Modification of Polyurethaneurea with PEG and YIGSR Peptide to Enhance Endothelialization Without Platelet Adhesion

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Abstract: Improved endothelialization without platelet adhesion is essential to enhance the long-term patency of synthetic vascular grafts and other blood-contacting devices. We have developed a dually modified polyurethaneurea by incorporating endothelial cell adhesive YIGSR peptide sequences as chain extenders and nonthrombogenic PEG as a soft segment (PUUYIGSR-PEG) in the polymer backbone. PUUYIGSR-PEG was successfully synthesized and characterized by proton NMR, FTIR, GPC, DSC, ESCA, and contact angle measurement. Despite having similar molecular weight, the peptide/PEG-modified polyurethaneurea (PUUYIGSR-PEG) showed superior mechanical properties compared to the control PEG-modified polyurethaneurea (PUUPPD-PEG). Virtually no platelet adhesion was observed on PUUYIGSR-PEG, while endothelial cell adhesion, spreading, and migration were significantly greater on PUUYIGSR-PEG compared to PUUPPD-PEG. Thus, this bioactive polymer may be an appropriate biomaterial for small diameter vascular grafts. © 2004 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 72B: 131–139, 2005

Keywords: vascular graft; endothelialization; polyurethaneurea; YIGSR; PEG

INTRODUCTION

Endothelialization has been proposed to improve the long-term patency of synthetic vascular grafts, as well as the performance of many cardiovascular devices.[1,2] The success of endothelialization depends on the adhesion of endothelial cells, retention of those endothelial cells under shear, and proliferation and migration of endothelial cells on the biomaterial surfaces. Modifications of biomaterials with adhesive peptide sequences such as YIGSR have shown improved attachment, spreading, and resistance of endothelial cells against shear stress.[3–6] In a previous study,[6] we synthesized a bioactive polyurethaneurea by incorporating GGGYIGSRGGGK peptide sequences into the polymer backbone. Improved endothelial cell adhesion, proliferation, migration, and extracellular matrix production were observed. However, although the incorporation of the YIGSR peptide did not enhance platelet adhesion, platelet adhesion to the polyurethane urea was significant. This will likely limit its clinical impact. Polyethylene glycol (PEG) has been an attractive biomaterial due to its resistance to protein adsorption, platelet adhesion, and bacterial adhesion.[7–9,16] These properties are believed to be caused by low interfacial surface energy, high chain motility, and molecular chain conformation.[8,10] To improve biocompatibility, various efforts have been made to introduce PEG into biomaterials by grafting,[9,11–13] physical blending,[14] covalent attachment to the surface mediated by proteins,[15,16] and synthesis of copolymers.[17] Covalent incorporation of PEG into polyurethaneureas as at least a portion of the soft segment is desirable for long-term stability and three-dimensional fabrication of a nonthrombogenic biomaterial.

In this study, we developed a YIGSR peptide/PEG-modified polyurethaneurea by incorporation of GGGYIGSRGGGK peptide sequences as a chain extender and PEG as a soft segment in the polymer backbone. The effects of the incorporated bioactive peptide sequences and hydrophilic PEG chain on adhesion of cells and platelets as well as bulk mechanical properties were evaluated.

MATERIALS AND METHODS

Synthesis of Polyurethaneurea (PUUPPD)

Prepolymer was synthesized by reacting methylene di(*p*-phenyl isocyanate) (MDI; Aldrich chemical Co., Milwaukee, WI) with poly(tetramethylene oxide) (PTMO; Aldrich chemical Co., Milwaukee, WI), and then extended with *p*-phenylene diamine (PPD; ACROS, New Jersey).[6] MDI was recrystallized in hexane, and PTMO was dried under vacuum for 48 h. A 10% (w/v) solution of MDI (4 mmol, MW: 250 g/mol) in 10 mL anhydrous *N,N*-dimethylformamide (DMF; Aldrich Chemical Co.) was prepared in a 100-mL three-neck

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round flask and stirred at room temperature. A 10% (w/v) solution of PTMO (2 mmol, MW: 2000 g/mol) in 30 mL anhydrous DMF was added, and the mixture was heated to 75°C and held there for 3 h under argon. The reactor was cooled to room temperature before PPD (2 mmol, MW:108 g/mol) in 3 mL anhydrous DMF was added as a chain extender. The polymer solution was then incubated 45°C for 3 h under argon gas. The polymer solution was cooled to room temperature, precipitated in methanol, and dried under vacuum.

Synthesis of PEG-Modified Polyurethaneurea (PUUPPD-PEG)

PTMO and polyethylene glycol (PEG, MW:4,600 g/mol; Aldrich Chemical Co., Milwaukee, WI) were dried for 48 h under vacuum and used as the soft segments (molar ratio of PTMO to PEG = 85/15). Polymer synthesis proceeded exactly as described above but using 85% PTMO/15% PEG mixture to form the soft segments.

Synthesis of YIGSR Peptide/PEG-modified Polyurethaneurea (PUUYIGSR-PEG)

Prepolymer was synthesized by reacting MDI with PTMO/PEG mixture (85/15 molar ratio) as described above and extended with a combination of GGGYIGSRGGGK peptide (Sigma-Genosys, Woodlands, TX) and PPD. Prepolymer was synthesized using a 10% (w/v) solution of MDI (1.5 mmol), PTMO (0.64 mmol), and PEG (0.11 mmol) at 75°C for 3 h under argon gas as above. Next, chain extension was performed using GGGYIGSRGGGK peptide (0.14 mmol) and PPD (0.61 mmol) in anhydrous DMF at 45°C for 3 h under argon. The polymer solution was cooled to room temperature, precipitated in ethyl acetate, and dried under vacuum.

Polymer Characterization

PUUPPD, PUUPPD-PEG, and PUUYIGSR-PEG were characterized via ^{1}H NMR using a 400-MHz NMR spectrometer (Advance 400, Bruker, Germany) in *N,N*-dimethylformamide-d_7 (DMF-d_7; Aldrich Chemical Co.). Molecular weight distributions were obtained by GPC with UV and evaporative light scattering detectors (Polymer Laboratories, Amherst, MA). Samples for GPC analysis were dissolved in HPLC-grade DMF at a concentration of 1 mg/mL and run at 70°C through PLgel 5-μm Mixed-C columns (Polymer Laboratories, Amherst, MA) at a flow rate of 1 mL/min. Calibration was performed using polystyrene standards (PS; Polymer Laboratories). DSC thermagrams were obtained using a Differential Scanning Calorimeter (Pyris 1, PerkinElmer, Wellesley, MA). Samples were cooled below −60°C and increased at 10°C/min to 300°C under nitrogen gas. Glass transition temperatures and melting temperatures were analyzed using Pyris software. Fourier transform infrared (FTIR) spectroscopy was performed using a Nicolet 500 spectrometer. Thin films of polyurethaneurea were prepared by mixing samples with KBr and pressed into pellets under vacuum. Sixteen scans were taken of each sample at a resolution of 4 cm^{-1}.

Preparation and Characterization of Polyurethaneurea Films

Polymers were dissolved in tetrahydrofuran (THF; 0.3 wt %) and sterilized using 0.2-μm pore size PTFE syringe filters (Whatman, NJ). Polymer films were prepared on glass coverslips (18 mm; Fisher Scientific, PA) by solvent casting at room temperature. Polymer films were held under vacuum for 48 h to ensure removal of the solvent. The equilibrium contact angles of DI water on PUUPPD, PUUPPD-PEG, and PUUYIGSR-PEG films were measured using a contact angle goniometer (CAM-Micro). Six measurements were taken to calculate average contact angles on the surface of each film.

ESCA analysis was performed using a Physical Electronics Model 5700 XPS instrument. Photo-emissions were produced through the use of a X-ray source (1486.6 eV) operated in the fixed retard ratio mode at a pass energy of 23.5 eV. Spectra were acquired over a 10–45° take-off angle range. Charge neutralization was accomplished via bombardment with a low energy beam.

Uniaxial mechanical testing was performed using an Instron model 5565 at a crosshead speed of 25 mm/min with a 5 kN load cell. Polymers were dissolved in DMF (Aldrich Chemical Co.) at 10 wt % and sterilized using 0.2-μm pore size PTFE syringe filters (Whatman, NJ). Polymer films were prepared in Teflon molds by solvent casting at 60°C under vacuum for 48 h. Test specimens were prepared according to ASTMD-638-VI. The tensile strength was calculated from the maximum load at break and the initial cross-sectional area of the specimen. Sample thickness was measured using a digital caliper (Mitutoyo, Hauppauge, NY).

Cell Maintenance

Bovine aortic endothelial cells (BAEC; Clonetics, San Diego, CA), passage 2–5, were used for this study. Dulbecco's Modified Eagle's Medium (DMEM; Sigma Chemical Co., St. Louis, MO) was prepared with 10% fetal bovine serum (FBS; BioWhitaker, Walkersville, MD), 2 m*M* L-glutamine, 1 unit/mL penicillin, and 100 mg/L streptomycin (GPS; Sigma Chemical Co.). Endothelial basal medium (EBM; Sigma Chemical Co.) was prepared with 10% endothelial medium supplement (Sigma Chemical Co., St. Louis, MO), which contained fetal bovine serum, basic fibroblast growth factor, heparin, epithermal growth factor, and hydrocortisone. BAECs were maintained on mixture of EBM and DMEM (25/75 volume ratio) at 37°C in a 5% CO_2 environment.

Adhesion and Spreading of BAECs on YIGSR Peptide/PEG-Modified Polyurethaneurea

To evaluate adhesion of endothelial cells, BAECs were seeded at a concentration of 17,000 cells/cm^2 in eight-well FlexiPerm chambers (Sigma Chemical Co.) attached to polyurethaneurea films. Cells were cultured at 37°C in a 5% CO_2

environment. To evaluate endothelial cell spreading, after 4 h of incubation, nonadherent cells were removed by rinsing, and fresh medium was added. Cells were observed by phase contrast microscopy (Zeiss Axiovert 135), and digital image processing (Scion Image) was used to determine the area per cell. To evaluate cell attachment, the medium was removed by rinsing with PBS, and adherent cells were detached with trypsin and counted using a Coulter Counter (Multisizer 3, Beckman Colulter).

Competitive inhibition of attachment and spreading of endothelial cells was also examined using soluble YIGSR peptides to ensure that improved BAEC adhesion and spreading was due to biospecific interactions with the YIGSR peptides that were incorporated into the polymer structure. Cells were seeded at a concentration of 17,000 cells/cm^2 in eight-well FlexiPerm chambers attached to polyurethaneurea films and incubated with soluble YIGSR peptide (Sigma Chemical Co.) at 0, 0.01, 0.1, and 1 m*M* in EBM/DMEM (25%/75%) at 37°C in a 5% CO_2 environment. After 4-h incubation, attachment and spreading of cells were evaluated as described above.

Migration of BAECs on YIGSR Peptide/PEG-Modified Polyurethaneurea

To assess migration, a fence-style assay was utilized. Polyurethaneurea films were placed in the six-well tissue culture plates, and double-walled round Teflon molds (inner diameter: 6 mm, outer diameter: 17 mm) were placed on top of the films. BAECs were seeded at a concentration of 35,000 cells/cm^2 in the inner walls. After 24 h, the original boundaries were recorded, and the inner walls were removed. Mitomycin C (Calbiochem, San Diego, CA) was added to the medium at 0.5 μg/mL to prevent cell proliferation. After 24 and 48 h of incubation at 37°C in a 5% CO_2 environment, the cells that had migrated over the original boundary were observed using a phase contrast microscope (Zeiss Axiovert 135).

Adhesion of Platelets on YIGSR Peptide/PEG-Modified Polyurethaneurea

PUUPPD-PEG and PUUYIGSR-PEG films were cast on glass coverslips (18 mm; Fisher Scientific, PA) as described above. A solution of 2.5 mg/mL collagen I (Sigma Chemical Co.) solution was prepared in 3% glacial acetic acid. Glass coverslips were incubated with the collagen I solution for 45 min in a humidified environment at room temperature to provide a highly thrombogenic reference material. Blood was obtained from a healthy volunteer with 10 U/mL heparin (Sigma Chemical Co.). Mepacrine (10 μM) (Sigma Chemical Co.) was added to fluorescently label the platelets. Surfaces of Collagen I (positive control), PUUPPD-PEG, and PUUYIGSR-PEG film were incubated with mepacrine-labeled whole blood at 37°C for 20 min and then rinsed with PBS. The number of adherent platelets per field of view (200×) was determined using a fluorescent microscope (Zeiss Axiovert 135, Thornwood, NY).

Statistic Analysis

Data were compared with two-tailed, unpaired *t* tests. *p*-Values less than 0.05 were considered to be significant. Data are presented as mean ± standard deviation.

RESULTS

Synthesis and Characterization of YIGSR Peptide/PEG-Modified Polyurethaneurea

The NMR spectra of polyurethaneurea were obtained and characterized as previously described.[6] The characteristic proton peaks of tyrosine (6.5–7.0 ppm) from the GGGYIGSRGGGK sequence were assigned, indicating the successful incorporation of the peptide sequence into the PUUYIGSR polymer. The peaks of prepolymer, PUUPPD-PEG, and PUUYIGSR-PEG were also assigned and characterized. Based on the number of protons calculated from peak intensities, the peptide concentration in the polymer was approximately 56 μmol/g. The surface peptide concentration of PUUYIGSR-PEG film was also estimated to be approximately 0.2 nmol/cm^2 using the polymer density and the thickness of the monolayer.[6,18–20]

FTIR spectra of the polyurethaneureas were also obtained and characterized. The incorporation of the PEG as a soft segment did not affect FTIR spectra compared to PUUPPD.[6] For both PUUPPD-PEG and PUUYIGSR-PEG, peaks for hard segments were observed at 1640 cm^{-1} (hydrogen-bonded urea carbonyl), at 1720 cm^{-1} (urethane carbonyl peaks for hydrogen bond), and at 1740 cm^{-1} (urethane carbonyl peaks for free bond). The CH stretch peaks of the soft segment, PTMO, appeared at 2850 and 2940 cm^{-1}, and the hydrogen-bonded NH peak also appeared at 3310 cm^{-1}.[6,21,22]

Thermal behaviors from DSC indicate successful incorporation of PEG as a soft segment. The glass transition temperature (T_g) of PUUPPD was observed at 9°C. However, two distinct T_g were observed at 14 and 40°C for PUUPPD-PEG and PUUYIGSR-PEG, likely caused by PTMO and PEG, respectively. The melting point (T_m) of PUUPPD and PUUPPD-PEG was determined about 180°C, and the T_m of PUUYIGSR-PEG was approximately 165°C.

The number-average molecular weight (M_n), the weight-average molecular weight (M_w), and the polydispersity index (PDI) were determined by GPC. PUUPPD-PEG and PUUYIGSR-PEG had similar molecular weights (PUUPPD: M_n = 113,489, M_w = 153,973, PDI = 1.36, PUUPPD-PEG: M_n = 92,645, M_w = 124,072, PDI = 1.34, and PUUYIGSR-PEG: M_n = 96,675, M_w = 116,103, PDI = 1.20).

The surface atomic concentration was determined using ESCA with various take-off angles (PUUPPD-PEG: C = 58.4%, N = 12.1%, O = 30.4% at 10 degree and C = 75.1%, N = 0.4%, O = 24.8% at 45 degree, PUUYIGSR-PEG: C = 58.7%, N = 12.0%, O = 29.0% at 10 degree and C = 73.0%, N = 0.9%, O = 25.9% at 45 degree). Nitrogen (N) and oxygen (O) concentrations are related to the urea linkages of the hard segments and amide linkages of the peptide se-

quences. The higher level of nitrogen and oxygen detected on the PUUYIGSR-PEG at 45 degree indicates that the GGGY-IGSRGGGK peptide sequences were successfully incorporated into the polymer backbone and present at the surface of the material.

Water contact angle measurement demonstrated that the contact angle dropped significantly on PEG-modified polyurethanurea, PUUPPD-PEG (59.6 ± 1.7) and PUUYIGSR-PEG (50.6 ± 1.3), compared to PUUPPD (81.6 ± 1.1). The contact angle of PUUYIGSR-PEG was also significantly lower than that of PUUPPD-PEG. Thus, the combination of the incorporated peptides and PEG made the surface of PUUYIGSR-PEG more hydrophilic and polar.

The incorporation of the PEG and peptide sequences into the polyurethaneurea backbone affected the mechanical properties. The mechanical properties of PUUPPD-PEG (elastic modulus: 0.44 ± 0.1 MPa, tensile strength: 3.1 ± 0.4 MPa, elongation: 370 ± 84.3%) was significantly lower compared to PUUPPD (elastic modulus: 1.1 ± 0.2 MPa, tensile strength 27.2 ± 1.2 MPa, elongation: 2580 ± 544.7%). However, the incorporation of the peptide sequences increased the mechanical properties of PUUYIGSR-PEG (elastic modulus: 0.21 ± 0.1 MPa, tensile strength 9.2 ± 1.8 MPa, elongation: 3532 ± 222.8%).

Adhesion and Spreading of BAECs on Polyurethaneurea Films

The effects of PEG and peptides incorporated into the polyurethaneurea on BAEC attachment and spreading were evaluated. BAECs were seeded on the PUUPPD, PUUPPD-PEG, and PUUYIGSR-PEG films, and adhesion and spreading were investigated after 4 h. The number of adherent cells on PUUPPD-PEG was significantly lower than on PUUPPD ($p < 0.005$) as shown in Figure 1(a). However, cell attachment increased dramatically on PUUYIGSR-PEG ($p < 0.001$). There was no statistical difference for endothelial cell attachment on PUUPPD versus PUUYIGSR-PEG. Similar results were also observed for cell area Figure 1(b) and percent of cell spreading Figure 1(c). Figure 2 also shows phase contrast micrographs of endothelial cells on PUUPPD-PEG and PUUYIGSR-PEG films. The incorporation of PEG decreased cell surface area and percent of cell spreading, but the incorporation of the peptides promoted cell spreading; Few cells attached, and most of them were round shape and did not spread on PUUPPD-PEG. However, a greater number of cells attached and completely spread on PUUYIGSR-PEG.

To ensure that improved cell adhesion and spreading were mediated by YIGSR specific cell adhesion receptors, competitive inhibition of endothelial cell attachment and spreading was studied using soluble YIGSR peptides in culture media. The adhesion of endothelial cells dramatically increased on PUUYIGSR-PEG compared to that on PUUPPD-PEG. However, adhesion of BAECs was reduced in the presence of soluble YIGSR peptides over the entire ranges of the soluble peptide concentrations (0.01, 0.1, and 1 m*M*) as shown in Figure 3. Similar results were also observed in cell

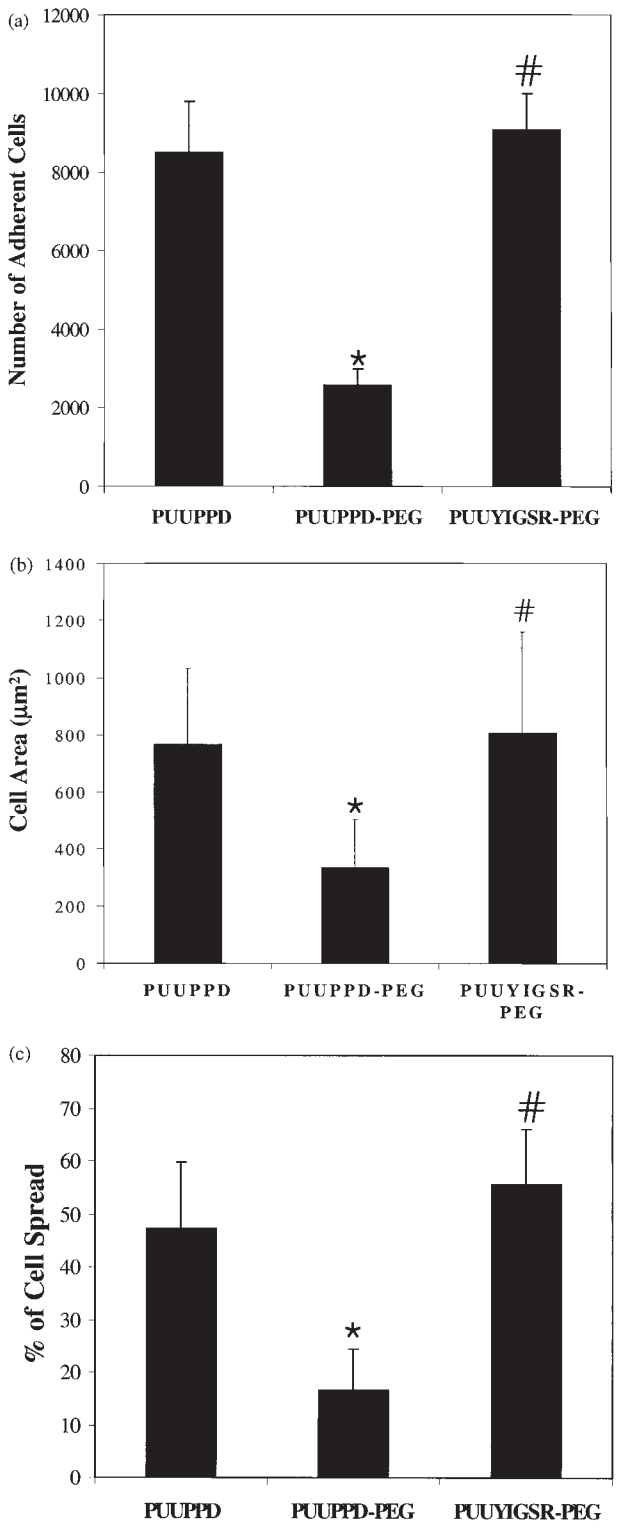

Figure 1. Attachment and spreading of BAECs on polyurethaneurea films after 4-h incubation. (a) Number of adherent cells. Data represent the mean of four samples. *$p < 0.005$ compared to PUUPPD, #$p < 0.001$, compared to PUUPPD PEG. (b) Cell surface area, and (c) percent of cells that were spread. Data represent the mean of 30 samples for cell surface areas and 10 samples for percent of cell spreading. #$p < 0.001$ compared to PUUPPD, #$p < 0.001$ compared to PUUPPD-PEG.

surface area and percent of cell spreading. Fewer cells attached, and most attached cells were round in shape over the entire range of soluble peptide concentrations as shown in Figure 4. These results suggest that adhesion of BAECs to this peptide-modified material is predominantly mediated by specific receptor–ligand interactions.

Migration of BAECs on PUUYIGSR-PEG

Because cell adhesion was extremely low on PUUPPD-PEG, migration of BAECs was evaluated only on PUUYIGSR-PEG. As shown in Figure 5, by 24 and 48 h after seeding, significant migration beyond the original boundary was observed. This suggests that these materials will be capable of supporting autologous endothelialization.

Adhesion of Platelets on YIGSR peptide/PEG-Modified Polyurethaneurea

To generate a platelet-resistant material, we synthesized polyurethaneureas that included PEG as a potion of the soft segment domains. The adhesion of platelets on polyurethanurea films was evaluated using mepacrine-labled whole blood (Figures 6 and 7). Adhesion of platelets on PUUPPD

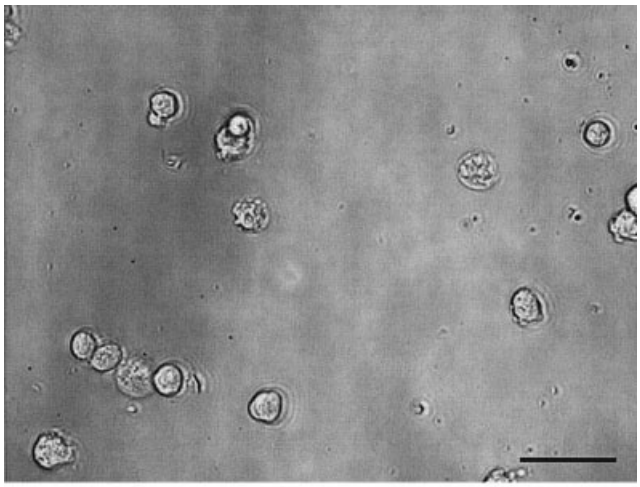

Figure 2. Phase contrast micrographs of BAECs on (a) PUUPPD-PEG and (b) PUUYIGSR-PEG after 4-h incubation. The length of the scale bar = 50 μm.

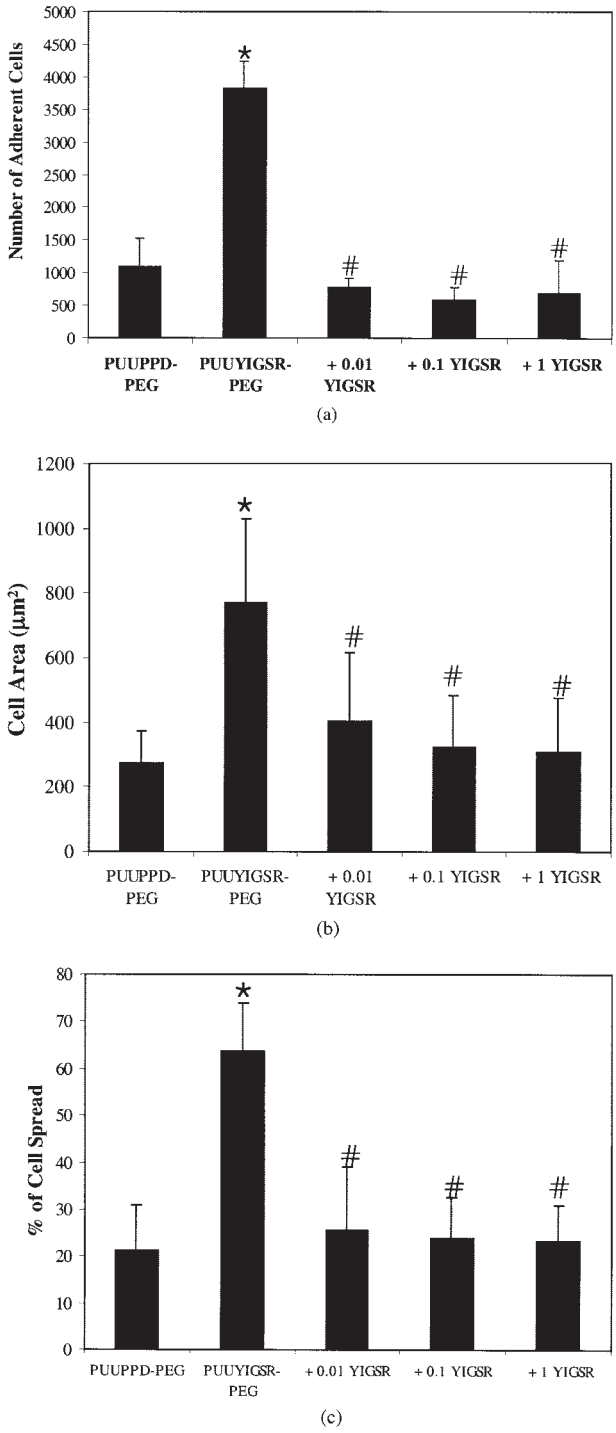

Figure 3. Competitive inhibition of attachment and spreading of BAECs by soluble YIGSR peptides after 4-h incubation. BAECs were incubated with soluble YIGSR peptides at three different concentrations (0.01, 0.1, and 1 m*M*). (a) A number of adherent cells. Data represent the mean of four samples. *$p < 0.001$ compared to PUUPPD-PEG, #$p < 0.001$ compared to untreated PUUYIGSR-PEG. Data represent the mean of 30 samples for cell surface areas (b) and 10 samples for percent of cell spreading (c). *$p < 0.001$ compared to PUUPPD-PEG, #$p < 0.001$ compared to untreated PUUYIGSR-PEG.

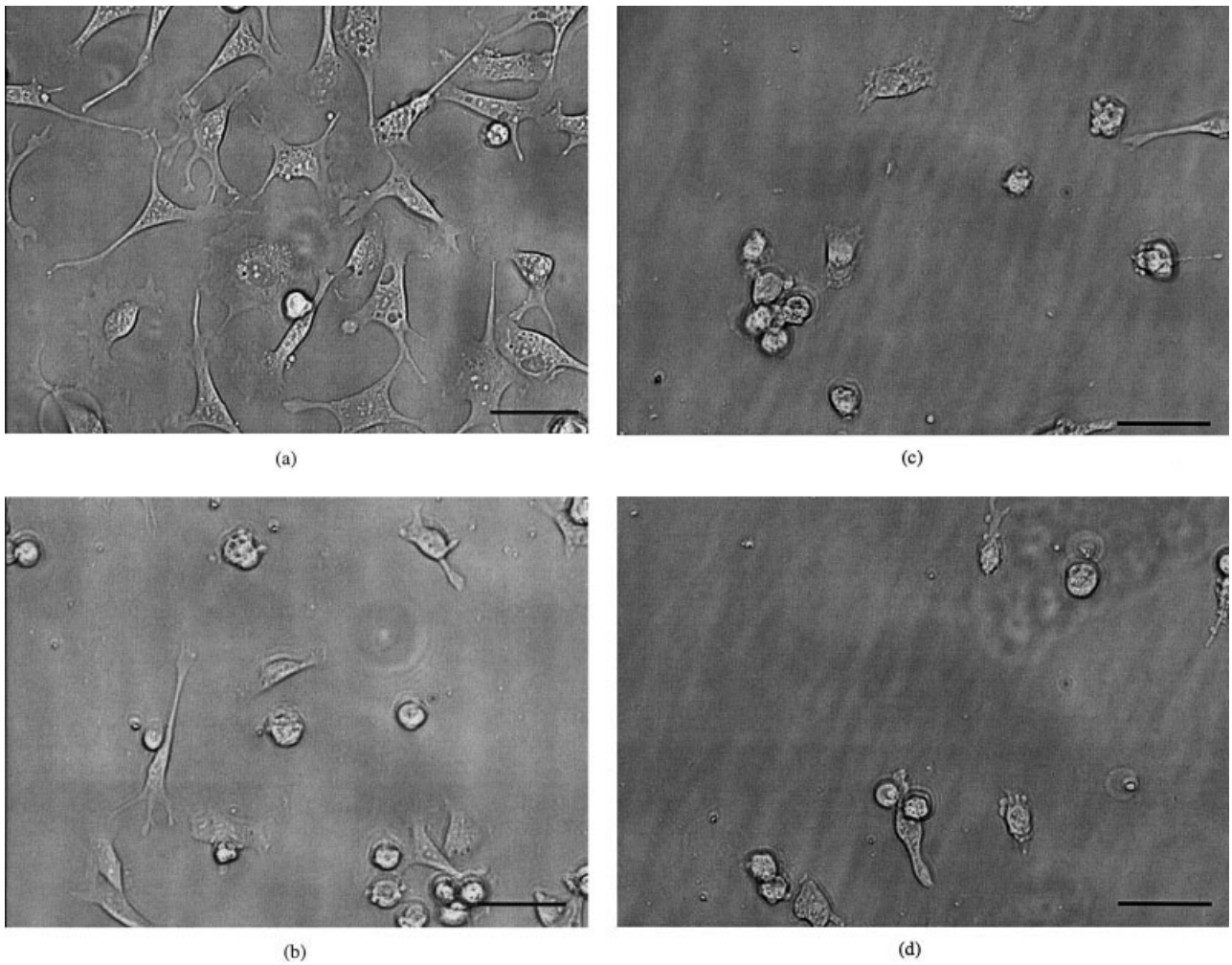

Figure 4. Phase contrast micrographs of competitive inhibition of attachment and spreading of BAECs incubated with soluble YIGSR peptides at three different concentrations. (a) PUUYIGSR-PEG (b) + 0.01, (c) + 0.1, and (d) + 1 YIGSR. The length of the scale bar = 50 μm.

(1696 ± 369 /mm^2) was lower than on Collagen I (4096 ± 324/mm^2) but still substantial. However, there was almost no adhesion of the platelets on PUUPPD-PEG (129 ± 48 /mm^2) or PUUYIGSR-PEG (99 ± 34/mm^2). Thus, PUUYIGSR-PEG is a material that supports robust endothelial cell attachment and growth while being highly resistant to platelet adhesion and resultant complications.

DISCUSSION

NMR, GPC, DSC, ESCA, and contact angle measurements confirmed successful incorporation of the peptide sequences and PEG. It was noted from the DSC results that two distinct peaks (T_g) were observed in PEG-modified polyurethaneurea caused by PTMO and PEG. Higher molecular weight and crystallization of PEG might induce higher T_g. The peptide sequences did not affect T_g but lowered T_m. These results were a little different from a previous study; introduction of the peptide sequences lowered T_g without affecting T_m.[6] In this study, the entanglement of the flexible PEG chain and the peptide sequences might affect thermal behavior; the high molecular weight PEG seems to prevent decrease of T_g but long peptide sequences also might restrict the crystallization of PEG.

The effect of PEG and the peptide sequences on the bulk properties of polymer has been clearly observed in mechanical properties. Despite similar molecular weight, the incorporation of the peptide sequences resulted in enhanced mechanical properties that were dramatically decreased by the presence of the PEG chain. In a previous study, the incorporation of the peptide sequence increased elongation without affecting tensile strength. However, in this study the peptide sequences enhanced both tensile strength and elongation. This may be caused by molecular interactions in polymer chains, the degree of distribution of hard segment domains in soft segment matrix, and restriction of motility of flexible PEG chain by long amino acid sequences. Thus, our study

suggests the possibility that the bioactive peptide sequences are able to control not only cellular behaviors but also bulk mechanical properties.

The hydrophilicity of polymer surfaces was evaluated using contact angle measurement. The order of hydrophilicity was PUUPPD > PUUPPD-PEG > PUUYIGSR-PEG. The lower contact angle of PUUYIGSR-PEG compared to PUUPPD-PEG indicated that peptides were also exposed on the surface with PEG. Endothelial cell attachment and spreading were evaluated to determine if the peptide sequences maintain their bioactivity on the surface without interference by PEG. Few endothelial cells attached on PEG-modified surfaces and most did not spread. On the other hand, a dramatic increase in cell attachment and spreading were observed on the peptide/PEG-modified surfaces. Competitive inhibition of cell attachment and spreading study confirmed that these results were mediated by specific YIGSR sensitive cell adhesion receptors.

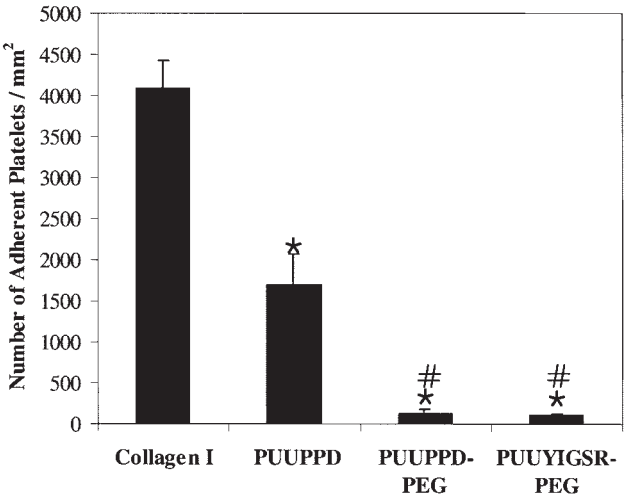

Figure 6. The number of adherent platelets on the surfaces of collagen I, PUUPPD, PUUPPD-PEG, and PUUYIGSR-PEG. Data represent the mean of five samples. *$p < 0.001$ compared to Collagen I, #$p < 0.001$ compared to PUUPPD.

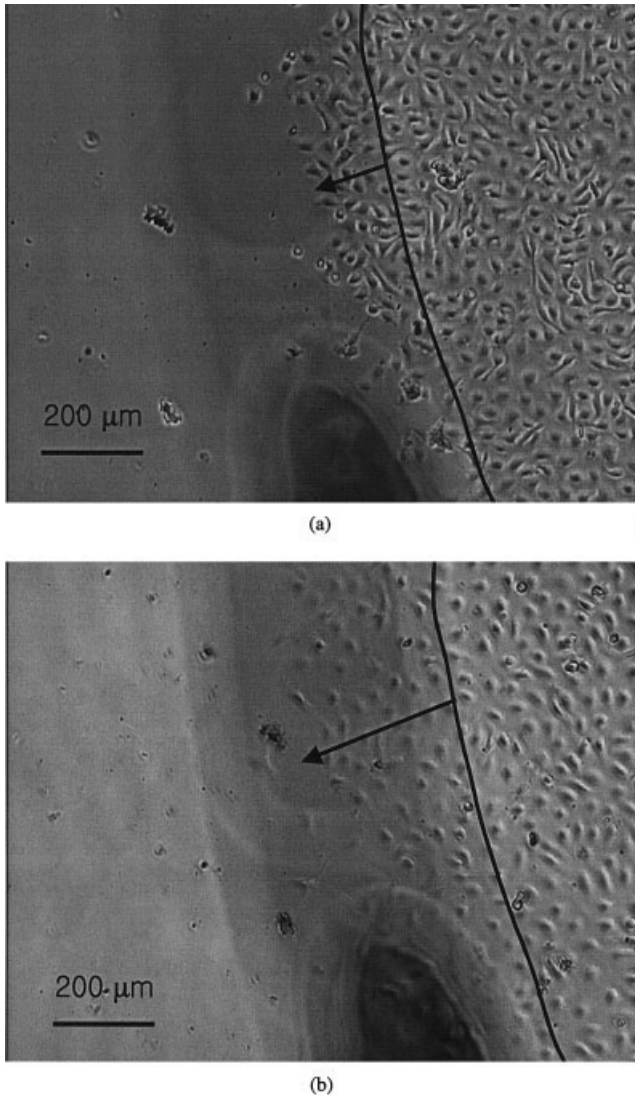

Figure 5. Migration of BAECs on PUUYIGSR-PEG after (a) 24 h and (b) 48 h. The length of the scale bar = 200 μm.

The main reason to modify biomaterials using hydrophilic PEG is to prevent platelet adhesion, and the results depend on modification methods, ratio of hard and soft segments, and molecular weight as well as contents of PEG.[11,15–17] In this study, 4600 mol/g PEG was incorporated at a 15% molar ratio as a soft segment. As shown above, this amount was enough to form a PEG rich phase on the surface, preventing cell attachment. Platelet adhesion to the surface was also evaluated using mepacrine-labled whole blood. There was no observable platelet adhesion on PEG- and PEG/peptide-modified polyurethaneurea surfaces in contrast to unmodified polyurethaneurea. This may be due to suppressed protein adsorption; adsorbed fibrinogen plays an important role in mediating platelet adhesion on surfaces by exposing the RGD peptide sequence, which binds to platelet integrin glycoprotein IIb/IIIa.[23,24] These results also confirmed the YIGSR peptide is an appropriate peptide sequence for cardiovascular applications. Therefore, there were the synergistic effects that improved mechanical properties and endothelialization by bioactive peptide sequences as well as suppressed platelet adhesion by hydrophilic PEG chains.

CONCLUSIONS

A bioactive peptide/PEG-modified polyurethanurea has been synthesized, and successful incorporation of the peptide sequences as a chain extender and PEG as a soft segment were confirmed. This material exhibited enhanced adhesion, migration, and proliferation of endothelial cells by a bioactive peptide sequences as well as suppressed platelet adhesion by hydrophilic PEG chains. Importantly, bioactive peptide sequences also supported improved me-

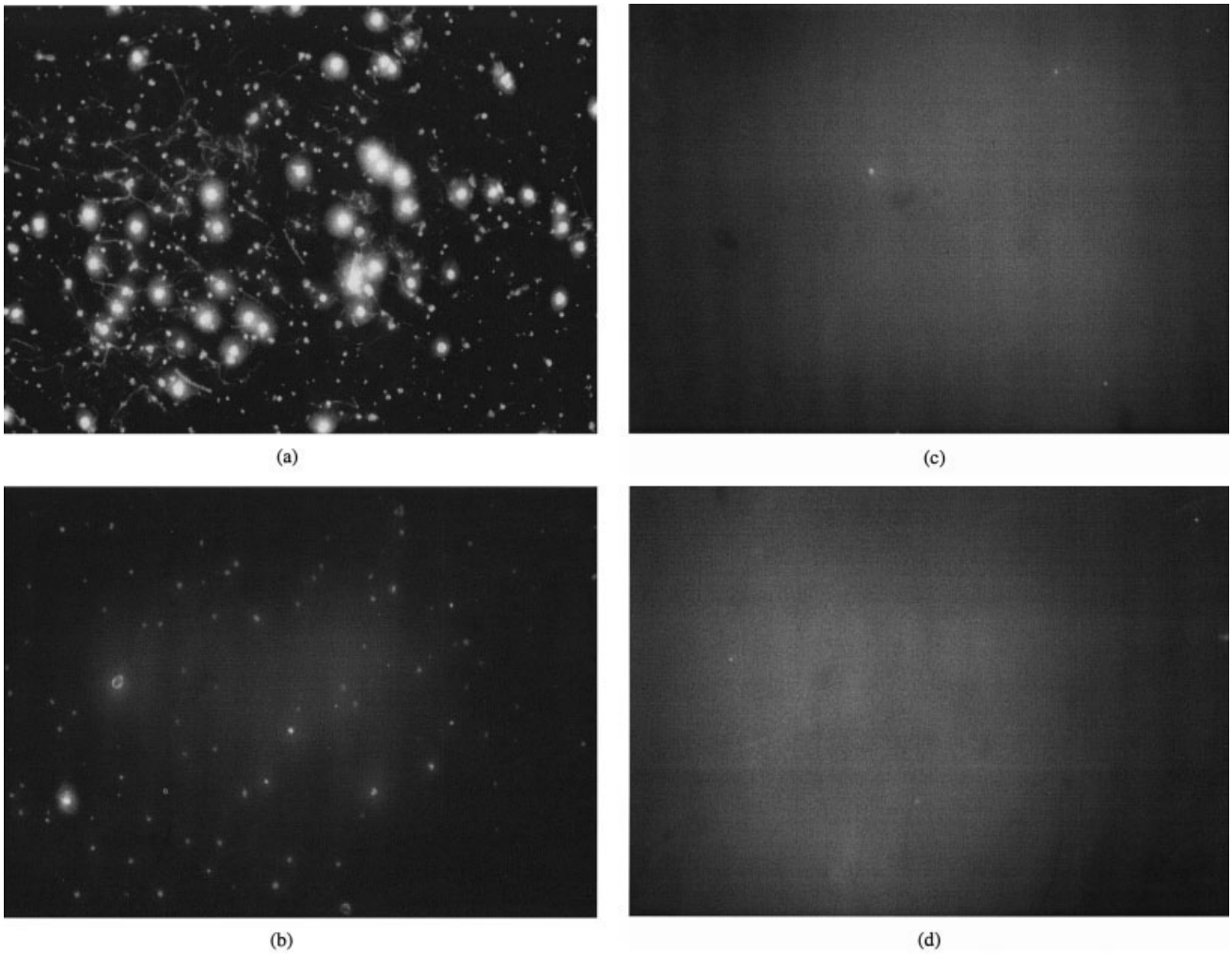

Figure 7. The fluorescent micrographs of platelets on collagen I and polyurethanurea films. Platelets were fluorescently labeled with mepacrine. (a) Collagen I, (b) PUUPPD, (c) PUUPPD-PEG, and (d) PUUYIGSR-PEG.

chanical properties. This material may be a good candidate for engineering small diameter vascular grafts.

REFERENCES

1. Clowes AW. Intimal hyperplasia and graft failure. Cardiovasc Pathol 1993;2:179S–186S.
2. Kobayashi H, Kabuto M, Ide H, Hojotani K, Kubota T. An artificial blood vessel with an endothelial-cell monolayer. J Neurosurg 1992;77:397–402.
3. Massia SP, Hubbell JA. An RGD spacing of 440 nm is sufficient for integrin $\alpha_v\beta_3$-mediated fibroblast spreading and 140 nm for focal contact stress fiber formation. J Cell Biol 1991;114:1089–1100.
4. Hubbell JA, Massia SP, Drumheller PD, Herbert CB, Lyckman AW. Bioactive and cell-type selective polymers obtained by peptide grafting. Polym Mater Sci Eng 1992;66:30–31.
5. Dee KC, Andersen TT, Bizios R. Cell function on substrates containing immobilized bioactive peptides. Mater Res Soc Symp Proc 1994;331:115–119.
6. Jun HW, West JL. Development of a YIGSR peptide-modified polyurethaneurea to enhance endothelialization. J Biomater Sci Polym Ed 2004;15:73–94.
7. Kim SW. Nonthrombogenic treatments and strategies. In: Ratner BD, Hoffman AS, Schoen FJ, Lemons JE, editors. Biomaterials science. San Diego: Academic Press; 1996. p 297–308.
8. Park KD, Kim YS, Han DK, Kim YH, Lee EHB, Suh H, Choi KS. Bacterial adhesion on PEG modified polyurethane surfaces. Biomaterials 1998;19:851–859.
9. Wesslen B, Kober M, Freij-Larsson C, Ljungh A, Paulsson M. Protein adsorption of poly(ether urethane) surfaces modified by amphiphilic and hydrophilic polymers. Biomaterials 1994;15:278–284.
10. Han DK, Park KD, Hubbell J, Kim YH. Surface characteristics and biocompatibility of lactide-based poly(ethylene glycol) scaffolds for tissue engineering. J Biomater Sci Polym Ed 1998;9:667–680.
11. Lee HJ, Matsuda T. Surface photograft polymerization on segmented polyurethane using the iniferter technique. J Biomed Mater Res 1999;47:564–567.
12. Park JH, Park KD, Bae YH. PDMS-based polyurethanes with MPEG grafts: Synthesis, characterization and platelet adhesion study. Biomaterials 1999;20:943–953.
13. Park KD, Piao AZ, Jacobs H, Okano T, Kim SW. Synthesis and characterization of SPUU-PEO-heparin graft copolymers. J Polym Sci Polym Chem 1991;29:1725–1737.

14. Park JH, Bae YH. Physicochemical properties and in vitro biocompatibility of PEO/PTMO multiblock copolymer/segmented polyurethane blends. J Biomater Sci Polym Ed 2002; 13:527–542.
15. Deible CR, Beckman EJ, Russell AJ, Wagner WR. Creating molecular barriers to acute platelet deposition on damaged arteries with reactive polyethylene glycol. J Biomed Mater Res 1998;41:252–256.
16. Deible CR, Petrosko P, Johnson PC, Beckman EJ, Russell AJ, Wagner WR. Molecular barriers to biomaterial thrombosis by modification of surface proteins with polyethylene glycol. Biomaterials 1998;19:1885–1893.
17. Silver JH, Myers CW, Lim F, Cooper SL. Effect of polyol molecular weight on the physical properties and haemocompatibility of polyurethanes containing polyethylene oxide macroglycols. Biomaterials 1994;15:695–704.
18. Blackwell J, Gardner KH. Structure of the hard segments in polyurethane elastomers. Polymer 1979;20:13–17.
19. Blackwell J, Lee CD. Hard-segment domain sites in MDI/Diol polyurethane elastomers. J Polymer Sci Polym Phys Ed 1983; 21:2169–2180.
20. Garrett JT, Sidelecki CA, Runt J. Microdomain morphology of poly(urethane urea) multiblock copolymers. Macromoleculaes 2001;34:7066–7070.
21. Sung CSP, Hu CB, Wu CS. Properties of segmented polyether poly(urethaneureas) based on 2,4-Toluene diisocyanate. 1. Thermal transitions, X-ray studies, and comparison with segmented poly(urethanes). Macromolecules 1980;13: 111–116.
22. Lee HK, Ko SW. Structure and thermal properties of polyether polyurethaneurea elastomers. J Appl Polym Sci 1993; 50:1269–1280.
23. Mazur C, Tschopp JF, Faliakou EC, Gould KE, Diehl JT, Pierschbacher MD, Connoly RJ. Selective $\alpha_{11b}\beta_3$ receptor blockage with peptide TP9201 prevents platelet uptake on dacron vascular grafts without significant effect on bleeding time. J Lab Clin Med 1994; 124:589–599.
24. Andrieux A, Rabiet MJ, Chapel A, Concord E, Marguerie G. A highly conserved sequence of the Arg-Gly-Asp-binding domain of the integrin β_3 subunit is sensitive to stimulation. J Biol Chem 1991;266:14202–14207.