Protein resistant surfaces based on hydrophilic polymer grafts: investigation by neutron reflectometry

Wei Feng[1], Mu-Ping Nieh[2], Shiping Zhu[1], John Katsaras[2], John L. Brash[1].
[1] Department of Chemical Engineering, McMaster University, Hamilton, Ontario, Canada L8S 4L7
[2] Canadian Neutron Beam Center, National Research Council, Chalk River, Ontario, Canada K0J 1J0

Introduction: Poly(ethylene glycol) (PEG) and phosphorylcholine (PC) have been recognized as highly effective surface modifiers for the prevention of protein adsorption. The mechanisms underlying PEG- and PC-mediated protein resistance are not clear, but it is believed that the amount and the structural arrangement of water resident in the polymer layers are important factors in determining the interactions of the surface with proteins.[1] The direct comparison of PEG- and PC-based systems, with respect to their protein-resistant behaviors, has been reported previously.[2] Silicon wafer surfaces grafted with oligo(ethylene glycol) methyl ether methacrylate (OEGMA, with PEG side chains of n = 4.5) and 2-methacryloyloxyethyl phosphorylcholine (MPC) were chosen as the basis for comparison. It was found that for a given graft density and chain length, the protein resistance of OEGMA and MPC surfaces was similar. The objective of the present work was to investigate the disposition of water in these grafted polymer layers. Using neutron reflectometry (NR), the depth profiles of polymer volume fraction in the grafted layers were determined and correlated to their protein resistance.

Methods: The preparation and characterization of OEGMA- and MPC-grafted silicon wafers was reported previously[2]. Surfaces are referred to as "x-y", where x is the polymer type (O=OEGMA, M=MPC) and y is the graft density (chains/nm^2). The graft chain length was fixed at 200 monomer units. NR measurements were performed in D_2O at the Canadian Neutron Beam Centre. The reflectivity data were analyzed using a two-layer + parabolic decay model. The parabolic function is:[3]

$$\Phi_{poly}(z) = \Phi_{0,poly}\left(1-\left(\frac{z}{h}\right)^2\right)^{\alpha}$$

where $\Phi_{poly}(z)$ is the polymer volume fraction at a distance z from the solid-solution interface; $\Phi_{0,poly}$ is the polymer volume fraction at distance 0; h is the cutoff thickness of the polymer brush in the solvent; and α is a fitting parameter. Protein resistance was assessed by measuring the adsorption of fibrinogen from Tris buffer using radiolabelling methods.[2]

Results and Discussion: Four surfaces were investigated. The depth profiles of polymer volume fraction (Φ_{poly}) in the layers, obtained from modeling the neutron reflectivity data, are shown in Fig. 1. The parameters estimated from the model: $\Phi_{0,poly}$, h, the average volume fraction of polymer chains ($\overline{\Phi}_{poly}$), and α are listed in Table 1. Also shown in Table 1 are fibrinogen adsorption data from solutions of concentration 1 mg/ml. For both polymer types, $\Phi_{0,poly}$, h, and α were lower at the lower graft density. For the higher density layers (O-0.39 and M-0.30), the cutoff thicknesses were 430.2 and 456.0Å respectively, both of which are close to the contour length

of 500 Å, indicating the brush-like nature of the layers and the high quality of water as a solvent for both graft types. From the values of $\overline{\Phi}_{poly}$, the number of water molecules associated with each PEG unit ($N_{w,EG}$) and each PC unit ($N_{w,PC}$) were calculated and are also listed in Table 1. The number of water molecules in the bound hydration layers of EG and PC moieties are $N_{hyd,EG}$ ~2.5[4] and $N_{hyd,PC}$ ~25.[5] Thus for the high graft densities (O-0.39 and M-0.30), the proportion of bound water is high compared to the low graft densities (O-0.07 and M-0.10). Given that the protein resistance is much higher for the high than for the low graft density layers (the differences perhaps greater than can be accounted for by the relatively small differences in chain density) it may be that the "water barrier" is more effective when most of the water is in the bound state. The greater extension of the chains away from the solid interface at high density (higher values of h) may also promote protein resistance.

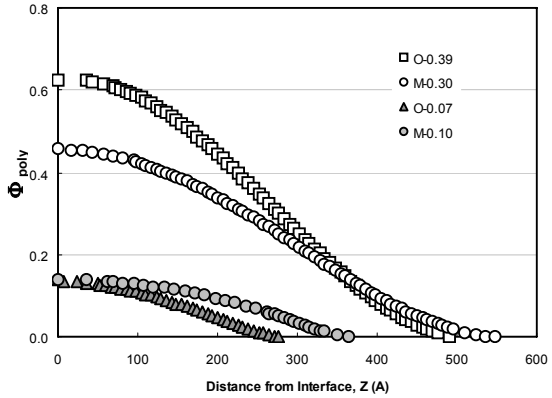

Fig. 1 Volume fractions of polymer layers in D_2O

Table 1. Properties of grafted polymer layers.

	O-0.39	M-0.30	O-0.07	M-0.10
$\Phi_{0,poly}$	0.63	0.47	0.14	0.14
h (Å)	430	456	194	277
α	3.2	2.2	1.4	1.1
$\overline{\Phi}_{poly}$	0.39	0.29	0.11	0.11
$N_{w,EG}$ or $N_{w,PC}$	4.9	30.4	30.6	98.9
Fibg ads (ng/cm^2)	8	7	99	62

References
1. R. Y. Wang, et al. 2005, J. Chem. Phys. 122: 164702.
2. W. Feng, et al. 2006, Biointerphases 1: 50.
3. M. S. Kent, et al. 1998, J. Chem. Phys. 108: 5635.
4. E. E. Dormidontova, 2002, Macromolecules 35, 987.
5. M. L. Berkowitz, et al. 2006, Chem. Rev. 106, 1527.

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