pH-control of the protein resistance of hydrogel gradient films †
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Introduction and aim

The polyethylene glycol (PEG) brush is the quintessential antifouling coating, and has proven successful in many biomedical applications. However, insufficient chemical stability has stimulated research in alternatives, and zwitterionic charge-balanced materials have emerged as interesting candidates. The excellent protein resistance properties of these materials is believed to follow from the lack of a net charge and thus absence of electrostatic interactions with proteins, and strong hydration due to the high density of charged groups. In polymers this can be accomplished by inclusion of zwitterionic groups or by co-polymerizing anionic and cationic monomers. We have approached this issue in a slightly different way; since these materials are ampholytic, the net charge will depend on the pH, and to investigate pH effects we have prepared layered polymer gradients, where a homogeneous layer of a charged polymer is covered by a thickness gradient of an oppositely charged polymer. We follow the variations in surface charge, swelling and protein resistance along the hydrogel gradients, and demonstrate that bulk pH variation leads to a pH-dependent region of charge neutralization on the polymer surface, and to pH-controllable protein resistance.

Hydrogel gradient formation

Self-Initiated Photo-Grafting and Photopolymerization (SIPGP) can be used to polymerize thin films on organic substrates, without initiators or catalysts. In addition, the process permits easy formation of patterns or gradients. Here, we use the monomers CEA (2-carboxyethyl acrylate) (anionic) and AEMA (2-aminoethyl methacrylate hydrochloride) (cationic), preparing first a uniform bottom layer of one component, and then a thickness gradient of the other on top.

Swelling profiles

Figure 2. Thickness profiles of the gradients in air, water (MQ), and at various pH values in 10 mM buffers, as determined by spectroscopic ellipsometry. The thicknesses of the dry gradients increase from left to right, as shown in the cartoons above the diagrams.

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Surface charge distribution

Colloidal-probe AFM was used to monitor the charge distribution on the polymer surfaces. The gold-coated probes were negatively charged by formation of a carboxylic acid-functionalized alkythiol self-assembled monolayer.

Protein adsorption assays

The protein adsorption profiles were monitored by imaging Surface Plasmon Resonance (SPR). Proteins were injected sequentially over the samples, with pepsin followed by lysozyme, at 0.5 mg/ml. The differences in the SPR wavelengths, ΔλSPR, before and after protein injection quantify the protein adsorption onto the surface.

Conclusions

• PCEA shows greater, and more pH-sensitive swelling than PAEMA.
• The net charge of the gradients is dependent on the pH, and the location of charge-neutral regions on the gradients can be controlled by pH.
• The proteins pepsin and lysozyme adsorb selectively to positively and negatively charged regions, while the neutral region remains protein-resistant.
• As the pH changes, proteins partially or completely desorb in regions where the pH-change induces a reversal of the surface charge.