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Retention of Endothelial Cells adhered on Polyurethane Surface under Flow Condition

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=Abstract=

Construction of the stable monolayer of endothelial cells onto physicochemically modified polymeric surface is one of the appropriate method to develop the small caliber vascular grafts with the long-term patency. In this study, we constructed the monolayer of endothelial cells on the fibronectin and the extracellular matrix-coated polyurethane surface derived from human fibroblast cells. To elucidate the adhesion strength of endothelial cells on the extracellular matrix-coated polyurethane, a laminar flow chamber apparatus was developed to exposure the shear stress on the apical membrane of endothelial cells. Endothelial cells show the strongest adhesion after two days of seeding onto the fibronectin-coated polyurethane surface, whereas endothelial cells on the extracellular matrix derived from the human fibroblast cells show the minimal doubling time of cellular growth.

Key words : Endothelial cell, Polyurethane, Retention, Laminar flow chamber

INTRODUCTION

Cellular adhesion on the extracellular matrix, the proper construction of tissue-matrix system, plays an important role in many biomedical applications including development of artificial organs[1], large-scale cell culture[2], and blood tissue-interactions of biomaterials[3] . Analysis of the cellular growth and the strength of cellular attachment force[4,5] can be useful to study the construction of tissue-matrix system, including the development of biomedical device where an intimate cell-biomaterial contact is needed [6] or, alternatively, must be avoided[7].

This endothelial cell-extracellular matrix system is very important in the development of cardiovascular prosthesis. Artificial prostheses are used to restore blood flow to ischemic tissue when autogenous vein grafts are not available[8]. Long-term survival of small caliber grafts has been limited by the high incidence of occlusion commonly caused by low flow rates and the thrombogenicity of available graft materials[9]. Establishment of a stable, nonthrombogenic lining on the luminal graft surface might improve the patency[10]. Toward this goal, efforts have been made to produce inherently nonthrombogenic materials [11] and to physiochemically modify polymeric surfaces[12,13]. An alternative approach is to develop a mechanically suitable artificial vessel that encourages the healing process to supply a monolayer of endothelial cells[14-16]. This would be presumably produce not only a nonthrombogenic but an antithrombogenic surface[17]. In the development of antithrombogenic vascular grafts, a firm attachment is needed[18] when endothelial cells are seeded on the inner wall of

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artificial grafts to create a natural lining, whereas without the presence of endothelial cells the inner wall has to be nonadhesive for platelets and erythrocytes.

The endothelium formed on the inner surface of the artificial graft bear the hemodynamic stress by changing the alignment of endothelial cells. Previous studies of cellular adhesion have been focused on the molecular biology of adhered proteins in cell attachment and movement[19]. In principle, the strength of attachment between the cell and substrate can be determined through breaking the contact by applying a known hydrodynamic force[20]. Micro-pipette aspiration[21], centrifugation of cells[22], and exposure of cells to fluid shear stress[7,23] are used to measure the strength of cellular adhesion. According to these studies, endothelial cells were oriented with the flow direction under the influence of shear stress and became more elongated when exposed to shear stress [24].

In this paper, we constructed the endothelial cell-extracellular matrix system onto polyurethane surface and expose endothelial cells to shear stress in a simple laminar flow chamber apparatus to investigate the cellular detachment and the strength of cellular adhesion of cells onto polyurethane polymer surface.

MATERIALS AND METHODS

Isolation and Culture of Endothelial Cells

Human umbilical vein endothelial cells were isolated from human umbilical vein[25] with modified collagenase digestion technique. Umbilical cords obtained after normal vaginal deliveries are placed in cord buffer (phosphate buffered saline (PBS), 137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_3PO_4 with 11 mM glucose) and stored at 4℃until used. The cord was removed and then cannulated at one end with a stainless-steel cannula (syringe adapter, A.H. Thomas, PA). The vein was flushed with warm (37℃) cord buffer to rinse out the blood and allowed to drain. The other end of the umbilical vein was cannulated with a stainless-steel cannula (needle adaptor, Thomas), and the vein was flushed with another warm cord buffer. The vein was finally filled with enough warm 0.2% collagenase (Clostridium histolyticum, type I, Worthington Diagnostic System Inc., Freehold, NJ) in cord buffer and incubated at 37℃ for 10 minutes. After incubation, the cord was gently kneaded twice between the fingers up and down its length and the collagenase solution was flushed out of the vein with cord buffer into a 50 ml conical plastic centrifuge tube containing 10 ml of culture medium (Medium 199 (Gibco BRL, Gaithersburg, MD) with HEPES (25 mM, Gibco BRL) containing 20 % fetal bovine serum (Gibco BRL), L-glutamine (2 mM, Gibco BRL), penicillin (100 units/ml, Gibco BRL), streptomycin (100 units/ml, Gibco BRL)). The tube was centrifuged for 7 minutes at 800 g and the supernatant decanted. The endothelial cells, form a small pellet in the bottom of the tube, were gently resuspended in 4 ml of culture medium and plated in a T-25 tissue culture flask. Endothelial cells were incubated at 37℃ under 5% CO_2. The culture medium was changed in every 2 days and endothelial cells of passage 2 to 3 were used for experiments.

Polyurethane Polymer Substrate Preparation

Polyurethane sheet was made with 12 % (w/v) polyurethane solution (PELLETHANE® 2360-80A, Dow Chemical Co., Midland, MI). PELLETHANE® solution (12g PELLETHANE®/100 ml DMAC (N,N-dimethylacetamide, Duk San Pharm., Yong In)) was cast into thin flexible sheet with the thickness of 0.3 mm on the clean glass surface. After evaporating the solvent, the sheet was immersed into ethanol for overnight and rinsed with deionized distilled water. Polyurethane sheet was assembled into Teflon™ culture ring apparatus and sterilized with ethylene oxide before the experiments.

Preparation of the Natural Extracellular Matrix from human foreskin fibroblast

Fibroblast cells isolated from human neonate foreskin. The tissue fragments were placed into a 100 mm-Petri dish containing a small amount of culture medium and cut into small pieces about 1 mm3 in size. Then, the tissue fragment placed into 35 mm-Petri dish containing the culture medium. A sterile

coverslip was placed over the tissue fragment, and 2 ml of culture medium was added carefully and incubated at 37℃ for 5 days. The tissue fragments anchor themselves to the culture dish. Human fibroblast cells (HFC) migrated from fragment to coverslip were cultured, and the remaining tissue fragments were removed. When the culture reached confluence, the culture medium rinsed once with PBS. Trypsin/EDTA (ethylenediaminetetraacetic acid) was then added to suspend HFCs from coverslip and culture dish was incubated at 37℃[25]. The suspension of HFCs was diluted with culture medium and transferred to 50㎠ tissue culture flask. HFCs were grown in Iscove's medium (IMDM, Gibco BRL, Gaithersburg, MD) with HEPES (25 mM, Gibco BRL) containing 10 % fetal bovine serum (Gibco BRL), L-glutamine (2 mM, Gibco BRL), penicillin (100 units/ml, Gibco BRL), and streptomycin (100 units/ml, Gibco BRL).

To produce natural ECM-coated polyurethane, HFCs among passage 7-10 were seeded at a density of 2.5×10^4 cells/㎠ in polyurethane sheet that assembled into Teflon™ culture ring apparatus. Cultures were maintained in Iscove's medium for seven days after confluence. Cultures were then washed with PBS and exposed for 3 minutes to 20 mM NH_4OH in distilled water followed by eight extensive washes with PBS to remove the gelatinous debris of HFCs [26].

Preparation of Fibronectin-coated Polyurethane

Fibronectin coating was performed by adding 0.5 ml of a 20 ㎍/ml solution of human fibronectin (Boehringer Mannheim, Mannheim, Germany) in PBS to polyurethane sheet for 2 hours at room temperature[27]. The final concentration of fibronectin on surface was 5 ㎍/㎠. The polyurethane sheet was then washed twice with PBS before using.

Parallel Plate Flow Chamber Apparatus and Experimental Setup

A parallel plate, channel flow chamber for flexible substrate, such as polyurethane, was developed to expose cultured endothelial cell with a known hydrodynamic shear stress[28]. The flow chamber for flexible substrate, shown in figure 1, consisted of a pair of parallel plates with a divergent entrance and a convergent exit channel, and plates were brass with glass windows for real-time microscopic studies. The flow path was formed by medical-grade silicon rubber gasket (150 in thickness). Teflon™ culture ring with endothelial cells was mounted on the elevator system for adjusting the thickness variation of polyurethane sheet. The sufficient space was installed beneath the polyurethane sheet to give assurance for the movement of flexible sheet. The upper plate was gently assembled into lower plate with bolts.

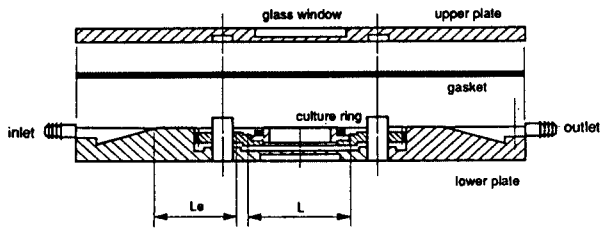

Fig. 1. Illustration of the chamber for flexible substrate

The flow chamber was mounted on the stage of a phase contrast microscope (CK-2, Olympus, Japan). The flow chamber was connected to the flow circuit by two 7.9 mm ports. Flow was generated by a roller pump (MasterFlex L/S Drive with Easy Load™ head, Cole-Pharmer Instrument Co., Chicago, IL) between two reservoirs. The upper reservoir emerged into the water bath to maintain the temperature of circulating fluid at 37℃. The temperature of circulating media was monitored with the needle type thermocouple (HYP2-21-1-1/2-T-G-48-OST-M, OMEGA Engineering, Inc., Stamford, CT) and digital indicator (DP-11 Series, OMEGA Engineering, Inc.). Air bubbles in the circulating fluid were trapped at the bubble trapper connected between the upper reservoir and the flow chamber. Before the experiment, the flow chamber, tubing, and reservoirs were steam sterilized.

Cell shape changes and motions were obsersved by a CCD camera (LK-636, TOSHIBA, Japan) attached to the microscope and connected to an image grabber (MIPS, medical image processing system, Choong Wae Medical, Seoul), IBM PC, monitor (SuperVision Pro 21", Dae Woo Electronics, Korea) and video recorder (Chromatic Series, Hitachi, Japan).

Theoretical Characterization of Laminar Flow Chamber

Simple relationship between flow rate and shear stress existed for fully developed flow between infinitely wide parallel plates. Generally, flow through straight capillaries of any cross-sectional shape is described by the classical Helmholtz-Smoluchowski equation[7,29]. This equation describes a linear relation between streaming potential changes and pressure drops. Deviations from linearity had lead to the following criterium for Le, entrance length :

$$Le \leq b \times L \qquad (1)$$

where b is a proportionality constant and L is the chamber length. Assuming Le to be 2 cm it could be concluded that the flow in the chamber used in this study is fully developed laminar flow up to flow rate of at least 500 ml/min, particularly when considering that Le will be smaller than assumed owing to the slowly converging entrance region. Consequently, the wall shear stress τ_w(dyne/㎠) in the center of the flow chamber could be calculated as follows :

$$\tau_w = \mu \frac{6Q}{wh^2} \qquad (2)$$

Shear Stress Exposure to Endothelial Cells

Adhesion/detachment experiments were accomplished with endothelial cells that were adhered on the fibronectin-coated polyurethane surface, the natural ECM(secreted from the human foreskin fibroblast cells)-coated polyurethane surface, and the pure polyurethane surface as a control. The seeding density of endothelial cells for experiments was 5×10^4 cells/㎠. Time durations for experiments were 1, 2, 4, 7, and 11 days after seeding the endothelial cells on the polyurethane sheet. Two different flow conditions were examined on the experiments. Very large shear stress, about 5 times larger than that of normal arteries, was applied to measure the adhesion strength and the detachment kinetics of endothelial cell adhered on the polymeric surfaces.

The first condition, the low shear stress condition was :

$$Q=3.17\text{ml/s},\ \tau_w=66\text{dyne/㎠},\ Re=298.6 \qquad (3)$$

and the second condition, the high shear stress condition, was :

$$Q=6.3\text{ml/s},\ \tau_w=131\text{dyne/㎠},\ Re=597.2 \qquad (4)$$

where Q, τ_w and Re represent flow rate, wall shear stress and Reynolds number, respectively. Flow exposure time to endothelial cells was 10 minutes and 250 ml of phosphate buffered saline (PBS) was used as the perfusate.

Detached endothelial cells were collected from the lower reservoir by the sampling of 10 ml perfusate at 1, 5, 10 minutes after flow begins. Then 5 ml of Isotone was added to the collected sample and the detached endothelial cells were counted three times with the Coulter Counter™ (Factory-grade, Coulter Counter Inc., England). The whole perfusate was circulated the flow circuit within just 2 minutes in case of low shear stress condition, detached cells were assumed to be well mixed in whole perfusate. To increase the mixing of detached cells in the perfusate, the outlet port to the roller pump was installed in the base of the lower reservoir. Remaining cells adhered on the polyurethane surface after shear exposure were isolated with trypsin/EDTA (Sigma Chemical Co., St. Louis, MO) and counted with Coulter Counter™.

Averaged cellular images were automatically saved on IBM PC by MIPS at 0, 10, 30 seconds, 1, 2, 4, 7, 10 minutes after flow begins. Experiments were repeated three times for statistical analysis. The schematic illustration of endothelial cell detachment experiments shown in figure 2.

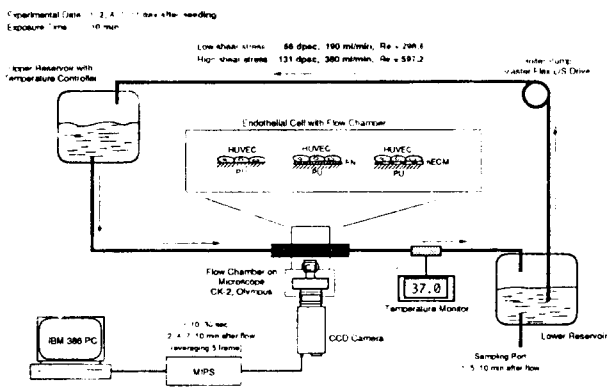

Fig. 2. Schmatic illustration of experimental setup for the flow chamber experiments

Table 1. Exponential curve fitting of growth curve and the doubling time of human umbilical vein endothelial cell on various coated polyurethane

Surface coating	Exponential curve fit of growth curve	Doubling time
Polyurethane control	$y=0.878\cdot\exp(0.239\cdot x)$	69.6hr
Fibronectin-coated polyurethane	$y=0.639\cdot\exp(0.289\cdot x)$	57.6hr
ECM-coated polyurethane	$y=0.595\cdot\exp(0.301\cdot x)$	55.3hr

*In the curve fit equations, x=incubation time(day) and y=number of cells/㎠(× 10^5cells)

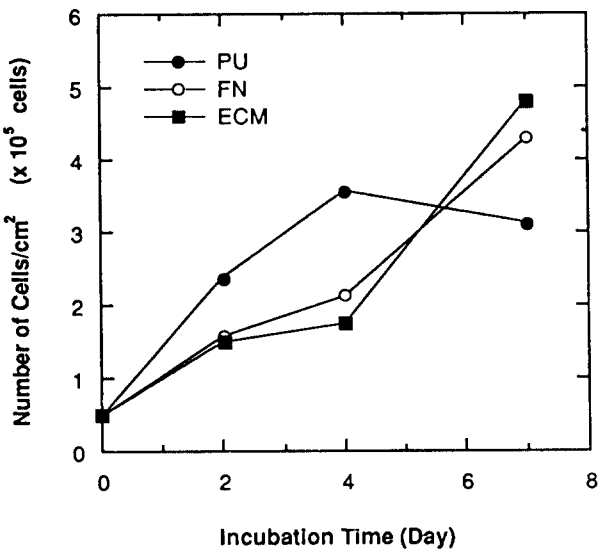

Fig. 3. Grownth of HUVEC cultured on various coated polyurethane expressed as the number of cells per ㎠. PU : polyurethane control, FN : fibronectin-coated PU, and ECM : ECM-coated PU derived from human fibroblast cells

RESULTS

Growth of Endothelial Cells on the Extracellular Matrix-coated Polyurethane

Figure 3 represents the cell growth curves of human umbilical vein endothelial cell (HUVEC) cultured on the polyurethane coated with various ECM. Endothelial cells grown on the ECM-coated polyurethane have a doubling time of 55.3 hours while the values of 57.6 and 69.6 hours were obtained for cell grown on fibronectin-coated polyurethane or control polyurethane surface, respectively. Table 1 shows the exponential curve fitting of cell growth and the doubling time of HUVEC on the polyurethane surface.

Kinetics of Endothelial Cell Retention on the Extracellular Matrix-coated Polyurethane with High Shear Stress

Endothelial cells adherent to deformable polymeric surface with or without the adhesive extracellular matrix protein were exposed to the shear stress of 66 dyne/㎠ and 131 dyne/㎠. The number of attached cells was monitored for 10 minutes. To minimize the variations of cell growth shown in figure 3, initial cell number on polymer surface at the time of experiments was taken as the summation of the detached cell number at 10 minutes and isolated cell number from the polyurethane surface after flow exposure. Kinetics of detachment of endothelial cell from polymeric surfaces was plotted in figures 4, 5, and 6 with the fraction of the initial number of adhered cells. For cells plated on control polyurethane, shown in figure 4, 60 % to 95 % of the cells detached during the first one minute of flow followed by a slower decrease in cell number with time, and the number of detached cells were proportional to the magnitude of applied shear stress. Analysis of image recordings of cell loss showed that the large decrease in the number of attached cells occurred immediately after the onset of flow. By 10 minutes, about 99 % of the cells were removed from the surface at 131 dyne/㎠.

When the cells were cultured on the polyurethane surfaces coated with fibronectin, cell detachment was reduced (figure 5). At 131 dyne/㎠, 55 % of the cells detached during the first one minute of flow at second day after seeding and, over the next ten minutes, an additional 20-25 % of the cells was removed from the polymeric surfaces. Figure 7 shows the serial images of cells adhered on fibronectin-coated polyurethane, which indicated the large decrease in attached cells within first one minute after the onset of flow. In case of ECM-coated polyurethane, the similar

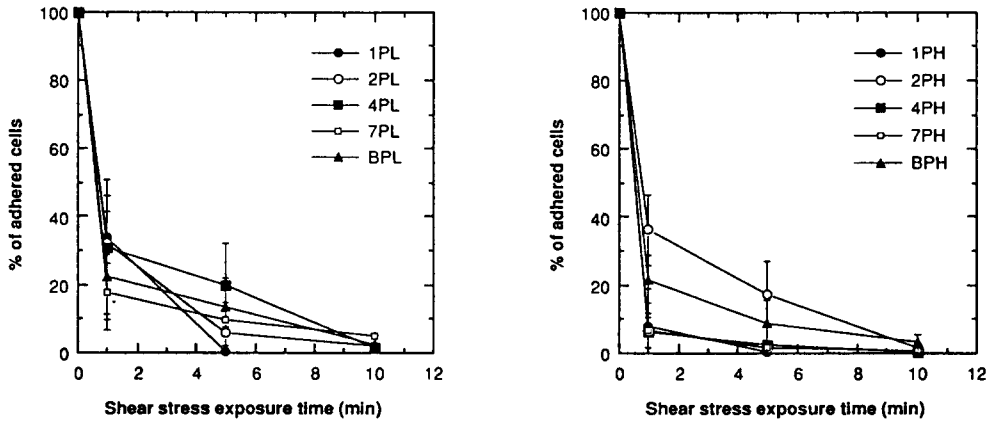

Fig. 4. Kinetics of detachment of endothelial cell from the control polyurethane surfaces. (A)the low shear stress condition, and (B)the high shear stress condition. In the legend, 1, 2, 4, 7, B(11)represent the incubation time of endothelial cells after seeding. L : low shear stress condition, and H : high shear stress condition

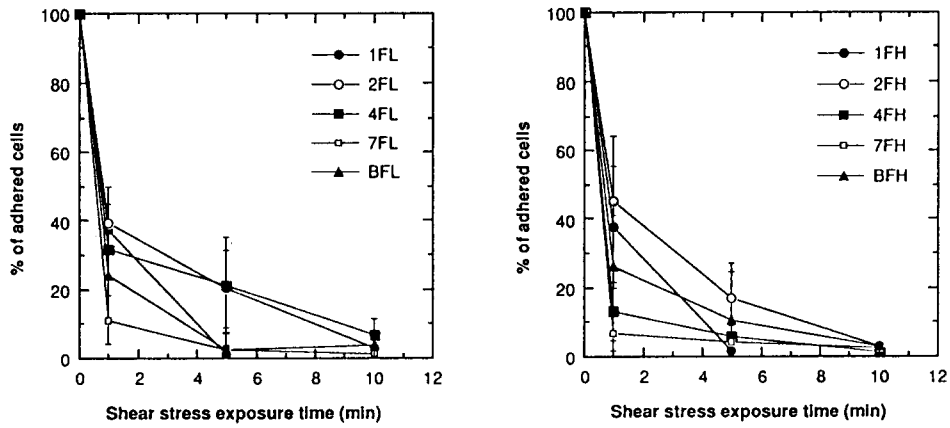

Fig. 5. Kinetics of detachment of endothelial cell from the fibronectin-coated polyurethane surfaces. (A)the low shear stress condition, and (B)the high shear stress condition

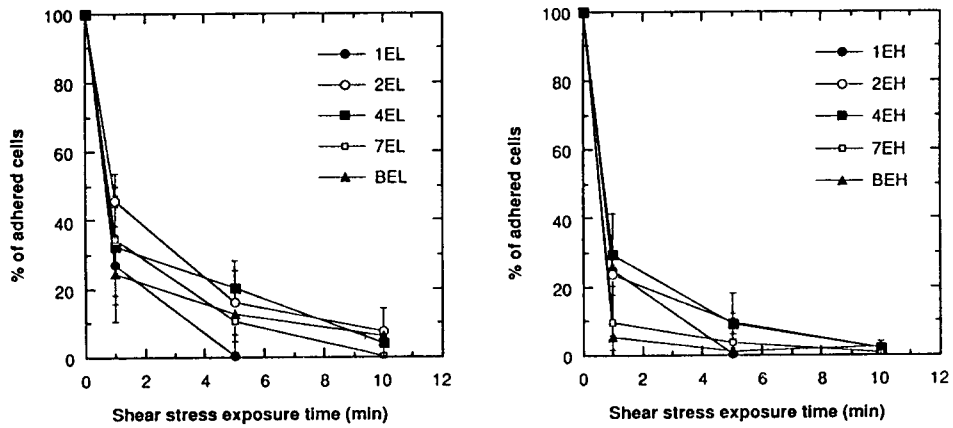

Fig. 6. Kinetics of detachment of endothelial cell from the ECM-coated polyurethane surfaces. (A)the low shear stress condition, and (B)the high shear stress condition

results were shown in figure 6.

Cell retention was measured up to eleven days after seeding after fluid flow exposure (figures 4, 5, and 6). At both flow conditions, the natural ECM-coated polyurethane and fibronectin-coated polyurethane gave better cell retention than the polyure-

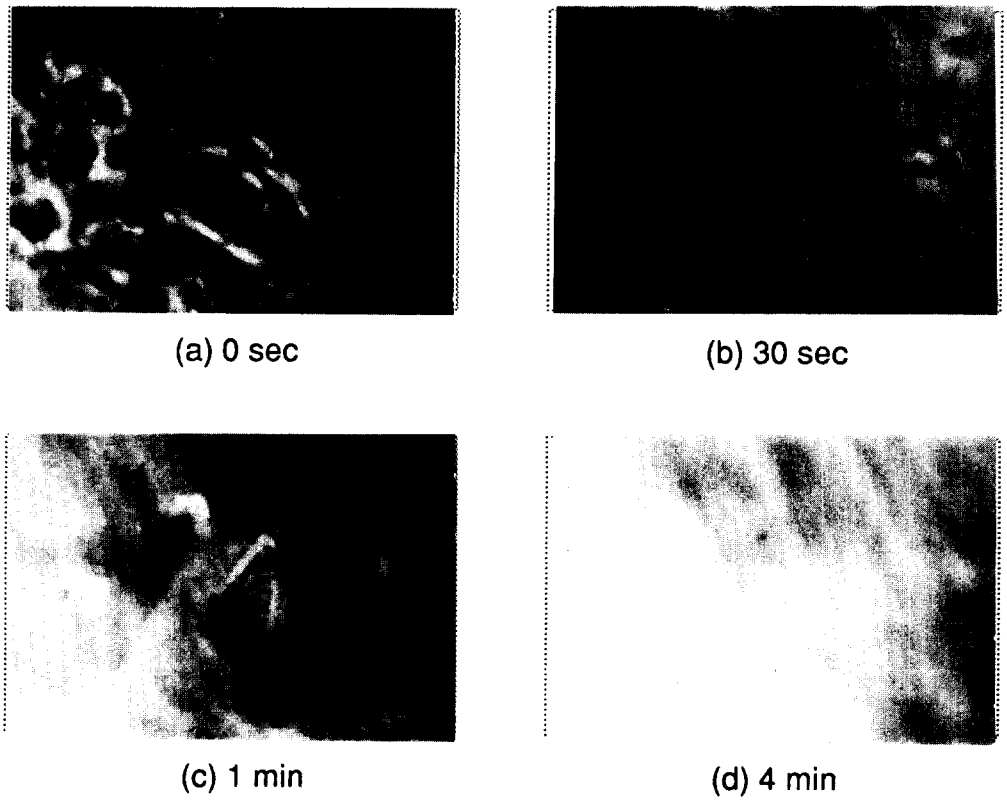

Fig. 7. Cellular detachment of endothelial cells from fibronectin-coated polyurethane, which indicated the large decrease in attached cells occurred within first 1 minutes after the onset of flow. Micrographs were taken at the experiments of seventh day after seeding. High shear stress condition was applied

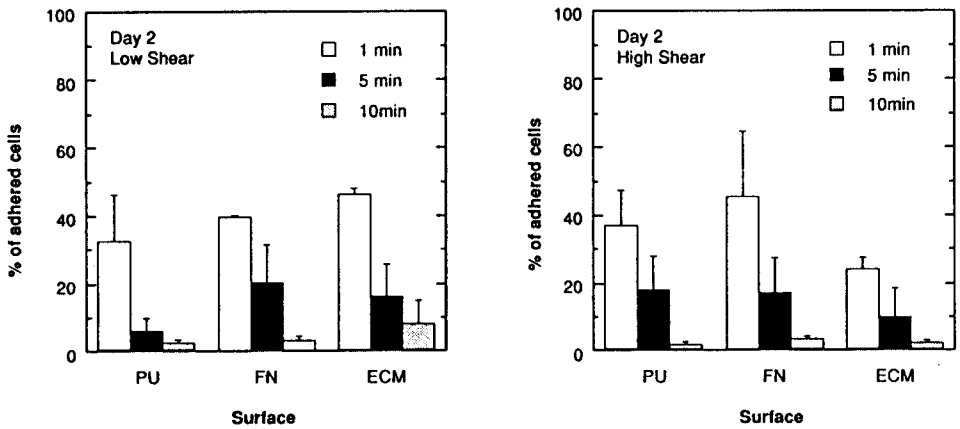

Fig. 8. Cell retention of the adhesive protein-coated polyurethane surface on the second day sfter seeding. (A)the low shear stress condition, and (B)the high sfear stress condition

thane control surface. The maximum retention of natural ECM-coated polyurethane occurred on the fourth day after seeding and the retention was decreased with second-order manner up to eleventh day (figure 6). On the other hand, maximum retention of fibronectin-coated polyurethane occurred on the second day after seeding, and for these two days, retention of endothelial cell on fibronectin-coated polyurethane was higher than that on the ECM-coated polyurethane. Figure 8 shows the cell retention of polyurethane surface on the second day after seeding. There was small difference in cell retention between two surfaces at the lower flow rates, but more cells were detached in the ECM-coated polyurethane at the

higher flow rate for the first two days.

DISCUSSION

Endothelial cell-extracellular matrix system was successively constructed on the various substrates. The existence of extracellular matrix reduces the doubling time of endothelial cell culture, as shown in Table 1.

An *in vitro* shear stress apparatus has been developed to investigate cellular adhesion and morphological changes of endothelial cells. Laminar flow through a rectangular channel was selected to produce fluid shear stress since this means would enable the generation of shear stress covering a wide physiological range. An image analysis system was used to observe the morphological changes of individual endothelial cells exposed to hydrodynamic flow condition.

Endothelial cell culture on the adhesive protein-coated polyurethane showed the importance of the extracellular matrix to facilitate endothelialization of biomaterials[9] that implanted without supplement of the growth factors[30]. The concept developed in this work was the use of the native ECM as a scaffold upon which HUVEC could attach, migrate, proliferate, and differentiate. Human fibroblast cells could easily grow in tissue culture and secrete an ECM and should closely simulate the native human ECM. ECM coating of synthetic material could facilitate endothelialization either spontaneously or in association with endothelial cell seeding[31]. Indeed the reduced doubling time of HUVEC grown on ECM was one of the major practical interest for the successful acceptance of the biomaterials[32].

Analysis of the retention of cells on polymeric surface after 10 minutes of flow indicated that the adhesive forces were well described by a biphasic distribution. It showed initial rapid isolation of receptors from ECM during first one minutes, and prolonged detachment after one minute. This initial detachment of cells correlated with cell spreading. Cells that had not spread were presumably attached weakly to the surface and easily removed following the onset of flow. Detachment following long-term exposure to flow was more complex, in part because fluid shear stress altered cell shape, orientation, mechanical properties of cell, and distribution of focal contacts[1]. Exposure of cells to flow for 10 minutes resulted in a decreased adhesion strength and detachment involved a combination of cell deformation, breakage of adhesive contact[19], and membranes rupture[33].

Cell adhesion and retention were maximal for fibronectin-coated polyurethane surface. Fibronectin is a large glycoprotein found in both blood and other body fluids and in an insoluble form in interstitial connective tissue and in or close to basement membrane[34]. It promotes the attachment and spreading of many cells on polymeric surface, and this is the basis of its use in coating vascular grafts before endothelial cell seeding[35,36]. Maximum adhesion strength of HUVEC seeded on fibronectin-coated polyurethane was occurred on the second day after seeding, and for these two days, adhesion strength of HUVEC on fibronectin was larger than that on the natural ECM-coated polyurethane. Higher adhesion strength implies that immobilized fibronectin offers higher ligand density to integrin than natural ECM [4]. Integrin β chains physically interconnect fibronectin with actin-filaments through actin-binding proteins on the inner surface of plasma membrane, elevated ligand binding on integrins evokes the secure rearrangement of cytoskeleton on adhered cell[37,38].

In summary, we have shown that the construction of endothelial tissue-extracellular matrix system on the polyurethane biopolymer. On the retention study with a laminar flow chamber apparatus, endothelial cells show the strongest adhesion after two days of seeding onto the fibronectin-coated polyurethane biopolymer, whereas endothelial cells on the extracellular matrix derived from the human fibroblast cells show the minimal doubling time of cellular growth. Further study is required to determine the role of integrin and extracellular matrix components in response to flow-mediated cellular retention.

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REFERENCES

1. J. S. Budd, K. E. Allen, and P. R. F. Bell, "*Effects of two methods of endothelial cell seeding on cell retension during blood flow*", British Journal of Surgery, vol. 87, pp. 878-882, 1991.
2. J. E. Rosenman, R. F. Kempczinski, W. H. Pearce, and E. B. Silberstein, "*Kinetics of endothelial cell seeding*", Journal of Vascular Surgery, vol. 2, pp. 778-784, 1985.
3. G. Ciapetti, E. Cenni, L. Pratelli, and A. Pizzoferrato, "*In vitro evaluation of cell/biomaterial interaction by MTT assay*", Biomaterials, vol. 14 (5), pp. 359-364, 1993.
4. S. C. Kuo, and D. A. Lauffenburger, "*Relationship between receptor/ligand binding affinity and adhesion strength*", Biophysical Journal, vol. 65, pp. 2191-2200, 1993.
5. E.-L. Florin, V. T. Moy, and H. Gaub, "*Adhesion forces between individual liang-receptor pairs*", Science, vol. 264, pp. 415-417, 1994.
6. M. R. Brunstedt, et al., "*Attachment and proliferation of bovine aortic endothelial cells onto additive modified poly(ether urethane ureas)*", Journal of Biomedical Materials Research, vol. 27, pp. 483-492, 1993.
7. G. A. Truskey, and J. S. Pirons, "*The effect of fluid shear stress upon cell adhesion to fibronectin-treated surfaces*", Journal of Biomedical Materials Research, vol. 24, pp. 1333-1353, 1990.
8. M. A. Rupnick, F. A. Hubbard, K. Pratt, B. E. Jarrell, and S. K. Williams, "*Endothelialization of vascular prosthetic surfaces after seeding or sodding with human microvascular endothelial cells*", Journal of Vascular Surgery, vol. 9, pp. 788-795, 1989.
9. S. K. Williams, and B. E. Jarrell, "*Thrombosis on endothelializable protheses*", Annals of the New York Academy of Sciences, vol. 516, pp. 145-157, 1987.
10. H. Miwa, et al., "*Improved patency of an elastomeric vascular graft by hybridization*", ASAIO Journal, vol. 38, pp. M512-M515, 1992.
11. K. Fujimoto, et al., "*Porous polyurethane tubes as vascular graft*", Journal of Applied Biomaterials, vol. 4, pp. 347-354, 1993.
12. T. Matsuda, M. J. Moghaddam, H. Miwa, K. Sakurai, and F. Ilda, "*Photoinduced prevention of tissue adhesion*", Transaction of American Society of Artificial Internal Organs, vol. 38, pp. M154-M157, 1992.
13. B. R. McAuslan, and G. Johnson, "*Cell responses to biomaterials I: Adhesion and growth of vascular endothelial cell on poly(hydroxyethyl methacrylate) following surface modification by hydrolytic etching*", Journal of Biomedical Materials Research, vol. 21, pp. 921-935, 1987.
14. S. K. Williams, T. Schneider, B. Kapelan, and B. E. Jarrell, "*Formation of a functional endothelium on vascular grafts*", Journal of Electron Microscopy Technique, vol. 19, pp. 439-451, 1991.
15. S. Niu, T. Matsuda, and T. Oka, "*Endothelialization on various segmented polyurethanes: Cellular behavior and its substrate dependency*", Transaction of American Society of Artificial Internal Organs, vol. 36, pp. M164-M168, 1990.
16. N. B. McKeown, et al., "*Facilitation of endothelial cell growth on hydroxylated ePTFE vascular grafts*", Transaction of American Society of Artificial Internal Organs, vol. 37, pp. M477-M478, 1991.
17. S. K. Williams, et al., "*Formation of a multilayer cellular lining on a polyurethane vascular graft following endothelial cell sodding*", Journal of Biomedical Materials Research, vol. 26, pp. 103-117, 1992.
18. J. Gerlach, H. H. Schauwecker, E. Henning, and E. S. Bucherl, "*Endothelial cell seeding on different polyurethanes*", Artificial Organs, vol. 13(2), pp. 144-147, 1989.
19. D. A. Hammer, and D. A. Lauffenburber, "*A dynamic model for receptor-mediated cell adhesion to surfaces*", Biophysical Journal, vol. 52, pp. 475-587, 1987.
20. C. H. Lundgren, M. B. Herring, M. P. Arnold, J. L. Glover, and P. J. Bendick, "*Fluid shear disruption of cultured endothelium: The effect of cell species, fibronectin cross-linking and supporting polymer*", Transaction of American Society of Artificial Internal Organs, vol. 32, pp. 334-338, 1986.
21. F. Moussy, A. W. Neumann, and W. Zingg, "*The forces of detachment of endothelial cells from different solid surfaces*", Transaction of American Society of Artificial Internal Organs, vol. 36, pp. M568-M572, 1990.
22. T. A. Horbett, J. J. Waldburger, B. D. Ratner, and A. S. Hoffman, "*Cell adhesion to a series of hydrophilic-hydrophobic copolymers studied with a spinning disc apparatus*", Journal of Biomedical Materials Research, vol. 22, pp. 383-404, 1988.
23. C. F. Dewey Jr., "*Effects of fluid flow on living vascular cells*", Journal of Biomechanical Engineer-

ing, vol. 106, pp. 31-35, 1984.
24. R. Nerem, "*Cellular engineering*", Annals of Biomedical Engineering, vol. 19, pp. 529-545, 1991.
25. E. A. Jaffe, Culture and identification of large vessel endothelial cells, in *Biology of endothelial cells* E. A. Jaffe, Ed. (Martinus Nijhoff Publishers, Boston, 1984) pp. 1-13.
26. P. Desgranges, M. Tardieu, D. Loisance, and D. Barritault, "*Extracellular matrix covered biomaterials for human endothelial cell growth*, The International Journal of Artificial Organs, vol. 15(12), pp. 727-726, 1992.
27. J. S. Budd, K. E. Allen, P. R. F. Bell, and R. F. L. James, "*The effect of varying fibronectin concentration on the attachment of endothelial cells to polytetrafluoroethylene vascular grafts*, Journal of Vascular Surgery, vol. 12, pp. 126-130, 1990.
28. J. K. Chang, J. Kim, J. H. Kim, D. C. Han, and B. G. Min, Morphological changes of human endothelial cell under high Reynolds number flow, N. A. Langrana, M. H. Friedman, E. S. Groods, Eds., Bioengineering Conference (The American Society of Mechanical Engineers, Breckenridge, Colorado, 1993), vol. BED-24, pp. 285-288.
29. T. G. van Kooten, J. M. Schakenraad, H. C. Van der Mei, and H. J. Busscher, "*Development and use of a parallel-plate flow chamber for studying cellular adhesion to solid surfaces*, Journal of Biomedical Materials Research, vol. 26, pp. 725-738, 1992.
30. E. Sirois, M. F. Cote, and C. J. Doillon, "*Erowth factors and biological supports for endothelial cell lining: in vitro study*, The International Journal of Artificial Organs, vol. 16(8), pp. 609-619, 1993.
31. W. K. Nicholas, D. Gospodarowicz, T. R. Kessler, and D. B. Olsen, "*Incresed adherence of vascular endothelial cells to biomer precoated with extracellular matrix*, Transaction of American Society of Artificial Internal Organs, vol. 27, pp. 208-212, 1981.
32. P. A. Schneider, S. R. Hanson, T. M. Price, and L. A. Harker, "*Durability of confluent endothelial cell monolayers on small-caliber vascular protheses in vitro*, Surgery, vol. 103(4), pp. 456-462, 1988.
33. A. Saterbak, S. C. Kuo, and D. A. Lauffenburger, "*Heterogeneity and probabilistic binding contributions to receptor-mediated cell detachment kinetics*, Biophysical Journal, vol. 65, pp. 243-252, 1993.
34. M. Paulsson, "*Basement membrane proteins : Structure, assembly, and cellular interactions*, Critical Reviews in Biochemistry and Molecular Biology, vol. 27(1), pp. 93-127, 1992.
35. H. M. H. Carr, et al., "*Fibronectin binding to gelatin-impregnated Dacron (Gelseal) protheses*, Artificial Organs, vol. 16(4), pp. 342-345, 1992.
36. G. Conforti, et al., "*Interaction of fibronectin with cultured human endothelial cells : Characterization of the surface receptor*, Blood, vol. 73(6), pp. 1576-1585, 1989.
37. M. D. Ward, and D. A. Hammer, "*A theoretical analysis for the effect of focal contact formation on cell-substrate attachment strength*, Biophysical Journal, vol. 64, pp. 936-959, 1993.
38. C. Wu, J. S. Bauer, R. L. Juliano, and J. A. McDonald, "*The $\alpha_5\beta_1$ integrin fibronectin receptor, but not the α_5 cytoplasmic domain, functions in an early and essential step in fibronectin matrix assembly*, Proceedings of National Academy of Science, vol. 268(29), pp. 21883-21888, 1993.

=국문초록=

오랜 기간동안 이식이 가능한 소구경 인조혈관을 개발하는데 적합한 방법중의 하나가 생체적합성 폴리머 표면에 혈관내피세포 단일층을 형성하는 것이다. 본 연구에서는 폴리우레탄 표면에 세포외기질 단백질중의 한 성분인 파이브로넥틴이나 사람의 섬유아세포에서 분리한 세포외기질을 접착시킨후 혈관내피세포층을 구축하였다. 폴리우레탄 표면에 부착된 혈관내피세포층의 부착능을 평가하기 위해서 층류발생기를 이용하여 세포층에 균일한 크기의 전단응력을 부가한후 세포의 이탈을 고찰하였다. 실험결과, 파이브로 넥틴상에 부착된 혈관내피세포층이 세포 배양 2일 후에 가장 강한 부착능을 나타냄을 알수 있었고, 섬유아세포에서 분리한 세포외기질상에 부착된 혈관내피세포는 가장 빠른 성장속도를 나타내었다.