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Patient-Specific Blood Vessel Scaffold for Regenerative Medicine

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Abstract— **In this research, we propose a method to construct an artificial blood vessel scaffold with patient-specific complex 3-dimensional shape and biocompatible porous polymer membrane adapted for cell cultivation, by introducing salt leaching technique into a fabrication technique for patient-specific 3-D blood vessel model proposed by authors. In this method, a membranous PVA (Poly Vinyl Alcohol) structure of desired 3-dimensional blood vessel shape was fabricated based on CT data. And the PVA structure was dip coated with polymer solution made by dissolving the Caprolactone (3wt%) and NaCl particle (27wt%) into chloroform (organic solvent). Finally, the NaCl particle and the membranous PVA structure were both eluted from the structure by dissolving these materials under water, and finally a patient-specific blood vessel scaffold with desired 3-D vascular shape was constructed. Presented scaffold has the mechanical compliance similar to human blood vessel and provides porous polymer structure appropriate for cell cultivation.**

Index Terms - Regenerative Medicine; Blood Vessel Scaffold; Patient Specific; Rapid Prototype; Salt Leaching

I. INTRODUCTION

The regenerative medicine is a new treatment method that reproduces greatly damaged organs by using cells. And in recent years, the regenerative medicine is now applied to various body areas such as the skins and the bones [1-3]. There is no problem of the rejection in the regenerative medicine because own cell manages the organ reproduction. Furthermore, by applying body absorptive materials as its scaffold material, there is an advantage that any artifact remains inside his body after certain period. As a result, the regenerative medicine attracts attention as presence that cancels the problem of inflammation and deterioration that was accompanied by the past artificial organs, and the problems of rejection and donor's shortages in organ transplant.

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In this situation, various researches are performed for the regenerative medicine of the artificial blood vessel, which is the target of this report. In this field, a blood vessel scaffold made of body absorptive polymer fiber with restoration function that vanishes after certain period inside human body and substituted by self-cells is the current majors concern [4-5].

However, long-term patency has not been achieved yet about the blood vessel of 5 mm or less in the inside diameter because of deteriorating, making to the lime and stenosis. Especially, the clot formation is often caused by the difference of mechanical compliance between the artificial blood vessel and adjoining living blood vessel, in the earliness after transplantation. As a result, clinical application of artificial blood vessel is actually limited to the large diameter blood vessel of about 10 mm or more in the inside diameter. Moreover, the diseased blood vessel is substituted by sewing straight artificial blood vessels made of polymer materials such as the polyester fibers and ePTEF, and resultant discontinuous blood vessel shape results in local stagnation of blood flow that may grow serious blood clout.

To solve such a problem, we propose a fabrication method for a patient-specific vascular scaffold with small diameter for regenerative medicine, as a solution for above problems. This fabrication technique is realized by introducing salt-leaching method that allows fabricating porous polymer membrane into the CT/MRI-based patient-specific blood vessel modeling technique, which is proposed by authors [6],[7]. Consequently, we realize an artificial blood vessel scaffold that provides; (1) patient-specific 3-dimensional structure appropriate for individual patient, (2) mechanical compliance similar to human blood vessel, and (3) body absorptive characteristic that allows the scaffold to be vanished in a certain period and replaced by self-cells.

2. REQUIRED CONDITIONS FOR ARTIFICIAL BLOOD VESSEL SCAFFOLD

In the achievement of the artificial blood vessel that provides a longterm in vivo patency by the regenerative medicine approach, achievement of the following item are indispensable.

1) Patient-Specific Blood Vessel Scaffold: A scaffold for sowing cells is necessary to develop the cell into the desired blood vessel shape. Currently desired artificial

blood vessel shape is constructed by suturing straight artificial vessel. However, the resultant discontinuous blood vessel shape may cause the stagnation of blood flow and may grow serious blood clot at that point. Therefore, it is ideal to be able to design a 3-dimensional blood vessel scaffold freely, to provide the best shape for each patient. Moreover, it is also useful to restore original blood vessel shape before the occurrence of his diseases, based on his CT or MRI information.

2) Porous polymer scaffold with body absorptive characteristics: In the construction of blood vessel scaffold, it is useful to achieve the porous polymer structure into which cells can go. And, it is also desirable to construct the blood vessel scaffold with the bioabsorbable polymer that disappears in human body within a certain period after its transplantation, and to exclude the necessity of the blood vessel resubstitution according to the patient's growth, by achieving complete substitution with self-cell.

3) Reproduction of mechanical compliance of human blood vessel: The reproduction of mechanical compliance of human blood vessel is important to realize a longterm patency after transplantation without causing stenosis by making to the lime and clot formation. Moreover, it is important to achieve the enough durability that might not be deteriorated after transplantation and be transformed.

3. Materials And Method

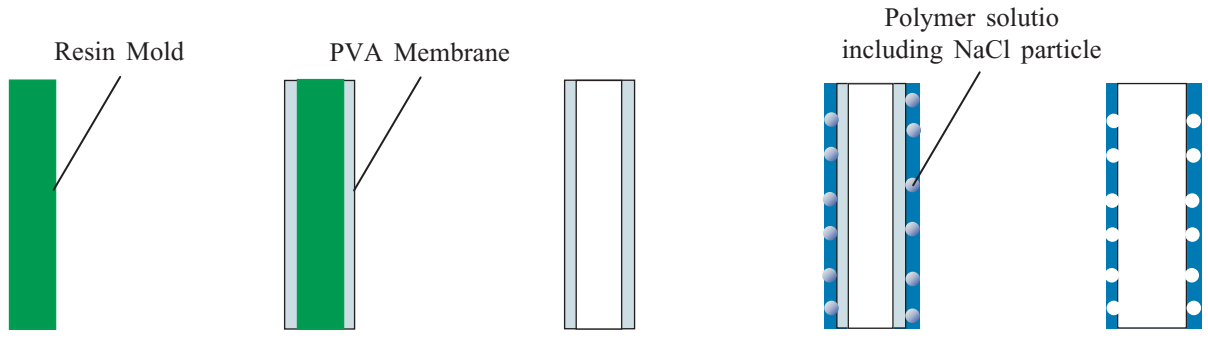
A. Formation of Porous Membrane with Salt Leach-ing Method

In this report, we applied the salt leaching method for the construction of blood vessel scaffold that provides micro porous structure appropriate for blood vessel scaffold. The salt leaching method is a method of forming the porous structure of polymer by using the NaCl particle [8]. In this method, a volatility high Polymer solution is prepared by dissolving polymer to an organic solvent, and NaCl powder of 10 mm in averaged particle size is mixed. Composite material of the high crystalline polymer and NaCl particle with desired shape is cast by pouring this mixture liquid into a mold with required shape. Afterwards, soaking this composite material under water and eluting the NaCl particle can obtain the porous polymer structure of the desired shape. In the salt leaching method, the pore size can control by changing the particle size of NaCl, and changing the volume ratio of NaCl with Polymer can control the porous degree. We confirmed that a scaffold with 500 μm in maximum pore size and this salt leaching method can achieve maximum porous degree of about 93%. Figure 1 shows the overview of proposed blood vessel scaffold fabrication process.

B. Vascular Scaffold Construction of Desired Shape

The blood vessel scaffold construction technique of desired shape based on the above-mentioned salt leaching method achieved by this report is described as follows. The blood vessel scaffold construction technique of desired shape based on the above-mentioned salt leaching method achieved by this report is described as follows.

To apply the salt leaching method, described in the former section, polymer solution of the low viscosity that was applicable to dipcoating film fabrication was prepared by mixing the Caproracton with NaCl powder in the chloroform selected as an organic solvent (Table I). The density of P(L-LA/CL) was assumed to be 3wt% to become an appropriate viscosity to the dipcoating, and, on the other hand, to obtain an appropriate porous



Step1: Mold of desired blood vessel shape made of sulfonamide resin

Step 2: Resin mold coated with PVA thin membrane

Step 3: Mold of desired blood vessel shape made of PVA resin obtained by eluting Resin mold

Step 4: PVA mold coated with polymer solution for salt leaching method

Step 5: Blood vessel scaffold with porous structure obtained by eluting PVA and NaCl particle

Figure 1 Salt leaching methed adapted for the fabrication of patient-specific blood vessel scaffold

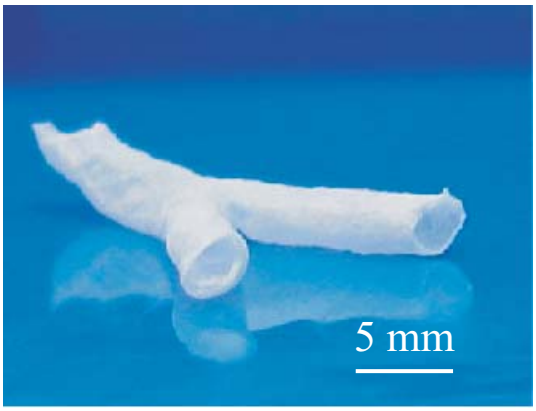


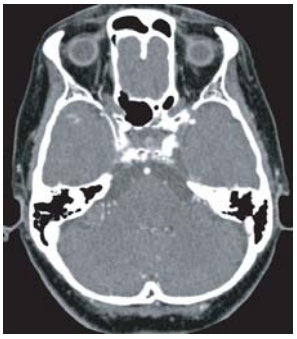
Figure 2 Blood vessel scaffold for regenerative medicine with Y letterform of 3 mm inside diameter, constructed by proposed fabrication technique

structure to the cell growth, the powdery NaCl, crushed into several mm of particle sizes, was mixed with this polymer solution at 27wt% density. Then, the sulfonamide resin was cast into desired blood vessel lumen shape (Fig. 2; in this example, Y letterform of 3 mm in the inside diameter) using casting mold. And, a mold with desired blood vessel lumen shape made of PVA film was constructed by soaking the entire structure in the acetone after PVA (polyvinyl alcohol) film of 100 μm in the film thickness had been formed on the surface of this resin mold by dip coating method. The composite material of P(L-LA/CL) and NaCl particle was formed on the surface of this PVA mold by withdrawing this PVA type at speed 1.0 mm/sec from the above-prepared polymer solution, and repeating the dip-coating process for 6 times. NaCl particle and the PVA structure were eluted simultaneously by soaking the entire structure under water after chloroform had been completely volatilized at room temperature atmosphere, as a result, blood vessel scaffold of desired shape that comprise of the porous polymer film was finally obtained (Fig. 2). Here, the PVA used as a mold material was selected, since it resists two kinds of organic solvents (acetone and chloroform) utilized in this process, and is finally elutable by water.

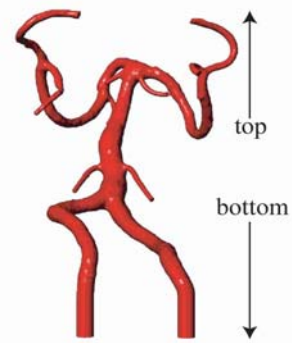
C. Patient-Specific blood vessel scaffold based on CT information

Patient-Specific blood vessel scaffold was constructed based on CT information, aiming to restore the blood vessel shape before the patient get the disease. Hereafter, this fabrication technique is described in detail.

We reconstructed the 3-dimensional blood vessel shape of the targeted artery (in this example, basilar artery) using 100 digital slice images obtained by helical CT scanning at regular 0.5 mm intervals and 0.3 mm/pixel resolution (Fig. 3 (a)). We virtually recreated



(a) One of CT image used for blood vessel scaffold construction



(b) Reconstructed 3-dimensional structure of targeted blood vessel

(c) Patient-specific blood vessel resin mold constructed by rapid prototyp-

(d) Patient-specific blood vessel PVA mold constructed by dip-coating

Figure 3 Fabrication process for patient-specific blood vessel scaffold based on CT information

each CT image 3-dimensionally on a PC as a 2-dimensional scalar CT field, based on the interval 0.5 mm, building up a 3-dmensional scalar field. Adding a specific scalar value conforming to the CT value on the boundary between the artery and its surroundings, we extracted 3-dimensional outlines of the arterial structure. Interpolating this 3-dimensionally, we created 3-dimensional iso-surfaces composed of identical CT values. This

Table I Condition for dip-coating the salt leaching polymer solution

Solvent	Chloroform
Polymer	P (L-LA/CL): Density: 3wt%
Salt	NaCl particle of about 10 mm diameter Density: 27wt%
Viscosity	6. 6 Pas

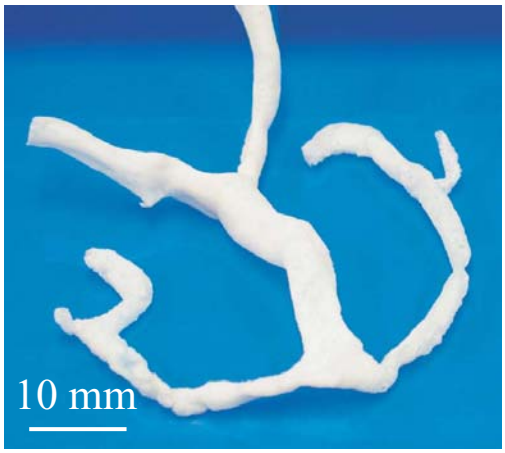


Figure 4 Patient-specific blood vessel scaffold of basilar artery for regenerative medicine constructed based on CT information. Scaffold is consists of porous P(L-LA/CL) membrane having artery-like mechanical compliance

iso-surface is then simplified by removing scattered irrelevancies part and ablating small branches, leaving only the basilar artery and aneurysm. This yielded the 3-dimensional structure of the individual cerebral artery from CT slice images. Figure 3 (b) shows the reconstructed arterial image.

We rapidprototyped a tree-like solid wax model of the targeted cerebral artery using the data from the above reconstructed 3-dimensional geometry (Fig. 3 (c)). Each fabricated layer was 13 μm thick. We used the fused deposition modality of rapid prototyping to fabricate of this wax model, which easily melts at relatively low temperature into a very low-viscosity liquid in the lost wax process. We used a chemical sulfonamide compound to construct it. This material has a melting point of about 100ºC and easily dissolves in solvent, especially acetone.

And, the uniform PVA membrane was formed on this RP model surface by soaking the RP model under 10wt% PVA solution, and withdrawing the RP model at 1.0 mm/sec for 6 times. Afterwards, by selectively dissolving the RP model using acetone, a blood vessel model with desired vascular lumen shape made of PVA was obtained (Fig. 3 (d)).

Here we selected PVA of 87-89 saponification level and 500 of polymerization level. The viscosity of solution rises when the polymerization level rises, and the PVA become easy to be melted when its saponification level increases. In this report, the above-mentioned saponification level was adopted to remove the PVA model by using the room temperature water without destroying P(L-LA/CL). And the above-mentioned polymerization level was selected because moderate viscosity was necessary when the RP model was dipcoated by the PVA solution. Finally, we constructed a patient-specific blood vessel scaffold as shown in figure 4, by applying the above-prepared PVA mold to the scaffold fabrication method presented in former section.

4. Results And Discussion

The fabrication technique that we propose has three features that make it attractive: (i) it can create patient-specific arterial scaffolds with more than 1 mm of inner diameter; (ii) it can reproduce complex configurations such as bifurcations other than simple cylindrical structure; (iii) it can reproduce a precritical original vascular structure from a patient's damaged vascular structure (e.g. we can remove aneurysms or widen the area of stenosis by a PC).

To evaluate the controllability of the thickness of the blood vessel wall of presented blood vessel scaffold, its membrane thickness was measured while changing the number of dip-coated layers from 4 layers to 7 layers at the dip-coating condition shown in table I. It was confirmed that the thickness of the fabricated blood vessel scaffold is proportional to the number of dipcoated layers, as shown in figure 5. As a result, it was confirmed to be able to adjust the membrane thickness of the blood vessel scaffold freely by adjusting the controlling frequency. Moreover, it was confirmed that a uniform membrane thickness is obtainable, as confirmed by figure 2. Figure 6 shows the SEM image of the blood vessel scaffold constructed with the proposed technique. It is confirmed from this figure to be able to form the polymer membrane with porous polymer structure according to the proposal technique.

On the other hand, to evaluate mechanical compliance of P(L-LA/CL), a bioabsorbable polymer adopted by this report, the tensile test based on JIS Z2201 was executed to this material. Experimental condition is shown in Table II. In this evaluation test, we prepared 4

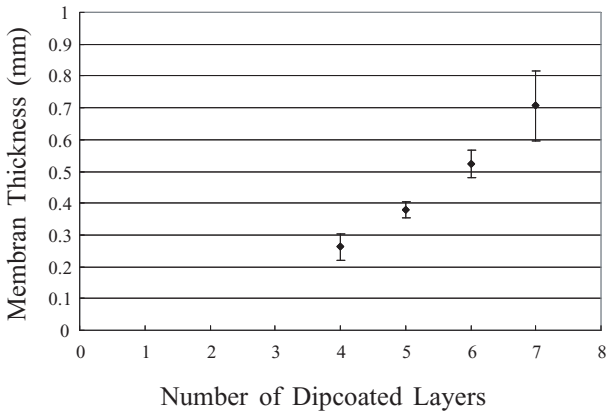


Figure 5 Result of the thickness controll experiment made for cyrindrical blood vessel scaffold

Table II Experimental condition for the tensile test shown in figure 5

Test Method	Tensile Test Velocity: 20 mm/min
Fabrication Method	Salt Leaching Caprolactone (P-(L/LA-CL)
Specimen Shape	JIS Z2201
Specimen Thickness	300 mm

kinds of scaffold specimen by changing its porous ratio from 0 % to 90 % by changing the ratio of NaCl particle. And by using these specimens compliance change according to the porous degree change of the scaffold was measured. The elastic modulus of each test piece calculated from this tensile test result is summarized in Figure 7 and Table III. As confirmed from this table, mechanical compliance of the scaffold can be adjusted by adjusting the porous degree, consequently, the best compliance can be achieved. Moreover, the elongation ratio for these 4 test pieces exceeded 100 %, and we confirmed that the constructed porous polymer membrane provides enough durability as artificial blood vessel scaffold.

On the other hand, by so wing cell, we evaluated the cell growth potential of this blood vessel scaffold. In this experiment, we sowed HUVEC (human umbilical vein endothelial cells) on this scaffold specimen (porous ratio: 90%, pore size: 10 μm, size: 5 x 5 x 0.5 mm, sheet-shaped). And, the FN coating was given to the surface of the scaffold to improve the cell bonding to the surface of the scaffold, by making it freeze-dry after this scaffold was soaked in the FN solution of 0.01% work stand. Afterwards, HUVEC was sown, and cultured on this test piece for 24 hours with $3.5x10^4$ cells/cm^2 under

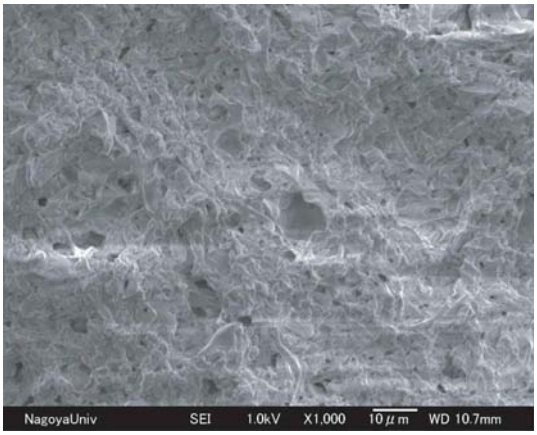


Figure 6 SEM image of the porous polymer structure of the blood vessel scaffold constructed by salt leaching method

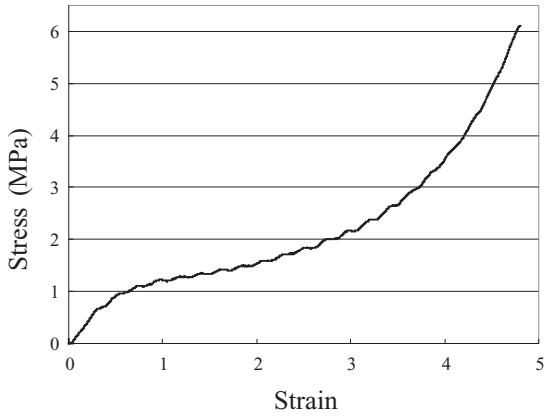


Figure 7 Tensile test result for the presented P(L-LA/CL) blood vessel scaffold. Experimental condition is described in Table II

Table III Experimentaly measured elastic modulus of presented blood vessel scaffold with variable polymer density

Polymer Density [%]	Young's Modulus [MPa]
10	0.17
15	0.19
20	0.23
100	0.32

$5\%CO^2$ environment 37ºC (Table IV). Figure 8 shows the SEM image in the cell bonding area obtained by this sowing examination. As confirmed from this figure, cell growth was confirmed on this blood vessel scaffold.

5. Conclusion

In this report, we constructed patient-specific blood vessel scaffold for regenerative medicine. On the other hand, the cell culture examination was executed, and bonding and the growth of HUVEC on the surface of constructed vascular scaffold.

It is a future work to evaluate the cell bonding on this scaffold under pulsatile stress field, and to optimize the porous degree and pore size of this scaffold to reproduce the mechanical compliance of living blood vessel. As a result, we aim at the achievement of a small diameter patient-specific artificial blood vessel with good biocompatibility.

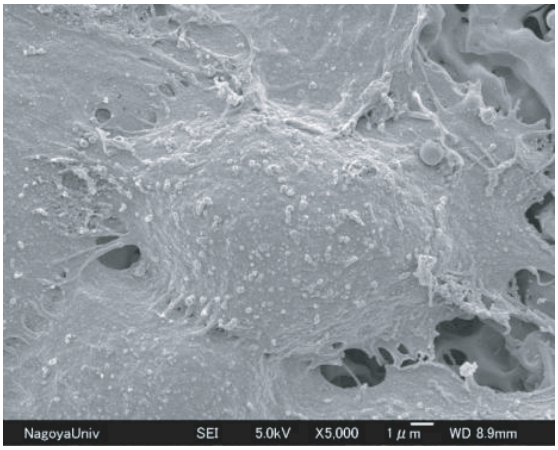


Figure 8 Experimetal result of cell culture on the pre-blood vessel scaffold consists of porous polymer membrane

Table IV Experimental condition for cell culture on presented blood vessel scaffold with porous polymer membrane

Applied Cell	HUVEC 2.3x104 cells/cm^2
Fabrication Method	at 37ºC, 5%CO2 for 24 hours
Caltivate Condition	Salt Leaching (P-(L/LA-CL) Cavity Ratio: 90 %
Surface Coating	Fibronectin, FN

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REFERENCE

[1] AG Mikos, Y. Bao, LG Cima, DE Ingber, JP Vacanti, and R. Langer, "Preparation of Poly (Glycolic Acid) Bonded Fiber Structures for Cell Attachment and Transplantation," J. Biomed. Mater. Res., Vol.27, pp.183-189, 1993

[2] G.R.D. Evans, K. Brandt, M.S. Widmer, A. Gurlek, et al. "Tissue Engineered Nerve Conduits: The Use of Biodegradable Poly-DL-lactic-co-glycolic Acid (PLGA) Scaffolds in Peripheral Nerve Regeneration," in Biological Matrices and Tissue Reconstruction, pp. 225-235 (1998)

[3] D.J.Mooney, C.L.Mazzoni, G.M.Organ, W.C.Puelacher, J.P.Vacanti, R.Langer, "Biomaterials for Drug and Cell Delivery, Vol. 331," Material Research Society (1994)

[4] Shirota T, He H, Yasui H, Matsuda T. Human endothelial progenitor cell (EPC)-seeded hybrid graft: proliferative and antithrombogenic potentials of EPC. Tissue Eng 9 (1): 127-136, 2003.

[5] Sonoda H, Takamizawa K, Nakayama Y, Yasui H, Matsuda T. Coaxial double-tubular compliant arterial graft prosthesis: time-dependent morphogenesis and compliance changes after implantation. J. Biomed Mater Res., 65A(2): 170-81, 2003.

[6] S. Ikeda, F. Arai, T. Fukuda, M. Negoro et al., "An in vitro patient-specific biological model of the cerebral artery reproduced with a membranous configuration for simulating endovascular intervention, Journal of Robotics and Mechatoronics, Vol.17, No.3 pp.327-334, 2005

[7] S. Ikeda, F. Arai, T. Fukuda, M. Negoro et al., "Rapid production of an in vitro anatomical model of human cerebral arteyies based on CT images, Proc. of IEEE MHS2002, pp.41-46, 2002

[8] M.S Widmer, G..R.D.Evans, K.Brandt et al., "Proceedings of the 1997 Summer Bioengineering Conference , Vol.35 ," The American Society for Mechanical Engineers (1997)