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The Effect of Fluid Shear Stress on Endothelial Cell Adhesiveness to Modified Polyurethane Surfaces

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Abstract : Generally vascular grafts with a relatively large inner diameter (> 5 mm) have been successfully employed for replacement in the human body. However, the use of small diameter grafts is limited, because these grafts rapidly occlude due to the thrombosis. The ideal blood-contacting surface of a prosthesis would be an endothelial cell (EC) lining, because the confluent monolayer of healthy ECs that culture natural blood vessels represents the ideal nonthrombogenic surface. For vascular graft application, the stable EC adhesion on surface under flow conditions is very important. In this study, the adhesive strength of ECs attached on polymer surfaces coated with collagen type IV (Col IV), fibronectin (Fn), laminin (Ln), and treated with corona was investigated onto polyurethane (PU) films. The EC-attached PU surfaces were mounted on parallel-plate flow chambers in a flow system prepared for cell adhesiveness test. Three different shear stresses (100, 150, and 200 dyne/cm^2) were applied to the flow chambers and each shear stress was maintained for 120 min to investigate the effect of shear stress and surface treatment condition on the EC adhesion strength. It was observed that the EC adhesion strength on the surface-modified PU films was in the order of Ln≅ Fn > Col IV > corona ≫ control. More than 70% of the adhered cells were remained on surface-modified PU surface after applying the shear stress, 200 dyne/cm^2 for 2 hrs, whereas the cells were completely detached on the control PU surface within 10 min after applying the same shear stress. It seems that the type of adsorbed proteins and hydrophilicity onto the PU surfaces play very important roles for cell adhesion strength.

Introduction

Surface-induced thrombosis remains as one of main problems in the development of blood contacting devices. Generally vascular grafts with a relatively large inner diameter (> 5 mm) have been successfully employed for replacement in the human body. However, the use of small diameter graft is limited, because these grafts rapidly occlude due to the thrombosis. The ideal blood-contacting surface of a prosthesis would be an endothelial cell (EC) lining, because the confluent monolayer of healthy ECs that cultures natural blood vessels represent the ideal nonthrombogenic surface. ECs in their resting state maintain a non-thrombogenic surface by various mechanism,

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including release of fibrinolytic factors (tissue-type plasminogen activator) and anti-platelet agent (prostacyclin) and by activation of protein C through thrombomodulin.[1] In the cardiovascular system, the cells are exposed constantly to hemodynamic forces due to the flow of blood. Endothelialization of the luminal surface reduced thrombogenicity of vascular prostheses, but most of the materials used to vascular prostheses are poor substrate for EC attachment under physiological shear stress, particularly those of small diameter.[2,3]

It is generally recognized that the behavior of the adhesion and proliferation of different types of cells on polymeric materials depended largely on the surface characteristics such as wettability (hydrophilicity/hydrophobicity or surface free energy), chemistry, charge, rigidity and topography. A large number of research groups including ours[4] have modified polymer surfaces and investigated the interactions of cultured cells and polymers with different surface properties.

In many case, surfaces supporting the proliferation of adherent cells have been coated with cell-adhesive proteins of polypeptides. Fibronectin (Fn) and vitronectin are well known cell-adhesive proteins existing in the serum. Their effects on cellular adhesion, spreading, and proliferation have extensively been investigated for many years.[5-24] Different types of collagen (I, III, IV, and V),[8-10,12,14,24] laminin (Ln),[10,12] and fibrinogen[8] have also been used to precoat polymer surfaces for improved cell adhesion. Most of these posses a distinct peptide sequence which is recognized specially and reversively by a set of cell-surface proteins, known as integrin, which promote cell-substratum adhesion.[8] Cationic polypeptides such as polylysine[23,24] and other basic polymers[6,7,10,11] have been used to improved cell adhesion since it is recognized that electrostatic interaction plays an influential role between negatively charged cell membrane surfaces and positively charged material surfaces. In our previous study, we compared the effects of protein and polypeptide types coated on the surfaces on the EC adhesion and proliferation behavior.[25] It was observed that the cells were well adhered and proliferated particularly on Col IV, Fn, and Ln adsorbed surfaces among the various proteins and polypeptides adsorbed surfaces.

Most of above studies on cell adhesion and proliferation have been carried out under static conditions. In many biomedical applications, particularly in cardiovascular applications, however, the adhesive strength of cells to a biomaterials is more determinant for the ultimate patency than adhesion or proliferation.[5,26-28] In this study, the adhesive strength of ECs attached on protein-adsorbed and corona-treated PU surfaces was investigated. The EC-attached PU surfaces were mounted on parallel-plate flow chambers in a flow system and different shear stresses were applied to the flow chambers to correlate the relationship of surface property and shear stress with the adhesion strength of ECs. The stable EC adhesion on surface under flow conditions is very important for vascular graft application.

Experimental

Substrates. Fifteen percents of PU (Pellethane®, 2363-80A, Dow Chemical Co., U.S.A) was dissolved in tetrahydrofuran (THF, Sigma Chem. Co., St. Louis, U.S.A). One gram of PU solution was cast onto Pyrex petri dishes (diameter; 100 mm) on a horizontal level in order to get ~200 μm thickness of PU films. After evaporation of the THF at room temperature, samples were cut into 5.0 × 7.0 cm size. The PU films were dried *in vacuo* for 7 days to remove residual THF and ultrasonically cleaned in ethanol. The pieces were stored in a vacuum oven until use.

Protein Adsorption onto PU Surfaces. Proteins, Col IV (human placenta; acid soluble), Fn (bovine plasma; 0.1% solution), and Ln (basement membrane of emgelbreth-holm-swarm mouse sarcoma) for precoating onto the PU films were purchased from Sigma Chem. Co. All the proteins were dissolved and diluted with Dulbecco's phosphate buffered saline (PBS; pH 7.3~7.4, Sigma Chem. Co.) free of Ca^{2+} and Mg^{2+} to make solution with a concentration of 0.1 mg/mL. Two milliliters of the protein solutions were placed into PU sampling holder. After 1 hr incubation at 37°C, the PU surfaces were gently rinsed with 3 mL of PBS twice to remove unadsorbed or weakly

adsorbed proteins, in a manner similar to that used in our previous study.[25]

EC Culture on PU Surfaces. CPAE bovine pulmonary artery ECs (KCLB 10209, Korean Cell Line Bank, Korea) were used to study the effect of surface modification on the EC adhesion strength.

The cells routinely cultured in tissue cultured polystyrene flasks (Corning, U.S.A.) at 37°C under 5% CO_2 atmosphere were harvested after the treatment with 0.25% trypsin (GIBCO Laboratories, U.S.A.). The surface-modified PU films were mounted in similar test chambers (dimension, 5.0 × 7.0 cm) to those described by van Wachem *et al.*[29] The films mounted in the test chambers were equilibrated with pre-warmed (37°C) PBS (pH 7.3~7.4) for 30 min. Then, the cells ($4\times 10^4/cm^2$) were seeded to the film surfaces. The culture medium used was RPMI (Rosewell Park Memorial Institute) 1640. The medium was contained 10% fetal bovine serum (FBS, GIBCO Laboratories), 100 units/mL penicillin (Sigma Chem. Co.), and 100 μg/mL gentamycin sulfate (Sigma Chem. Co.).

The cells were incubated on the surface-modified PU for the adhesion. After 1 day incubation at 37 °C under 5% CO_2 atmosphere, some of the PU surfaces were used for the cell adhesiveness test on parallel-plate flow chambers in a flow system as shown in Figure 1. Some others were examined by a scanning electron microscope (SEM; Model 2250N, Hitachi, Japan). For this, the film surfaces were washed with PBS and the cells attached on the surfaces were fixed with 2.5% glutaraldehyde (GIBCO Laboratories) in PBS for 30 min at room temperature. After through washing with PBS, the cells on the surfaces were dehydrated in ethanol graded series (50, 60, 70, 80, 90, and 100%) for 20 min each and allowed to dry on a clean bench at room temperature. The cell-attached surfaces were gold deposited in vacuum using gold sputter (Emscope, Model SC 500A, U.K.) and examined by a SEM with a tilt angle of 45 degree. Different fields for each section were randomly observed and analyzed. The results for cell adhesion after 1 day were expressed as the average number of cells attached per cm^2 of surface.

Flow Experiment. EC-attached PU surfaces were placed on parallel-plate flow chamber in a flow system for the cell adhesiveness test (Figure 1). The flow chamber consisted of a glass bottom plate, a polymethylmethacrylate (PMMA) top plate with inlet and outlet silicone rubber tubings (inner diameter, 1.5 mm), and silicon rubber gasket (thickness, 1 mm) inserted between the two plates. The effective chamber dimensions are 6.0 × 1.8 cm. EC-attached PU film was placed on the glass bottom plate. The flow chamber was held together by several clips. The flow chamber was mounted on an inverted microscope stage. Cell culture medium in 37 °C water bath was pumped into movable cell culture medium reservoir located above the flow chamber to provide a hydrostatic pressure difference, which can adjust the required flow rate. The shear stress τ_w (dyne/cm^2) was calculated using the following equations[30];

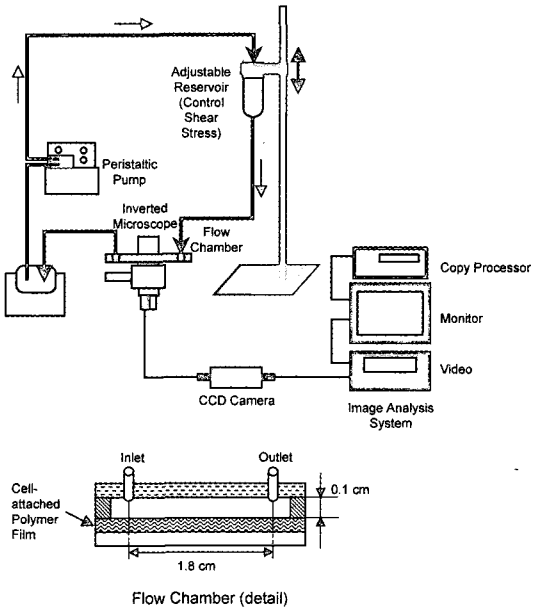

Figure 1. Schematic diagram of flow system for cell adhesiveness test.

$$\tau_w = 6Q\mu/wh^2$$

where Q is the volumetric flow rate (cm^3/sec), μ is the viscosity of the medium (0.1 dyne · sec/cm^2), w is the chamber width (1.8 cm), and h is the chamber height (0.1 cm). Three different shear stresses (100, 150, and 200 dyne/cm^2) were applied to the flow chambers by adjusting the

level of the movable medium reservoir, which are severe conditions than those of blood vessels of human body; physiological levels of venous and arterial shear stresses are 1~5 and 6~40 dynes/cm^2, respectively.[31] Each shear stress was maintained for 120 min to investigate the effect of shear stress on the EC adhesion strength. Under flow conditions the cells attached on the surface-modified PU film were observed through CCD camera attached in the inverted microscope and recorded in a video tape for the image analysis.

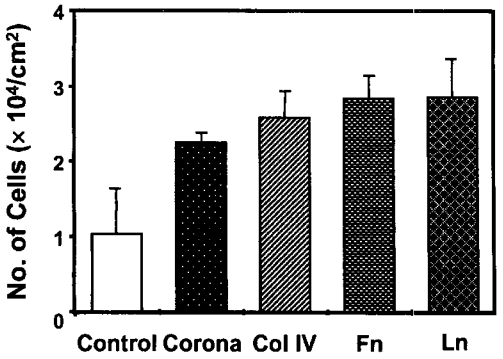

Figure 2. Numbers of endothelial cells adhered on surface-modified PU films(1 day culture).

Results and Discussion

Cell Adhesion on Surface-modified PU Films. Most cells attach poorly to hydrophobic substrates. The cell adhesion onto polymeric substrates is promoted by the adsorption of some serum proteins from the culture medium. We cultured ECs on the corona-treated and protein-adsorbed PU surfaces. The cell density on the PU surfaces was determined at 1 day after seeding 4×10^4/cm^2 cells. As shown in Figure 2, the ECs were adhered much more on the corona-treated and protein-adsorbed PU surfaces than the control PU surface. The cell adhesion after 1 day culture onto the corona-treated and protein-adsorbed PU surfaces varied between about 50 to 75% of the seeded cells, whereas that onto the control PU surface was less than 25%. The EC adhesion on the surface-modified PU films was in the order of Fn ≅ Ln ≅ ColIV > corona ≫ control. The ECs attached onto all the modified PU surfaces were spread well as shown in Figure 3. But the cells attached on control PU surface were not spread. Cells attached on a surface are spread only when they feel compatible on that surface.[4,25] The corona-treated hydrophillic PU surface (water contact angle; 50.3 ± 3.2°) and the protein adsorbed surfaces used in this study seem to have a positive effect of the cell adhesion and spreading. From this result, we expect that other materials such as vascular grafts can be preferably endothelialized by corona treatment or precoating of Col IV, Fn

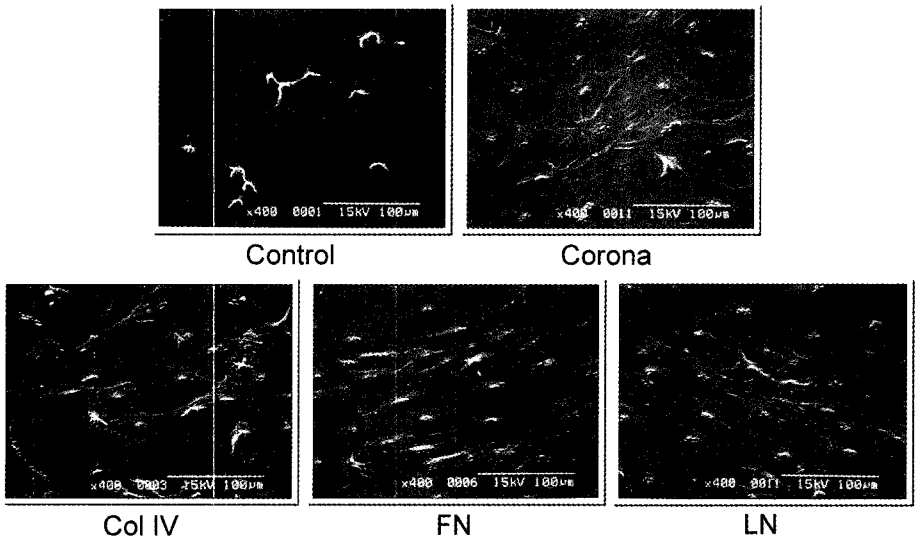

Figure 3. SEM microphotographs of endothelial cells adhered on surface-modified PU films(1 day culture, × 400).

and Ln on their surfaces.

Detachment of Cells from Surface-modified PU Films. After 1 day culture, EC-attached PU surfaces were mounted on parallel-plate flow chambers in a flow system. Three different shear stresses (100, 150, and 200 dyne/cm^2) were applied to the flow chambers and each shear stress was maintained for 120 min. The shear stresses applied on this study are much severe conditions than those of blood vessels of human body; physiological levels of venous and arterial shear stresses are 1~5 and 6~40 dynes/cm^2, respectively, as discussed earlier. We wanted to investigate the effects of shear stress and surface properties on the EC adhesion strength within short times.

Figure 4 shows the results of the cell detachment study on the modified PU surfaces under different shear stresses. The cells were detached more and faster on the control PU surface than on the modified PU surfaces, indicating that the cell adhesion strength is higher on the corona-treated and protein-adsorbed surfaces. More than 60% of the adhered ECs were remained on the modified PU surfaces after applying the shear stress, 200 dyne/cm^2 for 2 hrs, whereas the cells were completely detached on the control PU surface within 10 min after applying the same shear stress. In the control PU, about 40% of the adhered cells were remained at 100 dyne/cm^2 for 2 hrs, while the almost of ECs were detached at 150 and 200 dyne/cm^2 within 10 min. Figure 5 shows the inverted microscope images of ECs adhered on control and Fn-coated PU surfaces with different flowing time at 150 dyne/cm^2 of shear stress. It was observed that almost of ECs were remained on Fn-coated PU surface whereas the almost of the cells were detached on the control surface. Figure 6 shows a series of sequential inverted microscope images of ECs adhered on corona-treated PU surfaces with different flowing time at 200 dyne/cm^2 of shear stress. The morphology of the cells were changed from 60 min, that is to say, the cells were aligned along the flow direction.

The cell adhesiveness onto modified PU surfaces was in the order of Fn ≅ Ln > Col IV > corona ≫ control from Figure 4(c). This phenomenon seems closely related to the cell-adhesive proteins such as Fn and Ln interacted with the cells on the PU surface, providing the increased adhesive strength of the cells during exposure to flow. Also, PU surface was changed to hydrophillic after the corona treatment, resulting in the preferential adsorption of cell-adhesive protein such as Fn and vitronectin from culture medium.[21-24,32] However, it was observed that the cell adhesiveness of corona-treated PU surface was slightly lower than Fn and Ln coated PU surfaces. Other research

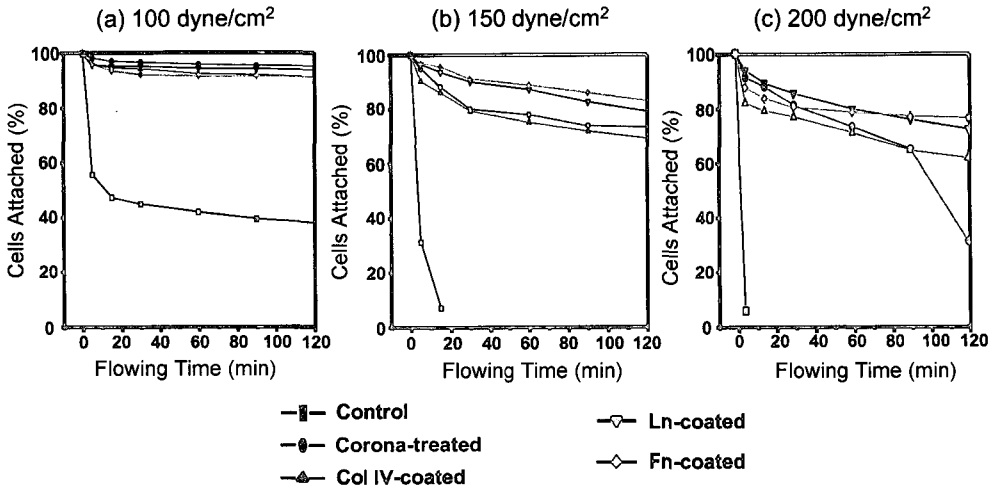

Figure 4. Endothelial cell adhesiveness on the surface modified PU films with different shear stresses (100, 150, and 200 dyne/cm^2).

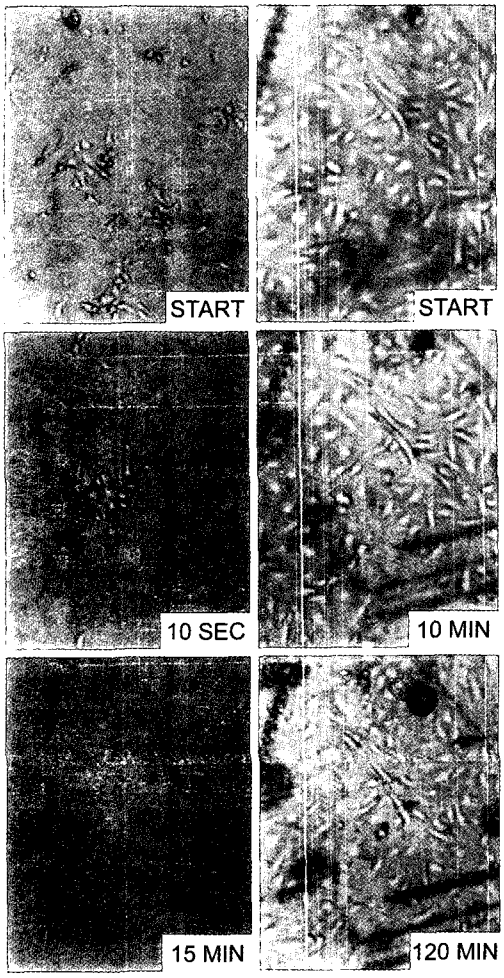

Figure 5. Inverted microscope images of endothelial cells adhered on (A) control and (B) Fn-coated PU surfaces with flowing time (shear stress: 150 dyne/cm^2).

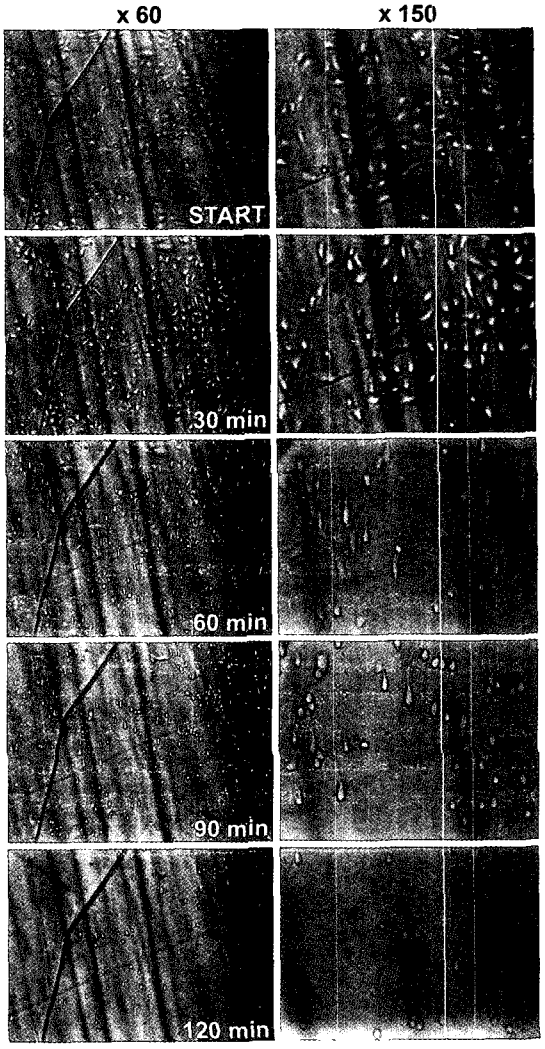

Figure 6. Inverted microscope images of endothelial cells adhered on corona-treated PU surfaces with flowing time (shear stress: 200 dyne/cm^2).

groups have also observed that cell adhesion strength after exposure to flow increased by preadsorbing Fn onto surfaces prior to cell attachement.[5]

Conclusion

It seems that adsorption of cell-adhesive protein and hydrophilic treatment onto the PU surfaces improve cell adhesion strength. From this result, we expect that the materials such as vascular grafts prepared from PU can be pre-ferably endothelialized and improved cell adhesiveness under the flow circumstance by simply preadsorption of Col-IV, Fn and Ln, or corona treatment on their surfaces.

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References

(1) K. Kotte-Marchant, A. A. Veenstra, and R. E. Marchant, *J. Biomed. Mater. Res.*, **30**, 209 (1996).

(2) P. Zilla, *Curr. Opinon Cardiol.*, **6**, 877 (1991).
(3) H. P. Isher and C. W. Tattersall, *J. Biomed. Mater. Res.*, **26**, 1383 (1992).
(4) H. B. Lee and J. H. Lee, *Biocompatibility of Solid Substrates Based on Surface Wettability,* in *Encyclopedic Handbook of Biomaterials and Bioengineering: Part A. Materials,* D. L. Wise, D. J. Trantolo, D. E. Altobelli, M. J. Yaszemski, J. D. Gresser, and E. R. Schwartz (eds.), Marcel Dekker, New York, 1995, Vol. 1, pp 371-398.
(5) J. S. Burmeister, J. D. Vrany, W. M. Reichert, and G. A. Truskey, *J. Biomed. Mater. Res.*, **30**, 13 (1996).
(6) A. Kikuchi, H. Taira, T. Tsuruta, M. Hayashi, and K. Kataoka, *J. Biomater. Sci., Polym. Edn.*, **8**, 77 (1996).
(7) J. G. Steele, B. A. Dalton, G. Johanson, and P. A. Underwood, *Biomaterials,* **16**, 1057 (1995).
(8) D. A. Dimilla, S. M. Albelda, and J. A. Quinn, *J. Colloid Interface Sci.,* **153**, 212 (1992).
(9) J. M. Li, M. J. Menconi, H. B. Wheeler, M. J. Rohrer, V. A. Klassen, J. E. Ansell, and M. C. Apple, *J. Vasc. Surg.*, **15**, 1010 (1992).
(10) A. Schneider, R. N. Melmed, H. Schwalb, M. Karck, I. Vlodavsky, and G. Uretzky, *J. Vasc. Surg.*, **15**, 649 (1992).
(11) J. G. Steele, G. Johnson, W. D. Norris, and P. A. Underwood, *Biomaterials*, **12**, 531 (1991).
(12) G. J. L. Thomson, R. K. Vohra, M. H. Carr, and M. G. Walker, *Surgery,* **109**, 20 (1991)
(13) P. A. Underwood and F. A. Bennett, *J. Cell Sci.*, **93**, 641 (1989)
(14) J. Kaehler, P. Zilla, R. Fasol, M. Deusch, and M. Kadletz, *J. Vasc. Surg.*, **9**, 535 (1989).
(15) P. B. van Wechem, B. W. L. Mallens, A. Dekker, T. Beugeling, J. Feijen, A. Bantjes, J. P. Detmers, and W. G. van Aken, *J. Biomed. Mater. Res.*, **21**, 701 (1987).
(16) K. A. Kesler, M. B. Herring, M. P. Arnold, J. L. Glover, H. M. Park, M. N. Helmus, and P. J. Bendick, *J. Vasc. Surg.*, **3**, 58 (1986).
(17) K. L. Bentley and R. J. Klebe, *J. Biomed. Mater. Res.*, **19**, 757 (1985).
(18) J. M. Seeger and N. Kingman, *J. Surg. Res.*, **38**. 641 (1985).
(19) R. Haas and L. Culp, *J. Cell. Physiol.*, **113**, 289 (1982).
(20) F. Grinnel and M. K. Feld, *J. Biol. Chem.*, **257**, 4888 (1982).
(21) F. Grinnel and M. K. Feld, *J. Biomed. Mater. Res.*, **15**, 363 (1981).
(22) R. J. Klebe, K. L. Bentley, and R. Schoen, *J. Cell. Physiol.*, **109**, 481 (1981).
(23) P. Desgranges, M. Tardieu, D. Loisance, and D. Barritault, *Int. J. Artif. Organs,* **15**, 722 (1992).
(24) K. J. Praat, S. K. Williams, and S. E. Jarrell, *J. Biomed. Mater. Res.*, **23**, 1131 (1989).
(25) J. H. Lee, D. K. Kim, G. Khang, and J. S. Lee, *Biomater. Res.*, **2**, 8 (1998).
(26) T. G. van Gooten, J. M. Schakenraad, H. C. van der Mei, and H. J. Busscher, *Biomaterials,* **13**, 897 (1992).
(27) T. A. Horbett, J. J. Waldburger, B. D. Ratner, and A. S. Hoffman, *J. Biomed. Mater. Res.*, **22**, 383 (1988).
(28) T. G. Ruardy, H. E. Moorlag, J. M. Schakenraad, H. C. van der Mei, and H. J. Busscher, *J. Colloid. Interface Sci.,* **188**, 209 (1997).
(29) P. B. van Wachem, T. G. Beugeling, J. Feijen, A. Bantejes, J. P. Detmers, and W. G. van Aken, *Biomaterials,* **6**, 403 (1985).
(30) R. B. Bird, W. E. Stewart, and E. N. Lightfoot, *Transport Phenomena,* Wiley, New York, 1960, pp 47.
(31) C. K. Zarines, D. P. Giddens, B. K. Bharadvaj, V. S. Sottiurai, R. F. Mabon, and S. Glagov, *Circ. Res.*, **53**, 502 (1992).
(32) J. H. Lee, G. Khang, J. W. Lee, and H. B. Lee, *J. Colloid Interface Sci.,* **205**, 323 (1998)