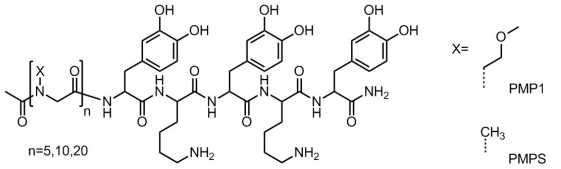
Long-Term Protein Adsorption Behavior on Polymer Brush Surfaces

K. H. Aaron Lau,[1,4] Erica Pollack,[1,4] Phillip B. Messersmith[1-6]

[1]Biomedical Engineering Department, [2]Materials Science and Engineering Department, [3]Chemical and Biological Engineering Department, [4]Chemistry of Life Processes Institute, [5]Institute for Bionanotechnology in Medicine, [6]Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Evanston, Illinois 60208.

Statement of Purpose: Long-term prevention of non-specific protein adsorption and cell adhesion on medical device surfaces is critical for their proper functioning. Since adsorbed proteins mediate cell-surface interactions, the development of surfaces resistant to protein adsorption is of major interest. However, protein adsorption is typically examined on experimental time-scales much shorter than device lifetimes. In this study, we evaluate the protein adsorption resistance of a set of peptidomimetic polymer (PMP) brush surfaces over 8 d. The PMPs are composed of N-substituted polyglycine peptoid brush segments joined to a pentapeptide mussel-mimetic adhesion motif for grafting onto substrate surfaces.[1] Peptoids are resistant to long-term enzymatic degradation[1] and different sidechains may be substituted to tailor the anti-fouling performance (Scheme 1). Fibrinogen (Fg) was chosen as the model protein due to the key roles it plays in the coagulation and inflammatory pathways. Adsorption was measured by *ex situ* ellipsometry and AFM, the latter of which could achieve very high sensitivities. Different brush chain lengths were also studied. Longer brush lengths generally confer higher protein resistance,[2, 3] but the long-term adsorption behavior has previously not been measured. The practical performance of different anti-fouling surfaces could also be more accurately defined through long-term evaluations.



Scheme 1. PMP chemical structure. PMP1 mimics PEG; Sarcosine units (PMPS) are reported to be anti-fouling.[4]

Methods: PMPs were synthesized by solid-phase synthesis using the submonomer protocol[3] and grafted onto TiO_2 films (3.5 nm; e-beam deposited on Si wafers) by 20 h immersion at 50°C (0.3 mM in 0.1 M MOPS, 3 M NaCl, pH 6). Fg was purchased from Sigma (~95% clottable) and dissolved in hepes buffer (1.2 mg/ml; pH 7.4). Adsorption was performed by incubation at 37°C in sterile conditions. Both positive (bare TiO_2) and negative controls (in unloaded buffer) were performed. Solutions were exchanged after 4 d to preclude protein degradation effects. Fg thickness was measured by spectroscopic ellipsometry after rinsing and drying in air, and scaled to the corresponding mass densities (Γ) according to the previously determined value on bare TiO_2 after 20 min adsorption (Γ_0 = 521 ng/cm^2).[3] Highly sensitive, complementary measurements of Fg surface coverages (θ) on PMP surfaces were measured by AFM in air, and Γ was estimated by equating θ =1 with Γ_0. Independent sample sets were prepared for each time point.

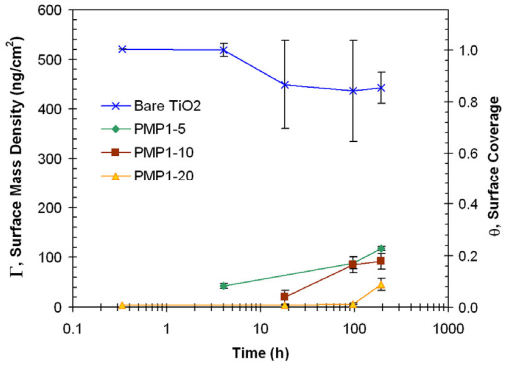


Figure 1. Fg adsorption on PMP-brush-coated TiO_2.

Results: Figure 1 shows the evolution of Fg adsorption over 192 h on PMP1 coated surfaces and on bare TiO_2. A full Fg layer was adsorbed on bare TiO_2 after 20 min adsorption and the adsorbed amount stayed relatively constant up to 8 d, indicating experimental conditions free from contamination and Fg aggregation. As anticipated, the longest PMP1 chain length ($PMP1_{20}$) was able to most effectively resist protein adsorption. Comparison with the adsorbed amount on bare TiO_2 indicated $PMP1_{20}$ was 99% effective in resisting Fg adsorption up to 96 h (6.3±1.7 ng/cm^2), whereas significant adsorption was already observed on $PMP1_5$ after 4 h (43±5 ng/cm^2). However, the amounts of Fg adsorbed on $PMP1_{10}$ and $PMP1_{20}$ coated surfaces were similar after 18 h. The difference in performance between $PMP1_{10}$ and $PMP1_{20}$ was obviously discernable only when the samples were measured at 96 h. Further, the fouling resistance of $PMP1_{20}$ slightly decreased only when measured at 192 h (90% effective, 46±12 ng/cm^2). PMP-coated control samples immersed in unloaded buffer showed only small losses in brush thickness up to 15 d (~10%; data not shown). The adsorption on $PMPS_{20}$ at 18 h has also been measured (8.8±2.4 ng/cm^2), which is not significantly different from the corresponding $PMP1_{20}$ value (3.9±1.2 ng/cm^2) at the 5% level. Long-term characterization of $PMPS_{20}$ is on-going.

Conclusions: It is shown that anti-fouling surfaces can be evaluated over the long-term to accurately characterize and differentiate their performances. Further long-term measurements and comparisons between different peptoid sidechain designs could provide insight into possible protein adsorption mechanisms and help improve the design of anti-fouling brush surfaces.

References:

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