Characterization of Biocompatible Thin Films Using Neutron Reflectometry

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Introduction

The development of surfaces that prevent nonspecific protein adsorption is important for many biomedical and biotechnology applications including biomaterials, biochips, and biosensors. For this purpose, the modification of biointerfaces with poly(ethylene oxide) (PEO) or with polymers based on phosphorylcholine (PC) has been found to be effective. The mechanisms of PEO- and PC-mediated protein resistance are presently not clear, but it is recognized that polymer chain length, graft density, and the structural arrangement of water molecules associated with the PEO- and PC containing layers are important factors in determining the interactions of the surface with proteins. Neutron reflectometry (NR) is nondestructive and powerful for the study of polymer-solvent interactions under a variety of conditions. Using NR, the objective of the present work was to determine the thickness and volume fraction profiles of poly(oligo(ethylene glycol) methyl ether methacrylate) (poly(OEGMA)) and poly(2-methacryloyloxyethyl phosphorylcholine) (poly(MPC)) thin films grafted on silicon wafers in both the dry and wet states. Additional motivation was the capability of NR to provide information on the average number of water molecules per EO or PC residue for possible correlation to protein adsorption behavior.

Experimental

NR measurements were performed using the C5 spectrometer. The neutron wavelength was 2.37 Å. The collimation slits were varied during the scan to ensure that the entire sample was "bathed" in neutrons as its footprint varied. Specular reflection was measured and plotted against neutron momentum transfer *Qz*. The range of *Qz* was from 0.006 to 0.2 $Å^{-1}$. Measurements were performed over three *Qz* regions, namely, 0.2–0.1, 0.1–0.04, and 0.04–0.006 $Å^{-1}$. The data were normalized using the incident beam intensity to account for variations due to slit widths and were corrected for background by setting the angle to 0.5° off specular reflection. Reflectometry experiments were carried out using samples in both the dry and wet states.

Prior to measurement in the dry state, samples were cleaned with methanol (HPLC grade) and dried over nitrogen to remove any water bound to the polymer grafts. Samples were then immediately placed in the sample cell taking care to keep the layers dry. For NR measurements in the wet state, both pure D_2O and a H_2O/D_2O mixture containing 8.1% D_2O by volume to give a solvent of null scattering length density "null-SLD water" were employed. Following the measurement in D_2O, the sample cell was flushed *in situ* with methanol and dried in a nitrogen stream for 1 h. Null-SLD water was then injected into the cell. For dry samples, the path of the incident neutron beam was from air to the sample to SiO_2 to silicon, while in the wet condition, the incident neutron path was from the backside of the sample, i.e., silicon to SiO_2 to the sample to D_2O (or null-SLD water). This arrangement ensures total reflection from silicon in both cases. PARRATT 32 (BENSC, Berlin) software was employed to fit the reflectivity data. In the dry state, a three-layer model was used, while a two-layer and parabolic decay model was used to fit the data in the wet state.

Results

Figure 1 shows the SLD profiles for the dry layers. NR data were fitted to a three-layer model using the PARRATT 32 software: SiO_2, the initiator layer, and the polymer layer. The SLDs of Silicon, SiO_2, the initiator layer, and air were kept constant at the bulk values. All other parameters were allowed to vary to satisfy the minimum sum of squares criterion. The various polymer layer thicknesses are also in good agreement with the values obtained by ellipsometry. The roughness parameters show that the SiO_2 and initiator layers are relatively smooth. They are consistent with our previous AFM measurements.

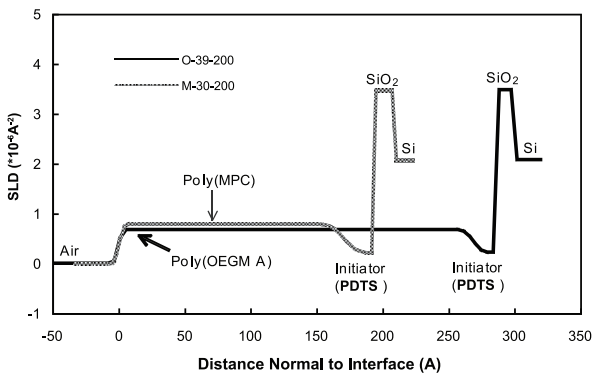

Fig 1. SLD profiles calculated using the best-fit data for surfaces.

The main objective of the present work was to investigate the conformational differences between poly(OEGMA) and poly(MPC) grafts in water, the medium most relevant to biomedical applications. Pure D_2O was chosen since it provides good neutron contrast for poly(OEGMA) and poly(MPC). Based on these considerations, the parabolic

profile equation was used to fit the wet state data in the present work. The SLD profiles in D_2O and in null-SLD water are shown in Figure 2. A noteworthy feature of the SLD profiles is the dramatic jump between the initiator layer and the innermost polymer film, suggesting that the initiator layer acts as a hydrophobic barrier, impermeable to water. From the SLD profiles, the polymer volume fraction as a function of distance through the film can be calculated. The polymer volume fraction profiles in D_2O for all the surfaces are shown in Figure 3. It is difficult to compare quantitatively the swelling behavior of poly(OEGMA) and poly(MPC) brushes in water because the polymer chains have different molecular distributions and graft densities. However, it appears that both poly(OEGMA) and poly(MPC) chains swell significantly in water, indicating strong interactions of these polymers with water.

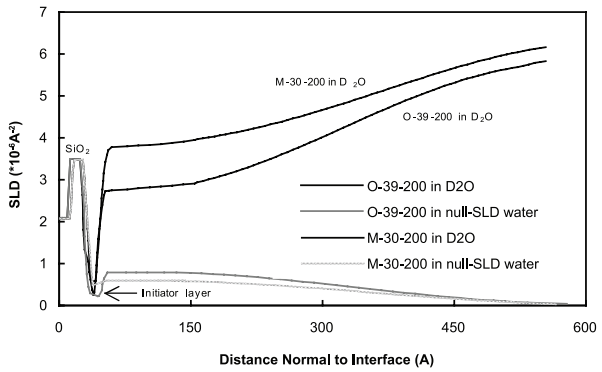

Fig 2. SLD profiles for surfaces in D_2O and in null-SLD water.

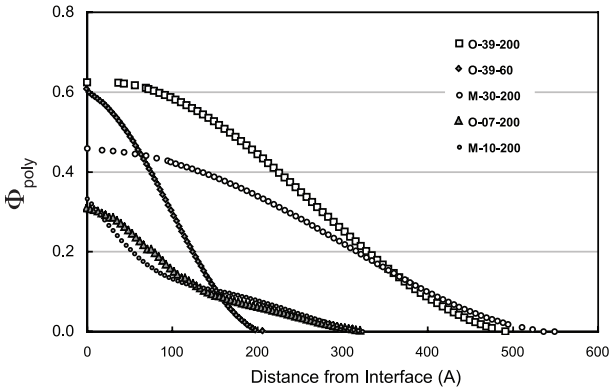

Fig 3. Polymer volume fraction profiles for surfaces in D_2O.

Conclusion

We investigated the structures of grafted poly(OEGMA) and poly(MPC) layers on silicon substrates (dry and wet state) using neutron reflectometry. For the dry surfaces, estimates of thickness and roughness from neutron reflectometry data are consistent with ellipsometry and AFM data, respectively. In the wet state, the best-fit NR data for the surfaces in D_2O and null-SLD water are in agreement. The parabolic model, with a stretched exponent, adequately describes the polymer fraction in the layer as a function of distance from the surface for both high and low graft densities. From estimates of the average number of water molecules per EO or PC moiety, it appears that the "water barrier" to protein adsorption is provided mainly by water in the bound state.

Publication

Wei Feng, Mu-Ping Nieh, Shiping Zhu, John L. Brash, Thad A. Harroun, and John Katsaras. Characterization of biocompatible acrylate polymer brushes bearing oligo(ethylene glycol) and phosphorylcholine side chains in water by neutron Reflectometry. Biointerphases. 2:34-43, 2007.