Notes

Thin Polymer Layers Formed Using Multiarm Poly(ethylene glycol) Vinylsulfone by a Covalent Layer-by-Layer Method

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Introduction

Hydrophilic materials have drawn a great deal of interest as a route to produce biocompatible materials.[1−5] PEG (poly(ethylene glycol)) materials and other hydrophilic materials have been shown to reduce protein adsorption and cellular adhesion, potentially improving biocompatibility.[6,7] However, long-term in vivo biocompatibility of hydrophilic materials is still elusive.[8] Ideally, biocompatible materials should not promote platelet activation/fragmentation, leukocyte adhesion/activation, or calcification, but hydrophilic materials can promote these processes, perhaps due to weakly adsorbed proteins.[9,10] For medical devices with complex topographies, such as endovascular stents, the production of poly(ethylene glycol) thin films can be approached in a number of ways. Self-assembly can generate monolayer-thick coatings of poly(ethylene glycol),[11−13] but long-term stability may not be sufficient and the films may be too thin for effective drug delivery. Surface-initiated polymerization of oligo(ethylene glycols) produce thin yet dense polymer brushes that effectively hinder protein adsorption and cell adhesion.[14,15] Layer-by-layer methods used to form polyelectrolyte multilayers produce thin films that effectively inhibit cell adhesion,[16,17] but the lack of covalent bonds will impact long-term stability.

Covalent bonds can be incorporated in preformed polyelectrolyte multilayer films simply by dehydrating salt bridges formed between amines and carboxylic acids[18,19] or cross-linking azo groups with UV light.[20] Additionally, covalent bonds can be formed at each step of the layer-by-layer reaction by using polymers containing isocyanates,[21] maleic anhydrides,[21,22] or EDC-activated carboxylates,[23] which can be reacted with polymers such as poly(ethylenimine)[22,24] or a poly(vinyl amine)-containing thermoresponsive copolymer.[23] A polymer layer can be cross-linked with small molecules including 2-aminoethanol,[21] terephthaloyl chloride,[25] adipoyl chloride,[26] and hexamethylene-1,6-bis(aminocarboxysulfonate).[27] Major and Blanchard have further demonstrated the rich variety of polymer cross-linking chemistries available for layer-by-layer synthesis.[28]

A particularly promising route was developed by the groups of Bergbreiter, Crooks, and Pishko. Thin films were formed by reacting α,ω-diaminopoly(*tert*-butyl acrylate) with activated carboxyl groups on a surface.[29] The *tert*-butyl groups were then deprotected to yield poly(acrylic acid). The numerous carboxylic acids on poly(acrylic acid) could then be activated for reaction with α,ω-diaminopoly(*tert*-butyl acrylate). Repeating the deprotection and activation steps, the large number of carboxyl groups on poly(acrylic acid) led to the formation of a hyperbranched surface coating. Reaction of the final activated poly(acrylic acid) layer with α-amino-ω-methoxypoly(ethylene glycol) produced films that were very resistant to cell adhesion and that could be easily patterned.[30−32] Pishko also produced thin films by successively reacting poly(allyl amine) with poly(ethylene glycol)−diacrylate or poly(ethylene glycol)−tetraacrylate via a Michael-type reaction,[33] producing films that should be degradable.[34] Hubbell et al. also studied the reaction of PEG−diacrylate with thiolated surfaces through a Michael-type addition, but they did not detect the presence of immobilized PEG.[35] We wished to produce stable thin films by a layer-by-layer method that had a higher proportion of poly(ethylene glycol) to aid in resisting protein adsorption and cell adhesion.

We produced vinylsulfone derivatives of poly(ethylene glycol) that were reacted sequentially with dithiothreitol (DTT), which contains two thiol groups. The dry thickness of the films increased linearly up to 10 layers, which then reached a plateau at about 50 nm for subsequent layers. We found a decrease in protein adsorption following the addition of 10−20 PEG layers to the glass, which correlated with a substantial reduction in cell adhesion on these films. To demonstrate that the reduction in cell adhesion was not due to the toxicity of the film, we then added cell adhesion peptide to the thin film, restoring cell adhesion to the substrate. While the current method reduces protein and cell adhesion, it does not eliminate it, suggesting defects in the PEG layer.

Materials and Methods

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. Clean borosilicate glass coverslips (Corning, Corning, NY) were incubated in a 2% solution of (3-aminopropyl)triethoxysilane in dry acetone for 1 h with gentle shaking. Aminophase glass coverslips were then cured at 100 °C for 3 h and stored dry until use. Multiarm PEG vinylsulfone was synthesized from four-arm or eight-arm PEG (molecular weight, 10 000; Nektar Therapeutics) using previously published protocols.[36] Aminophase glass was incubated with a solution containing 1% multiarm PEG−vinylsulfone overnight at pH 8.5 and 37 °C. The substrates were subsequently incubated with dithiothreitol (DTT) for 1 h at 37 °C. Substrates were then incubated with multiarm PEG−vinylsulfone for 1 h at 37 °C. This process was repeated to form multilayers up to 20 layers (Figure 1; one layer indicates multiarm PEG−vinylsulfone + DTT), with three phosphate-buffered saline (PBS) washes between each incubation step.

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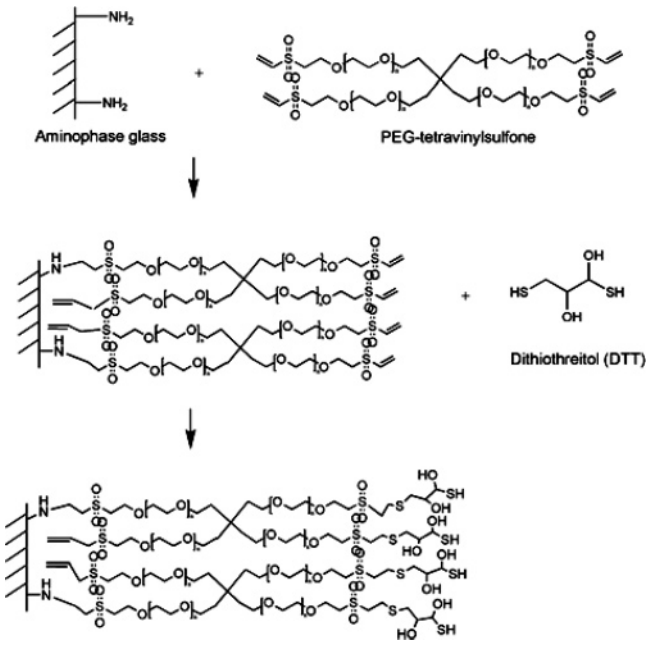

Figure 1. Schematic showing the procedure for modifying a surface by the layer-by-layer method using multiarm PEG−vinylsulfone and dithiothreitol (DTT). PEG chains with reactive vinylsulfone end groups were reacted with amine groups on aminophase glass followed by reaction with thiol groups on DTT through a Michael-type reaction, producing a thin, cross-linked PEG coating.

Ellipsometry was performed on a Gaertner L116C ellipsometer (Gaertner Scientific, Chicago, IL), using a 632.8 nm HeNe laser. Refractive indices and film thicknesses were analyzed by assuming a homogeneous three-layer model. Layer-by-layer methods were performed on Si/SiO_2 wafers modified by the same method described above for glass coverslips. After the formation of a certain number of layers, the substrates were washed with deionized water to remove salts from the surface and air-dried.

Chinese hamster ovary (CHO) cells (ATCC, Manassas, VA) were cultured in F-12K (Gibco, Carlsbad, CA) medium with 10% fetal bovine serum and 1% antibiotic (Gibco). CHO cells were removed from the culture substrates using trypin/EDTA (EDTA = ethylenediaminetetraacetic acid; 0.05%, Gibco) and centrifuged at 200*g* for 10 min. The cells were resuspended with the cell culture medium, and then the number of cells was counted using a hemocytometer. CHO cells were seeded on the PEG-layered glass at 2×10^4 cells/cm^2. The cells were cultured on the surfaces for 24 h and were then fixed with 10% formalin overnight. The substrates were then washed with PBS (pH 7.4), and the cells were imaged using a phase contrast microscope (Olympus CKX41). The density of cells was counted manually.

Glass Petri dishes (10 cm diameter, Corning) were used for the analysis of protein adsorption on the PEG-coated glass. The Petri dishes were aminosilanated, and films were formed as described above. Substrates were then incubated with 10 mL of Dulbecco's modified Eagle's medium (DMEM) containing 5% human serum for 1 h and washed with PBS three times to remove weakly adsorbed proteins. Proteins were eluted from surfaces using 500 μL of buffer containing 8 M urea, 4% CHAPS, and 40 mM Tris, rolling the liquid over the surface for 10 min as previously described.[37] The eluted proteins were concentrated by acetone precipitation and diluted with 20 μL of sample buffer containing 0.125 M Tris HCl with 0.4% sodium dodecyl sulfate (SDS) for analysis on SDS-PAGE gels. Protein bands were visualized with Sypro-Ruby stain (Bio-Rad, Hercules, CA).

The cell adhesion peptide, Ac-GCGYGRGDSPG, was synthesized on a peptide synthesizer (Applied Biosystems ABI 433A, Foster City, CA) using previously described protocols.[36] The peptides were dissolved in PBS (pH 8.5) at a concentration of 0.1 mg/mL. PEG-modified coverslips were placed into wells of a 24-well plate and were incubated with the peptide solution for 10 min at 37 °C and then rinsed with PBS three times to remove unreacted peptides. Human umbilical vein endothelial cells (HUVEC, Clonetics, Walkersville, MD) were grown in endothelial cell growth medium (EGM, Clonetics) on tissue culture polystyrene at 37 °C and 5% CO_2. The cells were briefly washed with 1 mL of 0.05% trypsin/0.02% EDTA solution and removed from culture flasks by adding 3 mL of the trypsin solution. Trypsinization was stopped by adding 3 mL of endothelial cell growth medium containing trypsin neutralizing solution. Cells were centrifuged at 200*g* for 5 min, resuspended in culture medium, and seeded onto the materials at 1×10^4 cells/cm^2 in 1 mL of complete EGM (containing 2% fetal bovine serum) for 24 h. Cells were imaged by phase contrast microscopy, and the number of cells was counted manually.

Data were expressed as means ± standard deviation. Statistical significance was assessed using ANOVA with the Scheffe posthoc test (Statistica, Tulsa, OK), and $p < 0.05$ was considered to be statistically significant.

Results and Discussion

Aminophase glass was produced using (3-aminopropyl)-triethoxysilane, leading to a small but detectable increase in the thickness of the surface layer of silicon wafers (Figure 2), similar to previous results.[38] Aminophase silicon wafers were then reacted sequentially with PEG−octavinylsulfone (PEG-OVS, molecular weight, 10 000) and DTT. DTT served to add free thiol groups (-SH) to the PEG layer via reaction with vinylsulfone groups, although both thiols on DTT could potentially react with PEG-OVS on the surface. Up to 20 layers (one layer = PEG-OVS + DTT) were added to the surfaces, with a measurable increase in thickness (Figure 2A). For up to 10 layers, the thickness of surface layers consistently increased. However, between 10 and 20 layers, the thickness was not significantly changed. The thicknesses of films fabricated by previous layer-by-layer techniques have been characterized by the use of ultraviolet absorbance, X-ray reflectometry, and ellipsometry.[16,39,40] Previous studies indicated that the thickness of layers increased linearly or exponentially, depending on the system.[16,40] The plateau in the thickness of PEG layers in this study is different from these two cases and might have occurred because the concentration of the cross-linker was not increased as the density of PEG on the substrate increased. This may have favored cross-linking in the upper layers when DTT was added, leaving few free thiol groups to react with subsequently added PEG−vinylsulfone. Additionally, DTT may have reacted with vinylsulfone groups in the underlying layers, which might serve to increase the density of PEG in the coating without increasing the height. Such questions could potentially be addressed with a sensitive measurement of film refractive index and thickness in water, e.g., with optical waveguide lightmode spectroscopy (OWLS).[41]

Attachment of PEG to the surface of a material can lead to a dramatic reduction in the amount of adsorbed protein (e.g., more than a 95% reduction in protein amount has been previously observed[42,43]). As the spacing between PEG chains

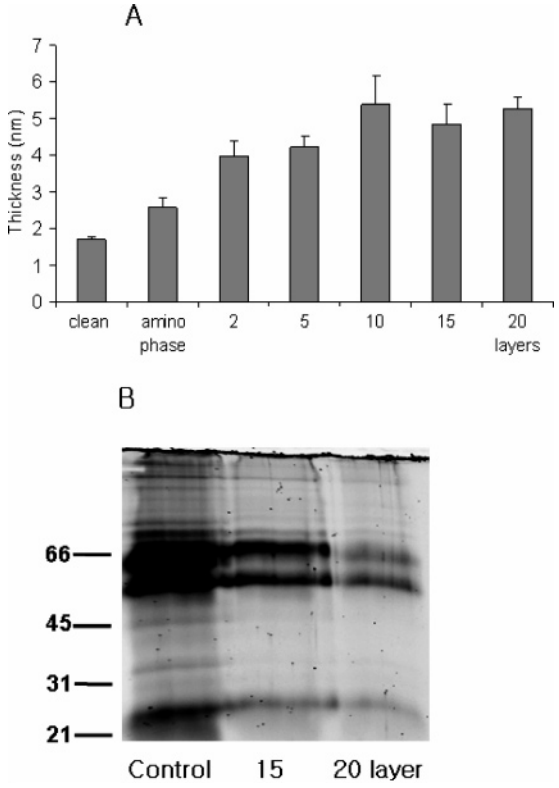

Figure 2. (A) Ellipsometric thicknesses ($n = 9$) of PEG layers made with 1% PEG−octavinylsulfone and 0.1% of DTT at pH 8.5 and 37 °C. The measurements were performed with the films dried in air. "Clean" = cleaned glass before aminosilanation. (B) SDS-PAGE gel of human serum proteins desorbed from PEG-derivatized aminophase glass. "Control" is aminophase glass. PEG-derivatized surfaces were produced using PEG−octavinylsulfone and DTT by the layer-by-layer method.

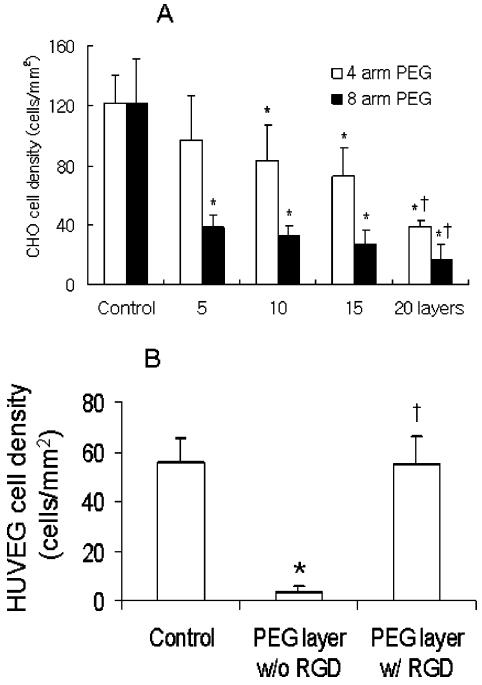

Figure 3. (A) CHO (Chinese hamster ovary) cell density on PEG layered surfaces. Aminophase glass surfaces ("control") were treated using the layer-by-layer method with 1% of either PEG−tetravinylsulfone (four arms) or PEG−octavinylsulfone (eight arms) and 0.1% of DTT at pH 8.5 and 37 °C, for up to 20 layers. Error bars represent standard deviations ($n = 6$). (∗) $p < 0.05$ compared to the cell density on the control. (†) $p < 0.05$ versus the cell density on five-layer PEG films. (B) HUVEC (human umbilical vein endothelial cell) density was measured after seeding on aminophase glass (control) or thin films formed from PEG−octavinylsulfone and DTT using the layer-by-layer method to produce five layers. (∗) $p < 0.05$ versus control. (†) $p < 0.05$ versus PEG layer without RGD containing peptide.

on a surface is reduced (i.e., density is increased), a reduction in protein adsorption is predicted.[7] We used human serum to investigate protein adsorption on our PEG-derivatized surface (Figure 2B). We previously demonstrated that adsorbed proteins can be effectively removed from surfaces using a small amount of liquid containing high concentrations of urea and CHAPS detergent.[37] Using SDS-PAGE, we compared adsorbed proteins from aminophase glass with 15 layers or 20 layers formed with eight-arm PEG-vinylsulfone and DTT. We found similar bands by SDS-PAGE for all of the substrates but with a large decrease in the amount of adsorbed protein on surfaces reacted with PEG. In our previous studies of protein adsorption on biomaterials,[37] we observed large differences in the composition of the adsorbed protein layer between hydrophilic (tissue culture polystyrene) and less hydrophilic surfaces (polypropylene, poly(ethyleneterephthalate), and poly(dimethylsiloxane)). In particular, we identified the protein fetuin in larger amounts than albumin on the hydrophilic surface. Our previous experience allows us to tentatively assign the major peaks on the basis of molecular weight as fetuin (about 60 KDa) and IgG (bands at about 50 and 25 KDa). The similarity between the bands present on the aminophase glass and the PEG-coated surfaces may indicate that proteins primarily interact with the underlying aminophase surface and that the total amount of adsorbed protein is controlled simply by the local density of PEG on the surface. This could result from large-scale defects in the coating or simply a broad distribution in the distance between attached chains, perhaps due to the density of aminosilane groups on the glass. A single layer of PEG−vinylsulfone was ineffective at preventing cell adhesion (data not shown), indicating that the density of amino groups on the surface was too low to achieve a dense PEG brush in one reaction step.

To determine if cell interactions were affected by thin layers of PEG, substrates were fabricated with up to 20 layers using four-arm or eight-arm PEG−vinylsulfone with DTT (Figure 3A). The substrates were incubated with CHO cells in cell culture medium containing 10% fetal bovine serum. The density of attached CHO cells decreased as the number of PEG layers increased. When the number of added PEG layers was the same, CHO cell adhesion on eight-arm PEG derivatized surfaces was significantly lower than on four-arm PEG layers. To demonstrate that the reduction in cell adhesion was not due to a toxic effect, we tested the ability of the coatings to resist HUVEC adhesion and the ability of a covalently attached cell adhesion peptide to restore adhesion to the films (Figure 3B). The peptide was covalently attached to the film using a Michael-type reaction between vinylsulfones in the film and a single cysteine in the peptide.[34] We found that HUVEC adhesion to the PEG coatings was less robust than CHO cell adhesion. A substantial reduction in cell spreading could be obtained with only five bilayers. However, HUVEC cell attachment was restored to the amount seen on aminophase glass following addition of the RGD peptide to the film.

Compared to previous PEG films produced by the layer-by-layer method, the current films contain a high fraction of PEG (with perfect cross-linking, about 95% of the dry films would be PEG chains, with the rest DTT and the pentaerythitol-based

cores of the mulitarm PEG). Given the highly cell adhesive nature of other polymers compared to PEG (for example, the underlying poly(acrylic acid) layer in the films produced by Crooks and Pishko is significantly more cell adhesive than the PEG layer),[30] the current films might be expected to present a more robust nonadhesive substrate. However, the large number of reactions required without eliminating cell adhesion suggests that major improvements to the method would be required for practical application.

Conclusion

A covalent layer-by-layer method was developed using Michael-type reactions to reduce nonspecific protein adsorption and cell response on the surfaces of materials. By repeating Michael-type reactions between vinyl sulfone groups and thiols, we fabricated thin PEG films on glass surfaces. By increasing the number of layers, we were able to greatly reduce protein adsorption and cell adhesion. Finally, we attached RGD-containing peptides to the PEG-layered surfaces and restored cell adhesion and spreading. Our modified layer-by-layer method may be readily extended to other functionalized surfaces including metals and plastics. However, one drawback of layer-by-layer techniques is the need for large numbers of reaction and washing steps. Such limitations potentially could be overcome using larger molecular weight polymers in each reaction, which we are currently exploring.

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References and Notes

(1) Hoffman, A. S. Hydrogels for Biomedical Applications. *Adv. Drug Deliv. Rev.* **2002**, *54* (1), 3−12.

(2) Elbert, D.; Hubbell, J. Surface Treatments of Polymers for Biocompatibility. *Annu. Rev. Mater. Sci.* **1996**, *26*, 365−394.

(3) Brandley, B. K.; Schnaar, R. L. Covalent Attachment of an Arg-Gly-Asp Sequence Peptide to Derivatizable Polyacrylamide Surfaces: Support of Fibroblast Adhesion and Long-Term Growth. *Anal. Biochem.* **1988**, *172* (1), 270−278.

(4) Lopez, G. P.; Ratner, B. D.; Tidwell, C. D.; Haycox, C. L.; Rapoza, R. J.; Horbett, T. A. Glow Discharge Plasma Deposition of Tetraethylene Glycol Dimethyl Ether for Fouling-Resistant Biomaterial Surfaces. *J. Biomed. Mater. Res.* **1992**, *26* (4), 415−439.

(5) Nguyen, K. T.; West, J. L. Photopolymerizable Hydrogels for Tissue Engineering Applications. *Biomaterials* **2002**, *23* (22), 4307−4314.

(6) Harris, J. M., *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; Plenum Press: New York, 1992.

(7) Jeon, S. I.; Lee, J. H.; Andrade, J. D.; De Gennes, P. G., Protein-Surface Interactions in the Presence of Polyethylene Oxide: I Simplified Theory. *J. Colloid Interface Sci.* **1991**, *142*, 149−158.

(8) Xue, L.; Greisler, H. P. Biomaterials in the Development and Future of Vascular Grafts. *J. Vasc. Surg.* **2003**, *37* (2), 472−480.

(9) Llanos, G. R.; Sefton, M. V. Does Polyethylene Oxide Possess a Low Thrombogenicity. *J. Biomater. Sci., Polym. Ed.* **1993**, *4* (4), 381−400.

(10) Sefton, M. V.; Gemmell, C. H.; Gorbet, M. B. What Really Is Blood Compatibility? *J. Biomater. Sci., Polym. Ed.* **2000**, *11* (11), 1165−1182.

(11) Elbert, D. L.; Hubbell, J. A. Self-Assembly and Steric Stabilization at Heterogeneous, Biological Surfaces Using Adsorbing Block Copolymers. *Chem. Biol.* **1998**, *5* (3), 177−183.

(12) Billinger, M.; Buddeberg, F.; Hubbell, J. A.; Elbert, D. L.; Schaffner, T.; Mettler, D.; Windecker, S.; Meier, B.; Hess, O. M. Polymer Stent Coating for Prevention of Neointimal Hyperplasia. *J. Invasive Cardiol.* **2006**, *18* (9), 423−426; discussion, 427.

(13) Irvine, D. J.; Mayes, A. M.; Griffith, L. G. Nanoscale Clustering of RGD Peptides at Surfaces Using Comb Polymers. 1. Synthesis and Characterization of Comb Thin Films. *Biomacromolecules* **2001**, *2*, 85−94.

(14) Andruzzi, L.; Senaratne, W.; Hexemer, A.; Sheets, E. D.; Ilic, B.; Kramer, E. J.; Baird, B.; Ober, C. K., Oligo(ethylene glycol) Containing Polymer Brushes as Bioselective Surfaces. *Langmuir* **2005**, *21*, 2495−2504.

(15) Ma, H.; Hyun, J.; Stiller, P.; Chilkoti, A., Non-Fouling Oligo(ethylene glycol)- Functionalized Polymer Brushes Synthesized by Surface-Initiated Atom Transfer Radical Polymerization. *Adv. Mater.* **2004**, *16* (4), 338−341.

(16) Elbert, D. L.; Herbert, C. B.; Hubbell, J. A. Thin Polymer Layers Formed by Polyelectrolyte Multilayer Techniques on Biological Surfaces. *Langmuir* **1999**, *15* (16), 5355−5362.

(17) Decher, G.; Eckle, M.; Schmitt, J.; Struth, B. Layer-by-Layer Assembled Multicomposite Films. *Curr. Opin. Colloid Interface Sci.* **1998**, *3* (1), 32−39.

(18) Harris, J. J.; DeRose, P. M.; Bruening, M. L. Synthesis of Passivating, Nylon-Like Coatings through Cross-Linking of Ultrathin Polyelectrolyte Films. *J. Am. Chem. Soc.* **1999**, *121*, 1978−1979.

(19) Richert, L.; Boulmedais, F.; Lavalle, P.; Mutterer, J.; Ferreux, E.; Decher, G.; Schaaf, P.; Voegel, J. C.; Picart, C. Improvement of Stability and Cell Adhesion Properties of Polyelectrolyte Multilayer Films by Chemical Cross-Linking. *Biomacromolecules* **2004**, *5*, 284−294.

(20) Sun, J.; Wu, T.; Liu, F.; Wang, Z.; Zhang, X.; Shen, J. Covalently Attached Multilayer Assemblies by Sequential Adsorption of Polycationic Diazo-Resins and Polyanionic Poly(acrylic acid). *Langmuir* **2000**, *16*, 4620−4624.

(21) Beyer, D.; Bohanon, T. M.; Knoll, W.; Ringsdorf, H.; Elender, G.; Sackmann, E. Surface Modification via Reactive Polymer Interlayers. *Langmuir* **1996**, *12*, 2514−2518.

(22) Huck, W. T. S.; Yan, L.; Stroock, A.; Haag, R.; Whitesides, G. M. Patterned Polymer Multilayers as Etch Resists. *Langmuir* **1999**, *15*, 6862−6867.

(23) Serizawa, T.; Nanameki, K.; Yamamoto, K.; Akashi, M. Thermoresponsive Ultrathin Hydrogels Prepared by Sequential Chemical Reactions. *Macromolecules* **2002**, *35*, 2184−2189.

(24) Kohli, P.; Blanchard, G. J. Design and Demonstration of Hybrid Multilayer Structures: Layer-by-Layer Mixed Covalent and Ionic Interlayer Linking Chemistry. *Langmuir* **2000**, *16*, 8518−8524.

(25) Zhang, F.; Jia, Z.; Srinivasan, M. P. Application of Direct Covalent Molecular Assembly in the Fabrication of Polyimide Ultrathin Films. *Langmuir* **2005**, *21*, 3389−3395.

(26) Major, J. S.; Blanchard, G. J. Covalently Bound Polymer Multilayers for Efficient Metal Ion Sorption. *Langmuir* **2001**, *17*, 1163−1168.

(27) Welsh, E. R.; Schauer, C. L.; Santos, J. P.; Price, R. R. In Situ Cross-Linking of Alternating Polyelectrolyte Multilayer Films. *Langmuir* **2004**, *20*, 1807−1811.

(28) Major, J. S.; Blanchard, G. J.; Strategies, for Covalent, Multilayer, Growth. 2. Interlayer Linking Chemistry. *Chem. Mater.* **2002**, *14*, 2574−2581.

(29) Zhou, Y.; Bruening, M. L.; Bergbreiter, D. E.; Crooks, R. M.; Wells, M. Preparation of Hyperbranched Polymer Films Grafted on Self-Assembled Monolayers. *J. Am. Chem. Soc.* **1996**, *118*, 3773−3774.

(30) Amirpour, M. L.; Ghosh, P.; Lackowski, W. M.; Crooks, R. M.; Pishko, M. V. Mammalian Cell Cultures on Micropatterned Surfaces of Weak-Acid, Polyelectrolyte Hyperbranched Thin Films on Gold. *Anal. Chem.* **2001**, *73*, 1560−1566.

(31) Ghosh, P.; Amirpour, M. L.; Lackowski, W. M.; Pishko, M. V.; Crooks, R. M. A Simple Lithographic Approach for Preparing Patterned, Micron-Scale Corrals for Controlling Cell Growth. *Angew. Chem., Int. Ed.* **1999**, *38* (11), 1592−1595.

(32) Lackowski, W. M.; Ghosh, P.; Crooks, R. M., Micron-Scale Patterning of Hyperbranched Polymer Films by Micro-Contact Printing. *J. Am. Chem. Soc.* **1999**, *121*, 1419−1420.

(33) Russell, R. J.; Sirkar, K.; Pishko, M. V. Preparation of Nanocomposite Poly(allylamine)−Poly(ethylene glycol) Thin Films Using Michael Addition. *Langmuir* **2000**, *16*, 4052−4054.

(34) Elbert, D. L.; Hubbell, J. A. Conjugate Addition Reactions Combined with Free-Radical Cross-Linking for the Design of Materials for Tissue Engineering. *Biomacromolecules* **2001**, *2*, 430−441.

(35) Heggli, M.; Tirelli, N.; Zisch, A.; Hubbell, J. A., Michael-Type Addition as a Tool for Surface Functionalization. *Bioconjug. Chem.* **2003**, *14*, 967−973.

(36) Wacker, B. K.; Scott, E. A.; Kaneda, M. M.; Alford, S. K.; Elbert, D. L. Delivery of Sphingosine 1-Phosphate from Poly(ethylene glycol) Hydrogels. *Biomacromolecules* **2006**, *7*, 1335−1343.

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(37) Kim, J. K.; Scott, E. A.; Elbert, D. L. Proteomic Analysis of Protein Adsorption: Serum Amyloid P Adsorbs to Materials and Promotes Leukocyte Adhesion. *J. Biomed. Mater. Res., Part A* **2005**, *75* (1), 199−209.

(38) Lom, B.; Healy, K. E.; Hockberger, P. E. A Versatile Technique for Patterning Biomolecules onto Glass Coverslips. *J. Neurosci. Methods* **1993**, *50* (3), 385−397.

(39) Tronin, A.; Lvov, Y.; Nicolini, C. Ellipsometry and X-Ray Reflectometry Characterization of Self-Assembly Process of Polystyrenesulfonate and Polyallylamine. *Colloid Polym. Sci.* **1994**, *272* (10), 1317−1321.

(40) Lvov, Y.; Decher, G.; Mohwald, H., Assembly, Structural Characterization, and Thermal-Behavior of Layer-by-Layer Deposited Ultrathin Films of Poly(vinyl sulfate) and Poly(allylamine). *Langmuir* **1993**, *9*, 481−486.

(41) Voros, J.; Ramsden, J. J.; Csucs, G.; Szendro, I.; De Paul, S. M.; Textor, M.; Spencer, N. D. Optical Grating Coupler Biosensors. *Biomaterials* **2002**, *23* (17), 3699−3710.

(42) Huang, N.; Michel, R.; Voros, J.; Textor, M.; Hofer, R.; Rossi, A.; Elbert, D.; Hubbell, J.; Spencer, N. Poly(L-lysine)-*g*-poly(ethylene glycol) Layers on Metal Oxide Surfaces: Surface-Analytical Characterization and Resistance to Serum and Fibrinogen Adsorption. *Langmuir* **2001**, *17*, 489−498.

(43) Tosatti, S.; De Paul, S. M.; Askendal, A.; VandeVondele, S.; Hubbell, J. A.; Tengvall, P.; Textor, M. Peptide Functionalized poly(L-lysine)-*g*-poly(ethylene glycol) on Titanium: Resistance to Protein Adsorption in Full Heparinized Human Blood Plasma. *Biomaterials* **2003**, *24* (27), 4949−4958.

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