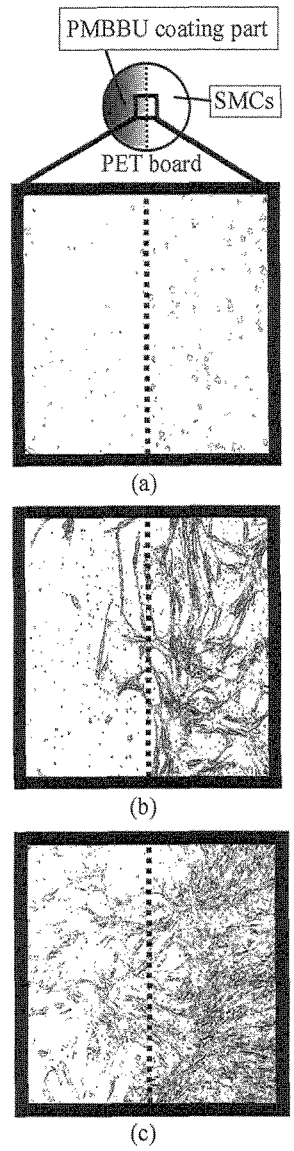


Fig.7.SMC proliferation in borderline
(a) After 30 minutes
(b) After 6 days
(c) After 2 weeks

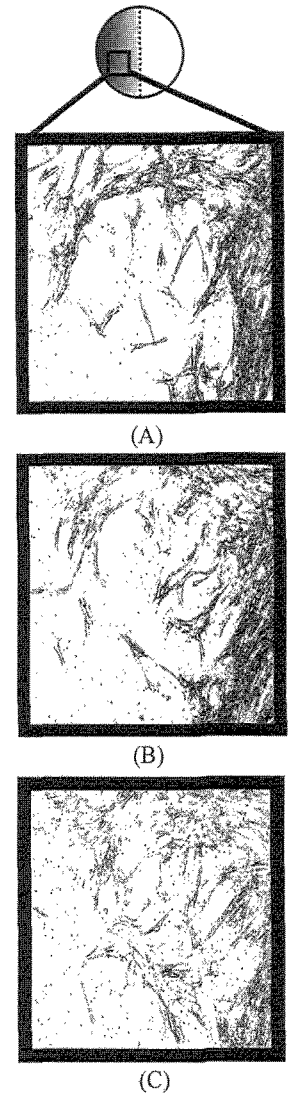


Fig.8. SMC proliferation in interfacial neighborhood
(A) After 12 days
(B) After 14 days
(C) After 16 days

4.CONCLUTIONS

The SPU hollow fibers were prepared by the dry-jet wet spinning process from DMF or DMSO solutions. Young's modulus in the low load region of J-curve when making it at 60°C in temperature of the outer coagulant solvent at SPU:DMSO:EtOH = 20:75:5 (wt%), became smaller than that of the SPU(DMF) hollow fiber, breaking strength increased, and an ideal J-curve was obtained. The problem in the future is more detailed clarification of the relationship between the structure of the hollow fiber and J-curve. The appearance where SMC invade on PMBBU coated on the PET board was observed. The problems in the future are establishment of a more quantitative experiment system and to get the appearance where HUVEC invade on PMBBU coated on the SPU.

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