Biomimetic polymer brushes to control protein-surface interactions

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Statement of Purpose: Molecular and interfacial considerations are important regardless of the method used to create nanoscale polymer films: spin-coating, layer-by-layer deposition, various grafting methods, and interfacial transfer. Our research focuses on the creation of brush-like monolayer and bilayer block copolymer films by the use of an interfacial technique known as the Langmuir-Blodgett (LB, vertical) and Langmuir-Schaefer (LS, horizontal) methods. The LB and LS techniques have received attention to potentially create chemical sensors, ranging from integrated circuits to biomimetic surfaces. A major strength of LB and LS methods is the ability to externally impose molecular conformation and density of amphiphiles through compression at the air/water interface and transfer these properties onto a substrate. Through selection of interfacial conditions and the LB/LS technique, we have created brush-like block copolymer films of desired surface density, height, and structure. Such bilayer film properties suggested non-fouling capabilities and potential as a biomimetic surface. Therefore, we tested and confirmed their resistance protein adsorption.

Methods: 1,2-Polybutadiene-b-poly(ethylene oxide) (PBd-PEO, Mw=6-4k, respectively) was obtained from Polymer Source, Inc. (Canada) and fibrinogen was obtained from Sigma. Langmuir Films: The subphase used was reverse osmosis water. PBd-PEO was characterized as surface films at the water interface by pressure/area (Π-*A*) isotherms. LB/LS films: After application of a PBd-PEO solution, the monolayer was compressed by two mobile barriers to a target pressure and held constant. The first monolayer was transferred onto a hydrophilic SiO_2 wafer by the LB method with the PEO facing towards the wafer (hydrophilic-hydrophilic interactions). Once the initial monolayer deposition has dried, the LS deposition was performed to transfer a second monolayer onto the initial monolayer thereby forming a bilayer. The bilayer was kept submerged for protein adsorption studies. Protein adsorption studies: The media of the surfaces was exchanged with either phosphate buffer solution (control) or fibrinogen solution and incubated. After the incubation period, the substrate removed from the well and dried. Ellipsometry was used to measure changes in thickness of films.

Results: The interfacial behavior, i.e. conformation, orientation and density, of PBd-PEO can be controlled by compression at the water surface to give a surface pressure vs. area (Π-*A*) isotherm. With this isotherm we were in position to transfer our copolymer film onto a substrate by the LB method. Figure 1a plots the ellipsometric thickness *h* of transferred monolayers as a function of the air/water interfacial density. The interfacial air/water density is the inverse of the area per molecule, $\sigma_{AW} = 1/A$. The increase in thickness with density is linear, as expected from conservation of volume, and indicates polymer chain extension in the normal direction. Figure 1b represents a good agreement between experimental and calculated densities and implies a high efficiency of film transfer by the LB method. Taken together, a high transfer efficiency and high surface density strongly suggest that this monolayer maintains its brush-like character upon transfer with the PBd (hydrophobic) at the topmost of the surface.

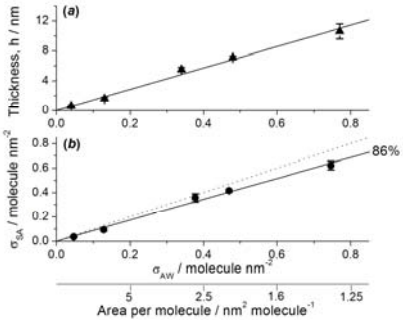

Figure 1. (a) Transferred film thickness h as a function of air/water interfacial density σ_{AW}, determined by ellipsometry. The linear increase is expected from conservation of volume. (b) "Substrate" interfacial density σ_{SA} as a function of σ_{AW}. Overall transfer efficiency is determined by the best-fit solid line, with perfect transfer represented by the dotted line.

The high interfacial density of the initial monolayer is crucial to transferring a second monolayer from the interface. Due to a large contact area of favorable hydrophobic-hydrophobic interactions, the LS method successfully transfers a second monolayer. As opposed to an unstructured film, the topmost surface of our bilayer presents a dense PEO brush, shown to be resistant to fibrinogen adsorption from solution (Figure 2). By contrast, a monolayer film presents a hydrophobic and attractive interface to fibrinogen. The changes in thickness reveal substantial protein adsorption on monolayer films and significantly less on bilayer films.

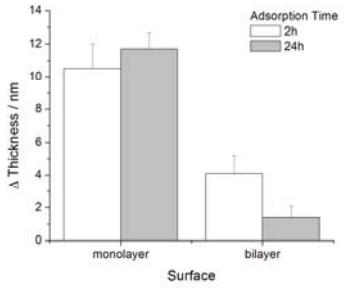

Figure 2. Change in thickness of monolayer and bilayer films following incubation with fibrinogen. Enhanced resistance to protein adsorption is found for the hydrophilic bilayer relative to the hydrophobic monolayer.

Conclusions: We expect our biomimetic bilayer films with PEO at the topmost of the surface will be useful to inhibit non-specific cell adhesion. Future work will involve: (1) end-labeling our polymers with adhesion ligands such as the tripeptide arginine-glycine-aspartate (RGD), so as to use polymeric bilayer films to control the extent of cell adhesion, spreading, and possibly migration and (2) fluorescent surface labeling to study lateral diffusivity to gain insight in cell-surface interactions.