Understanding How Nanopatterning Affects Protein Deposition

B.J. Casey[*], J.S. Silverstein[**], P. Kofinas[***] and B.J. Dair[****]

[*]United States Food and Drug Administration, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Division of Chemistry and Materials Science, 10903 New Hampshire Avenue, Silver Spring, MD, USA, brendan.casey@fda.hhs.gov
[**] United States Food and Drug Administration, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Division of Chemistry and Materials Science, 10903 New Hampshire Avenue, Silver Spring, MD, USA, joshua.silverstein@fda.hhs.gov
[***]University of Maryland, College Park, MD, USA, kofinas@umd.edu
[****]United States Food and Drug Administration, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Division of Chemistry and Materials Science, 10903 New Hampshire Avenue, Silver Spring, MD, USA, benita.dair@fda.hhs.gov

ABSTRACT

A photochemical, thiol-ene based modification scheme was utilized to functionalize block copolymers (BCPs) from the parent polymer poly(styrene)-block-poly(1,2-butadiene) (PS/PB). The films were then processed using suitable solvent systems, spin-coated, and dried which resulted in the development of functionalized, nanopatterned films. Protein (fibrinogen, albumin, cytochrome C, IgG) deposition experiments were conducted in both static and dynamic conditions utilizing a Q-Sense E4 Auto quartz crystal microbalance with dissipation technology (QCM-D). Results indicate that the functional group, pattern, and experimental conditions (e.g. static or dynamic) all influence protein adsorption dynamics. The results have direct implications for the development of testing protocols to assess the safety and efficacy of medical devices consisting of surfaces that have been manipulated on the nanoscale.

Keywords: block copolymer, thiol-ene, nanopattern, poly(styrene)-block-poly(1,2-butadiene), protein adsorption

1 INTRODUCTION

With the advent of the nanotechnological age there is a growing trend in the medical device industry to manipulate the surface nanotopography of devices to promote or elicit desirable physiological responses such as better integration, faster regeneration, and/or quicker repair [1-5]. The mechanism by which cells and tissue detect and respond to nanotopographical features is still not fully resolved. *In vitro* studies have demonstrated that nanofeatures often have a significant effect on cell morphology, adhesion, motility, proliferation, endocytotic activity and gene regulation. Specific proteins in physiological fluids, such as blood plasma or bone marrow proteins, have been shown to mediate adhesion, differentiation and growth of cells on implant surfaces [1,6]. Furthermore, studies have shown that surfaces with nanofeatures have the capability to drastically affect the adsorption and conformation of these proteins [7-9]. Since the protein layer on a medical device directly impacts a number of physiological processes and is a main indicator of how the body reacts to a foreign substrate it is crucial to understand how such nanotextured surfaces influence protein adsorption dynamics, specifically blood protein adsorption dynamics. In this experiment, modified BCPs were used to create nanopatterned films to investigate the impact that chemistry and nanoscale features have on protein adsorption.

2 METHODS

2.1 Polymer Nanopattern Processing

PS/PB BCPs were modifed with amide, anionic, cationic, and pharmaceutical (captopril) functional groups using thiol-ene photochemistry. After purification and drying, the polymers were dissolved at 0.5 wt% in suitable solvents, or co-solvent mixtures, and spin-coated at 2000 rpm onto either silicon wafers or gold coated quartz crystals. Films were imaged using an Asylum MFP-3D atomic force microscope (AFM).

2.2 Quartz Crystal Microbalance Experiments

In a typical experiment, the polymer film was spin-coated on the quartz crystal sensor and placed in the QCM-D module. The films were equilibrated under flow (100 µL/min) with phosphate buffered saline (PBS) at pH 7.4 for at least 30 minutes until a stable baseline was achieved. After equilibration a 50 µg/mL protein PBS solution (albumin, fibrinogen, cytochrome c, or IgG) was introduced to the film for 60 minutes. Finally, the films were washed with fresh PBS to remove weakly bound proteins.

3 RESULTS

3.1 Polymer Nanopattern Processing

Nanopatterned polymer films were created by modifying the vinyl group on the PB block of PS/PB BCPs using thiol-ene photochemistry. Figure 1 below shows the structure of the PS/PB parent polymer. The modification scheme could be implemented to functionalize the PS/PB polymer with a number of functionalities to create a variety of daughter polymers each with unique chemistry. Furthermore, the modification scheme required no harsh reaction conditions and was completed within 1 hour.

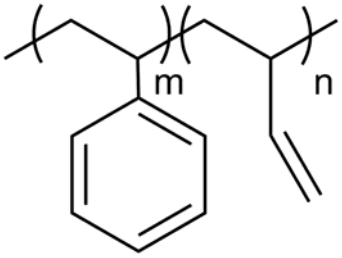

Figure 1: Structure of parent polymer poly(styrene)-block-poly(1,2-butadiene) (PS/PB) used to create functional nanopatterned surfaces.

Nuclear magnetic resonance spectroscopy (^{1}H NMR) was used to determine the functionalization degree. Once functionalization was confirmed, the various daughter polymers were solvated in tetrahydrofuran, dimethylformadide, propylene glycol monomethyl ether acetate, dimethyl sulfoxide or combination thereof. Atomic force microscopy was used to confirm that each film was phase separated and nanopatterning was achieved. Figure 2 below shows an AFM image of a nanopatterned, amide-functionalized BCP. This image is representative of typical nanopatterning that was achieved with various other functionalities, e.g. amine and carboxylic acid.

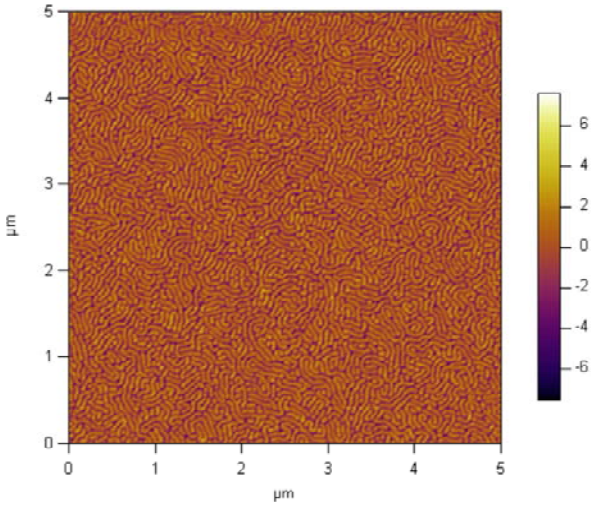

Figure 2: AFM image of a nanopatterned, amide-functionalized PS/PB BCP. Image is representative of the typical nanopatterning achieved for various types of functionalities.

3.2 Quartz Crystal Microbalance Experiments

The functionalized, nanopatterned films were utilized to investigate the influence that nanopatterned chemistry have on protein (albumin, fibrinogen, cytochrome c, IgG) adsorption. Protein deposition was analyzed under both static and dynamic conditions. Along with various functionalized block copolymers several control samples including the stock PS/PB, PS, and a random copolymer containing electrostatic charge with no nanopatterning were tested. Figure 3 below is a graph of typical raw data obtained from the QCM-D investigating the adsorption of fibrinogen onto a nanopatterned polymer film. The observed exponential frequency decrease corresponds to protein adsorption. The experiments indicated that chemistry, pattern, and the experimental conditions (static or dynamic) can drastically influence protein adsorption.

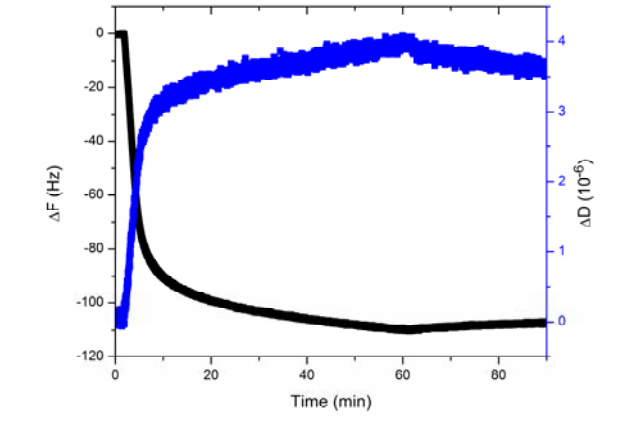

Figure 3: Raw data from QCM experiment investigating the adsorption of fibrinogen onto a functionalized, nanopatterned PS/PB BCP film. The black curve (left axis) corresponds to change in frequency while the blue curve (right axis) corresponds to change in dissipation.

4 CONCLUSION

In conclusion, a photochemical, thiol-ene based modification scheme was developed which allowed for the facile functionalization of a PS/PB BCP to produce a chemically diverse set of daughter polymers. These polymers were then processed with suitable solvents and spin-coated to produce nanopatterned films. These films were used to investigate the dependence that chemistry and nanopatterning have on protein adsorption. The results indicate that chemistry, nanopatterning, and experimental conditions (static or dynamic) all influence protein adsorption. The results have implications in the proper development of protocols to assess the protein adsorption of medical devices with nanoengineered surfaces.

Disclaimer: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

REFERENCES

[1] T.J. Webster, L.S. Schadler, R.W. Siegel and R. Bizios, "Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin," Tissue Engineering, 7, 291-301, 2001.

[2] L. Le Guehennec, A. Soueidan, P. Layrolle and Y. Amouriq, "Surface treatments of titanium dental implants for rapid osseointegration," Dental Materials, 23, 844-854, 2007.

[3] E. Stratkis, A. Ranella and C. Fotakis, "Biomimetic micro/nanostructured functional surfaces for microfluidic and tissue engineering applications," Biomicrofluidics, 5, 013411-1 – 013411-31, 2011.

[4] K.R. Milner and C.A. Siedlecki, "Fibroblast response is enhanced by poly(L-lactic acid) nanotopography edge density and proximity," International Journal of Nanomedicine, 2, 201-211, 2007.

[5] A. Thapa, T.J. Webster and K.M. Haberstroh, "Polymers with nano-dimensional surface features enhance bladder smooth muscle cell adhesion," Journal of Biomedical Materials Research Part A, 67A, 1374-1383, 2003.

[6] G. Kumar, C.K. Tison, K. Chatterjee, P.S. Pine, J.H. McDaniel, M.L. Salit, M.F. Young and C.G. Simon, "The determination of stem cell fate by 3D scaffold structures through the control of cell shape," Biomaterials, 32, 9188-9196, 2011.

[7] A. Hunter, C.W. Archer, P.S. Walker and G.W. Blunn, "Attachment and proliferation of osteoblasts and fibroblasts on biomaterials for orthopedic use," Biomaterials, 16, 287-295, 1995.

[8] N. Ferraz, B. Nilsson, J. Hong and M.K. Ott, "Nanoporesize affects complement activation," Journal of Biomedical Materials Research Part A, 87A, 575-581, 2008.

[9] F.A. Denis, P. Hanarp, D.S. Sutherland, J. Gold, C. Mustin, P.G. Rouxhet and Y.F. Dufrene, "Protein adsorption on model surfaces with controlled nanotopography and chemistry," Langmuir, 18, 819-828, 2002.