Macromolecular Research, Vol. 17, No. 7, pp 458-463 (2009)

Fabrication of Endothelial Cell-Specific Polyurethane Surfaces co-Immobilized with GRGDS and YIGSR Peptides

Won Sup Choi, Jin Woo Bae, Yoon Ki Joung, and Ki Dong Park*

Department of Molecular Science and Technology, Ajou University, Suwon 443-749, Korea

Mi Hee Lee and Jong-Chul Park

Department of Medical Engineering, Brain Korea 21 Project for Medical Science, Yonsei University, College of Medicine, Seoul 120-749, Korea

Il Keun Kwon

Department of Oral Biology & Institute of Oral Biology at Graduate School of Dentistry, Kyung Hee University, Seoul 130-701, Korea

Received June 11, 2008; Revised August 11, 2008; Accepted August 12, 2008

Abstract: Polyurethane (PU) is widely used as a cardiovascular biomaterial due to its good mechanical properties and hemocompatibility, but it is not adhesive to endothelial cells (ECs). Cell adhesive peptides, GRGDS and YIGSR, were found to promote adhesion and spreading of ECs and showed a synergistic effect when both of them were used. In this study, a surface modification was designed to fabricate an EC-active PU surface capable of promoting endothelialization using the peptides and poly(ethylene glycol) (PEG) spacer, The modified PU surfaces were characterized *in vitro*. The density of the grafted PEG on the PU surface was measured by acid-base back titration to the terminal-free isocyanate groups. The successful immobilization of peptides was confirmed by amino acid analysis, following hydrolysis, and contact angle measurement. The uniform distribution of peptides on the surface was observed by scanning electron microscopy (SEM) and atomic force microscopy (AFM). To evaluate the EC adhesive property, cell viability test using human umbilical vein EC (HUVEC) was investigated *in vitro* and enhanced endothelialization was characterized by the introduction of cell adhesive peptides, GRGDS and YIGSR, and PEG spacer. Therefore, GRGDS and YIGSR co-immobilized PU surfaces can be applied to an EC-specific vascular graft with long-term patency by endothelialization.

Keywords: polyurethane, GRGDS, YIGSR, endothelial cells, endothelialization.

Introduction

For last decades, polyurethane (PU) has been widely applied for blood-contacting devices such as catheters, stents, artificial hearts, and vascular prosthesis due to its relatively good blood compatibility and mechanical properties.[1-6] However, with undesirable clinical results which are caused by lack of blood compatibility of conventional PU, many researchers have focused on a series of biological events that may occur at PU surface under continuous blood stream. As the results, blood-contacting synthetic biomaterials have been found out to generally induce thrombus formation, which is initiated by absorbed plasma proteins on the surfaces, followed by platelet adhesion and activation along coagulation pathways.[7,8]

*Corresponding Author. E-mail: kdp@ajou.ac.kr

Based upon these phenomena at the interface, various approaches in terms of blood compatibility have been readily attempted and the progresses for modifying surface properties of PU include (1) chemical modification by the grafting of hydrophilic components, like poly(ethylene glycol) (PEG) or biomembrane structure;[4,9-11] (2) surface modification by incorporating bioactive agents such as fibrolytic enzymes (t-plasminogen activator, urokinase), various prostaglandins (PGE_1), and potent anticoagulant (heparin and hirudin), through either physical or chemical coupling[9,12-15] and (3) biological modification using protein or endothelial cells (ECs) seeding.[16,17] Among them, the most promising one can be a biomimetic approach that takes advantage of the highly thromboresistance of EC layer, which presents as the inner surface of the natural vessel wall that constitutionally perform a regulatory role in hemostasis. Thus, ECs seeding technologies can improve blood compatibility of PU by creat-

ing a new micro-environment of endothelium on the surface.

For effective ECs attachment and proliferation as the initial stage for endothelialization, a variety of methods have been developed until now and the use of EC-adhesive molecules that are derived from extracellular matrix (ECM) by ECs is preferentially adopted.[18,19] Arg-Gly-Asp (RGD) peptide was found to be the major functional amino acid sequence, presenting in all major ECM proteins and it has been known to be associated with the attachment and spreading of ECs.[20-23] As an another EC-adhesive molecule, laminin derived Tyr-Ile-Gly-Ser-Arg (YIGSR) peptide was often used for promoting endothelialization.[24,25]

As previously mentioned, PEG has been frequently utilized to improve blood compatibility of biomaterials because it has high dynamic motions, a low interfacial free energy and non-adhesive property.[10,11,26] Other results demonstrate that PEG can provide more opportunities for binding with biological matters such as proteins or cells due to the high mobility on water interface if it can act as the spacer arm with a biofunctionality.[9,27,28]

In this study, PU surface was firstly modified with PEG as a spacer for an effective EC activity, and then two kinds of ECs-adhesive peptides (GRGDS and YIGSR) were chemically immobilized. With surface characterizations of the modified PU, EC activities on these surfaces were investigated *in vitro*.

Experimental

Materials. Polycarbonate urethane (PU, Bionate 90A) was supplied by The Polymer Technology Group Inc (Berkeley, CA). Hexamethylene diisocyanate (HMDI) (Aldrich, Milwaukee, WI) was distilled under a reduced pressure before use. Poly(ethylene glycol) (PEG, Mw = 1 kDa) (Sigma, St. Louis, MO) was dissolved in chloroform, precipitated in diethyl ether, and dried under a reduced pressure at room temperature. Anhydrous *N,N*-dimethylacetamide (DMAc), methyl ethyl ketone (MEK), dibutyltin dilaurate (DBTDL), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Aldrich (Milwaukee, WI). Cell adhesive peptides, such as GRGDS and YIGSR were purchased from Chem-Impex International Inc (Wood Dale, IL). Other reagents and solvents were used without further purification.

Preparation of Aminated PU Surfaces. PU was dissolved in DMAc, precipitated in water, dried and extracted with methanol for 24 h to remove the low molecular weigh components. And then PU was completely dried under the reduced pressure. The purified PU was dissolved in DMAc: MEK (1:2) at 20% (w/v) and cast on teflon mold at 60 °C and dried under the reduced pressure for 48 h. The thickness of PU film was measured by a digital caliper (0.2 mm approximately).

Aminated PU surface was prepared as follows. Firstly, HMDI (0.67 g, 4 mmol) was dissolved in toluene and PEG (2 g, 2 mmol) solutions were added dropwisely. The reaction was carried out under N_2 gas at 60 °C for 3 h and then HMDI-PEG-HMDI was purified through repeated precipitation in diethyl ether. HMDI-PEG-HMDI was grafted onto the PU surface through the allophanate reaction between the urethane urea-nitrogen proton and the terminal isocyanate group of isocyanate-derivatized PEG in the presence of DBTDL with 0.1 v/v% in toluene at 50 °C. And then, PEG grafted films (PU-PEG-NCO) were thoroughly washed with dried toluene. Finally, the PU-PEG-NCO films were soaked into distilled water for 4 h, allowing for converting terminal isocyanate groups into amine groups. The molar amount of the grafted PEG was measured by acid-base back titration of free isocyanate groups on the surface, prior to the preparation of PU-PEG-NH_2.

Surface Immobilization of Cell Adhesive Peptides. Cell adhesive peptides (GRGDS and YIGSR) were chemically immobilized onto the aminated PU surface with PEG spacer. Carboxyl groups of peptides were activated by using EDC and NHS at a room temperature for 10 min (molar ratio of Pep-COOH: EDC: NHS = 1: 1.2: 0.5). The peptides solution (0.1 μmol) was added into 40 mL of distilled water, which contained PU film (3×2 cm^2), and then gently agitated at a room temperature for 24 h. The modified film was thoroughly rinsed with distilled water and dried under the reduced pressure for 48 h. The schematic procedure for peptide immobilized PU was described in Figure 1.

Surface Analysis. The surface morphology of the modified PU films was observed by a scanning electron microscopy (SEM) (JSM-6380, JEOL, Ltd., Tokyo, Japan) after sputter-coating with gold and an atomic force microscopy

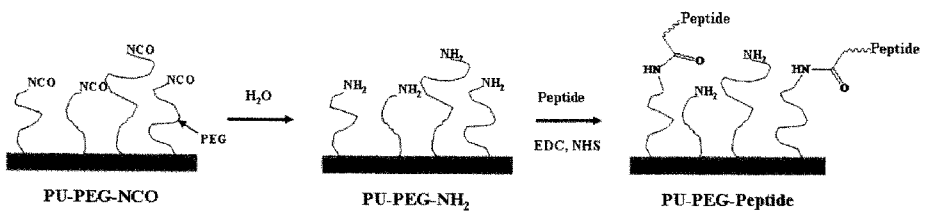

Figure 1. Preparation of PU-PEG-peptide: covalently bind peptide to an aminated polymer surface using ECD and NHS as coupling agents.

(AFM) (PSI, AutoProbe CP), equipped with a 50 μm piezoelectric scanner. AFM images were obtained in non-contact mode with a NCHR cantilever (resonance frequency at approximately 310 kHz). The surface wettability of the series of PU films was evaluated by measuring the static contact angles toward deionized water using the sessile drop method with a goniometer (GBX, Scientific Instrument, Romans, France).

Amino Acid Analysis. The amount of peptides on the modified PU film was quantitatively analyzed by amino acid analysis (AAA).[29,30] The film was placed into a glass tube, added 1 mL of 6 N HCl, and vacuum sealed. The tube was hydrolyzed at 110 °C for 24 h. Free amino acids were analyzed by a high-performance liquid chromatograph (HPLC, Hewlett Packard 1100 Series, Waldbom, Germany), equipped with a reverse-phase column (Waters symmetry C18).

***In vitro* Cell Culture.** Human umbilical vein endothelial cells (HUVEC, Cambrex Bioscience, Walkersville, MD) were seeded and cultured in the dish in an endothelial cell basal medium-2 (EBM-2, Clonetics, Walkersville, MD) supplements with 2% fetal bovine serum (Lonza), 1% antibiotic antimycotic solution (Sigma) and EC growth supplements (Sigma) at 37 °C in a humidified 5% CO_2 atmosphere. The modified PU films (Diameter: 0.9 cm), which sterilized under an ethylene oxide (EO) gas prior to use were placed a 48-well tissue culture plate (Corning, NY). Culture wells were inoculated 0.5 mL growth medium containing of the HUVECs (1×10^5). The attachment and proliferation of cells on each film were measured by the mitochondrial dependent reduction of 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT reagent, Sigma, St. Louis, MO) to formazan. After cell culture for 3 days, 50 μm of MTT reagent was added to the samples. After 4 h of incubation at 37 °C in humidified atmospheres of 5% CO_2, the produced formazan crystals were dissolved in 200 mL of dimethyl sulfoxide and the solution was transferred to a 96 well plate (Falcon, NJ, USA). The absorbance of the resulting solution was measured using an ELISA reader (Spectra Max 340, Molecular Device Co., Sunnyvale, CA, USA) at a wavelength of 570 nm. The absorbance is directly proportional to the mitochondrial activity which is related to the number of living cells present in the culture wells.

Statistical Analysis. All the quantitative values were expressed as a mean ± standard deviation. Statistical analysis was carried out using a student's T-test ($p < 0.05$).

Results and Discussion

Surface immobilization of EC adhesive peptides onto PU has been established for improving its blood compatibility through the induction of endothelial layer that prevents spontaneous blood clotting in the blood vessel. To accomplish this strategy, PU surface was firstly functionalized by using the allophanate reaction between secondary amine groups and diisocyanates as previously mentioned, and the molar amount of residual isocyanates (PU-PEG-NCO) on the surface was about 8.2×10^{-7} mol/cm^2.

Table I. Surface Characterization of Treated or Non-Treated PU Film

Surfaces	Peptide Density[a] (nmol/cm^2)	Contact Angle[b] (degree)	Roughness (*Ra*)[c] (nm)
PU		56.2±2.3	3.3±0.3
PU-PEG		35.3±1.2	4.7±0.4
PU-PEG-GRGDS	0.39	34.2±1.7	11.0±1.2
PU-PEG-YIGSR	0.53	33.1±2.1	10.9±0.9
PU-PEG-GRGDS/YIGSR	0.32/0.47	34.4±1.9	11.2±1.7

[a]Measured by an amino acid analysis. Free amino acids were analyzed by a HPLC. [b]Saturated water contact angle was measured by a Sessil Drop method (mean ± S.D., n=10). [c]Values of roughness parameter (*Ra*) for the surfaces was measured by scanning mode of an atomic force microscopy (mean ± SD., n=5).

The surface characteristics of the modified PU were summarized in Table I. It has been reported that EC behaviors on the polymeric surface with EC adhesive peptides depend on the surface density of immobilized peptides, which resulted in the enhanced EC activity when it ranged 0.1 to 0.2 nmol/cm^2.[24,31] Accordingly, the surface density of peptides on PU-PEG surface has been considered and each density of GRGDS and YIGSR were measured to be 0.39 and 0.53 nmol/cm^2, respectively. In the case of co-immobilization of these peptides, the similar values were obtained by the feed amounts of peptides.

EDC/NHS coupling chemistry was carried out according to previously reported references. Of course, the reaction between peptides could be occurred, but the reaction could be minimally diminished by the control of the reaction time, feed ratios of reagents, and order of reagent addition. For example, the end of PEG chain was firstly activated with EDC/NHS before adding peptides.

Although the surface immobilization of cell adhesive peptides has been known to play a crucial role for *in vitro* and *in vivo* cellular responses, various parameters such as hydrophilicity/hydrophobicity and the morphological changes can also affect to cell attachment and proliferation. The contact angle of PU-PEG surface showed a decreased value due to grafting of hydrophilic PEG chains, compared to PU control. After the immobilization of peptides, they did not change significantly in contact angles. There have been some results that hydrophilicity of polymeric surfaces with RGD or YIGSR peptide increased by the presence of ionic peptide molecules on the surface.[24,32] However, the grafted PEG spacer was much more hydrophilic than the peptides which are coupled to PEG spacer.

To characterize the surface disturbance of the modified PU films, the morphology of films was observed by SEM

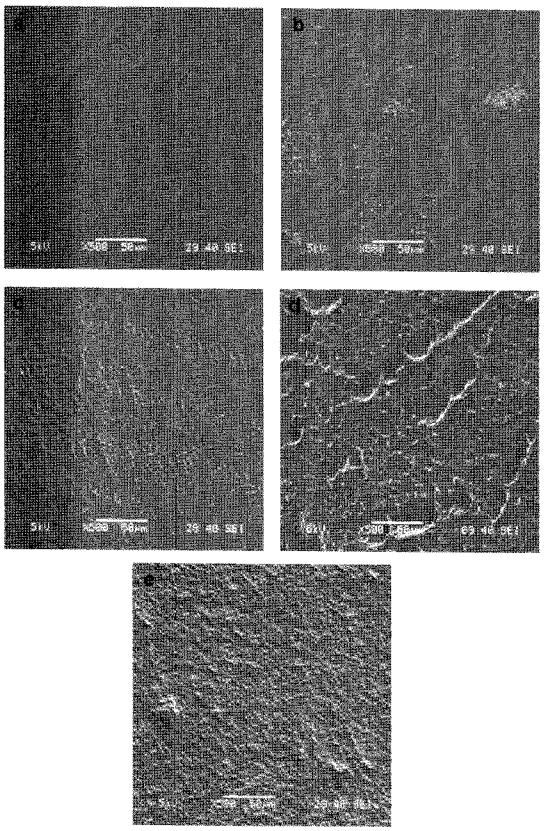

Figure 2. SEM micrographs of the modified PU surfaces. (a) PU, (b) PU-PEG, (c) PU-PEG-GRGDS, (d) PU-PEG-YIGSR, and (e) PU-PEG-GRGDS/YIGSR.

and AFM. As shown in Figure 2, SEM micrograph for the PU control film was smooth but some ripples were observed after grafting of PEG and peptides onto the PU. For further investigating their surface disturbance, AFM topographies in non-contact mode were shown by three-dimensional (3-D) images of films and *Ra* values as a parameter for surface roughness were presented (Figure 3). The *Ra* value of PU-PEG surface increased, indicating that its roughness is higher than that of the non-treated film. After surface immobilization of peptides, the *Ra* value significantly increased as compared to PU-PEG (Table I). However, there were no significant differences in *Ra* results between two kinds of peptides due to their similarities with both surface density and molecular weights.

There have been attempts to introduce the ECM-derived proteins (fibronectin, vitronectin, laminin) or specific peptide sequences onto various surfaces for higher cytocompatibility and cell adhesion. Since the finding of the signaling domains that are composed of several amino acids along the long chain of ECM proteins, the short peptide fragments, like RGD and YIGSR, have been used for surface modification in numerous studies. The RGD sequence is derived from fibronectin and laminin, whereas, the YIGSR sequence is derived from only β chain of laminin. Therefore, we hypothesized that as all ECM proteins play a role in cell-ECM interactions, more than one peptide sequences are required and complementary for cell-materials interactions. For example, when RGD and YIGSR were combined, it is possible that the rate of assembly and disassembly of the focal adhesion sites might have been influenced by the YIGSR-driven association of laminin binding protein with the RGD, focal adhesion sites.

The early attachment and the proliferation tests of EC were performed in order to investigate the effect of immobilized peptides on surface affinity of EC. The effects of cell adhesive peptides (GRGDS and YIGSR) immobilized onto the PU surfaces on the adhesion and proliferation (1 and 3 days, respectively) of HUVECs were analyzed with MTT assay. The attachment of HUVECs depending on modified PU surfaces was shown in Figure 4. The attached cell numbers on all peptides-immobilized PU surfaces increased as comparing to that on PU surface at early stage of incubation (4 h). This result indicates that modified surface environments affect the early attachment of HUVECs, mainly attributed to the presence of immobilized peptides. Figure 5 shows the surface-dependent HUVEC proliferation at 1 and 3 day incubations. Peptides-immobilized PU surfaces had higher HUVECs viability than those of unmodified PU surface at both incubation times. Especially, there was significantly increase in HUVECs population on GRGDS grafted PU-PEG surface at 1 day. The presence of the tripeptide RGD sequence, and its critical role in the cell-attachment process were found in many adhesion plasma and extracellular matrix proteins, such as von Willebrand factor, vitronectin, fibronectin, and collagen.[33] In addition, there are a lot of researches that the peptide have an important part of mediating cell attachment and spreading.[34,35]

It is well established that high attachment of cell is an evident prerequisite for a number of important cell-function processes, such as cell proliferation or cell migration.[33,36] In this study, however, YIGSR grafted PU-PEG surfaces increased much more than GRGDS modified PU-PEG surface at 3 days, although lower cell attachment rate. Interestingly, the combination of GRGDS plus YIGSR was found to significantly increase in HUVEC proliferation rate relative to GRGDS and YIGSR alone at 3 days.

The major extracellular matrix proteins (laminin, fibronectin and collagen) that directly interact with cells have been found to contain several distinct and different regions.[37] Fibronectin molecules have different cellular and ECM protein binding capabilities that can bind to collagen, glycosaminoglycans and also to cells through a RGD sequence.[38] On the other hand, laminin contains RGD in the α helix and YIGSR in the β sheet which facilitate cellular attachment.[39]

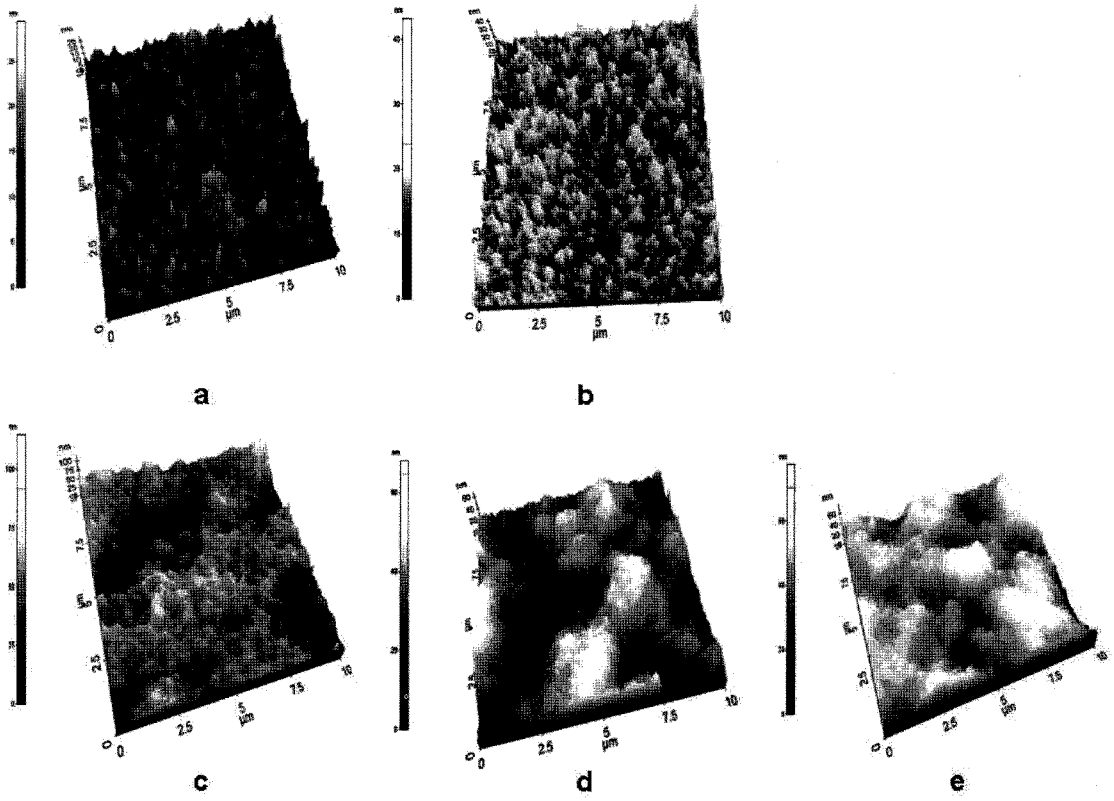

Figure 3. AFM 3-D images of the modified surfaces. (a) PU, (b) PU-PEG, (c) PU-PEG-GRGDS, (d) PU-PEG-YIGSR, and (e) PU-PEG-GRGDS/YIGSR.

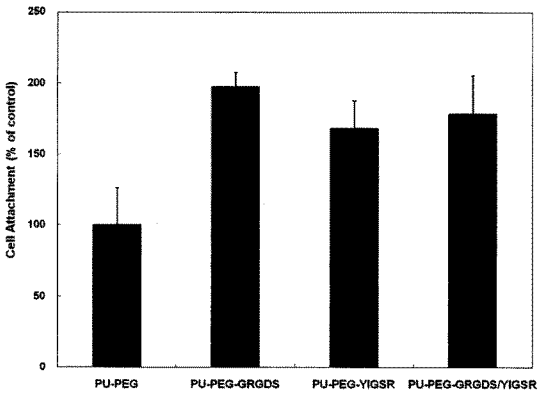

Figure 4. Relative numbers of HUVECs attached on surfaces of peptide-modified PU films after incubation for 4 h. Mean ± S.D ($n = 4, p < 0.05$).

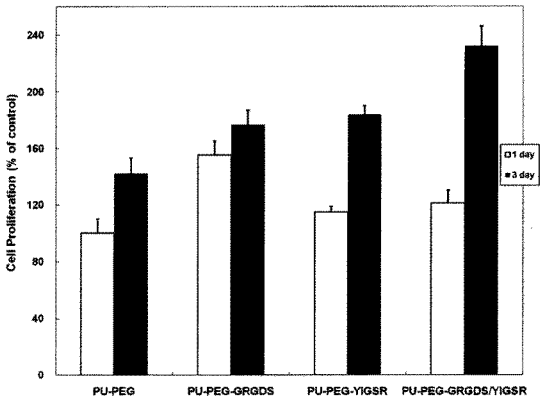

Figure 5. Time-dependent number of cells (HUVECs) adhered on a series of peptide modified PU films. Mean ± S.D ($n = 4, p < 0.05$).

And YIGSR has been shown to mediate attachment and spreading on a laminin substrate in endothelial cells,[40] hepatocytes,[41] and melanoma cells.[42] It is, therefore, reasonable to assume that combination of these two peptides will be needed to support increased biological activity of HUVEC to varying degrees.

There are some researches that introduction of hydrophilic PEG spacer onto surfaces hindered rather helped peptide or cell adhesion. In our previous study, peptides were

modified after chemically grafting with hydrophilic PEG and the same tendency also exhibited. The adhesion rate of HUVECs for the PEG-PU surface is lower than that of no hydrophilic group grafted PU surface. In proliferation result, however, the result for PEG modified PU surface was much higher than unmodified surface after 3 days (data not shown). As stated above, the advantage of using PEG onto surface include high mobility that can act as the spacer arm with a biofunctionality so provide more opportunities for binding with proteins or cells was conformed.

Conclusions

In this study, bioactive polyurethane surface was prepared by chemically immobilizing peptides which enable its cell compatibility to improve simultaneously. Our results demonstrated that PU surfaces were successfully modified with peptides (GRGDS and YIGSR) for enhancing endothelialization. These bioactive PU surfaces revealed higher HUVEC activities than unmodified PUs *in vitro*. In addition, the combination of GRGDS plus YIGSR was found to synergistically increase HUVEC proliferation rates compared to single peptide immobilized surfaces. Therefore, peptides immobilized PU surfaces as EC-specific material can be applied to various cardiovascular devices, including a novel vascular graft with long-term patency.

Acknowledgement. This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A050082).

References

(1) D. I. Cha, K. W. Kim, G. H. Chu, H. Y. Kim, K. H. Lee, and N. Bhattarai, *Macromol. Res.*, **14**, 331 (2006).
(2) K. H. Lee, B. S. Lee, C. H. Kim, H. K. Kim, K. W. Kim, and C. W. Nah, *Macromol. Res.*, **13**, 441 (2005).
(3) A. V. Raghu, H. M. Jeong, J. H. Kim, Y. R. Lee, Y. B. Cho, and K. Sirsalmath, *Macromol. Res.*, **16**, 194 (2008).
(4) H. J. Yoo and H. D. Kim, *Macromol. Res.*, **16**, 596 (2008).
(5) J. K. Yun, H. J. Yoo, and H. D. Kim, *Macromol. Res.*, **15**, 22 (2007).
(6) H. D. Park, J. W. Bae, K. D. Park, T. Ooya, N. Yui, J. H. Jang, D. K. Han, and J. W. Shin, *Macromol. Res.*, **14**, 73 (2006).
(7) M. Tirrell, E. Kokkoli, and M. Biesalski, *Surface Science*, **500**, 61 (2002).
(8) J. H. Lee, Y. M. Ju, and D. M. Kim, *Biomaterials*, **21**, 683 (2000).
(9) K. D. Park, A. Z. Piao, H. Jacobs, T. Okano, and S. W. Kim, *J. Polym. Sci. Part A: Polym. Chem.*, **29**, 1725 (1991).
(10) K. D. Park, Y. S. Kim, D. K. Han, Y. H. Kim, E. H. Bae, H. S. Lee, and K. S. Choi, *Biomaterials*, **19**, 851 (1998).
(11) Y. H. Kim, D. K. Han, K. D. Park, and S. H. Kim, *Biomaterials*, **24**, 2213 (2003).
(12) K. Doi and T. Matsuda, *J. Biomed. Mater. Res.*, **34**, 361 (1997).
(13) H. S. Yang, K. Park, and J. S. Son, *Macromol. Res.*, **15**, 256 (2007).
(14) C. M. Jung, Y. C. Bae, and J. J. Kim, *Macromol. Res.*, **15**, 682 (2007).
(15) C. Y. Tan and Y. H. Kim, *Macromol. Res.*, **16**, 481 (2008).
(16) J. Guan, M. S. Sacks, E. J. Beckman, and W. R. Wagner, *J. Biomed. Mater. Res.*, **61**, 493 (2002).
(17) C. Fields, A. Cassano, C. Allen, A. Meyer, K. J. Pawlowski, G. L. Bowlin, S. E. Rittgers, and M. Szycher, *J. Biomat. Appl.*, **17**, 45 (2002).
(18) J. S. Park, J. M. Kim, and S. J. Lee, *Macromol. Res.*, **15**, 424 (2007).
(19) S. J. Lee, Y. Son, and C. H. Kim, *Macromol. Res.*, **15**, 348 (2007).
(20) Y. S. Lin, S. S. Wang, T. W. Chung, Y. H. Wang, S. H. Chiou, J. J. Hsu, N. K. Chou, K. H. Hsieh, and S. H. Chu, *Artificial Organs*, **25**, 617 (2001).
(21) S. P. Massia and J. Stark, *J. Biomed. Mater. Res.*, **56**, 390 (2001).
(22) D. A. Wang, J. Ji, Y. H. Sun, J. C. Shen, L. X. Feng, and J. H. Elisseeff, *Biomacromolecules*, **3**, 1286 (2002).
(23) M. S. Bae, K. Y. Lee, and Y. J. Park, *Macromol. Res.*, **15**, 469 (2007).
(24) H. W. Jun and J. West, *J. Biomater. Sci.-Polym. E.*, **15**, 73 (2004).
(25) E. Genove, C. Shen, S. Zhang, and C. E. Semino, *Biomaterials*, **26**, 3341 (2005).
(26) O. H. Kwon, Y. C. Nho, K. D. Park, and Y. H. Kim, *J. Appl. Polym. Sci.*, **71**, 631 (1999).
(27) K. M. Park, Y. K. Joung, and K. D. Park, *Macromol. Res.*, **16**, 517 (2008).
(28) Y. J. Jun, K. M. Park, Y. K. Joung, S. J. Lee, and K. D. Park, *Macromol. Res.*, in press.
(29) M. J. Ernsting, G. C. Bonin, M. Yang, R. S. Labow, and P. Santerre, *Biomaterials*, **26**, 6536 (2005).
(30) J. A. Neff, K. D. Caldwell, and P. A. Tresco, *J. Biomed. Mater. Res.*, **40**, 511 (1998).
(31) C. C. Larsen, F. Kligman, K. K. Marchant, and R. E. Marchant, *Biomaterials*, **27**, 4846 (2006).
(32) J. J. Yoon, S. H. Song, D. S. Lee, and T. G. Park, *Biomaterials*, **25**, 5613 (2004).
(33) K. C. Dee, T. T. Andersen, and R. Bizios, *Mater. Res. Soc. Symp. Proc.*, **331**, 115 (1994).
(34) E. Ruoslahti and M. D. Pierschbacher, *Science*, **238**, 491 (1987).
(35) S. E. Dsouza, M. H. Ginsberg, and E. F. Plow, *Trend. Biol. Sci.*, **16**, 246 (1991).
(36) S. M. Sagnella, F. Kligman, E. H. Anderson, J. E. King, G. Murugesan, R. E. Marchant, and K. K. Marchant, *Biomaterials*, **25**, 1249 (2004).
(37) Y. Yamada and H. K. Kleinman, *Curr. Opin. Cell. Biol.*, **4**, 819 (1992).
(38) J. Labat-Robert, *Pathologie et Biologie*, **51**, 563 (2003).
(39) K. M. Malinda and H. K. Kleinman. *Int. J. Biochem. Cell Biol.*, **28**, 957 (1996).
(40) D. S. Grant, K. Tashiro, B. Segui-Real, Y. Yamada, G. R. Martin, and H. K. Kleinmam, *Cell*, **58**, 933 (1989).
(41) B. Clement, B. Segui-Real, P. Savagner, H. K. Kleinman, and Y. Yamada, *J. Cell. Biol.*, **110**, 185 (1990).
(42) Y. Iwamoto, J. Graf, M. Sasaki, H. K. Kleinmam, D. R. Greatorex, G. R. Martin, F. A. Robey, and Y. Yamada, *J. Cell. Physiol.*, **134**, 287 (1988).