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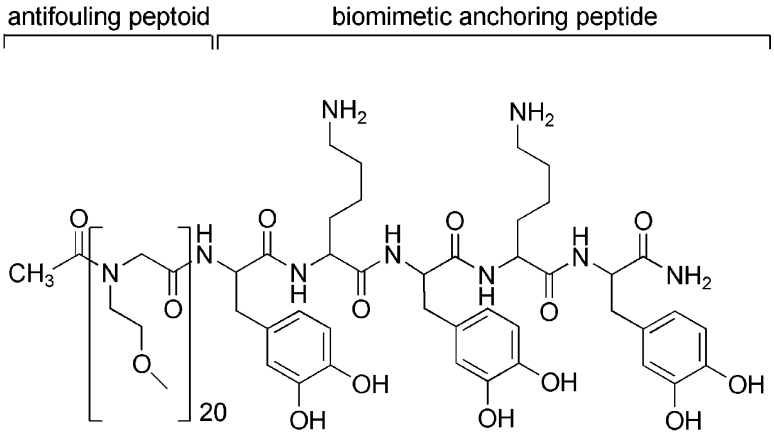

PMP1

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New Peptidomimetic Polymers for Antifouling Surfaces

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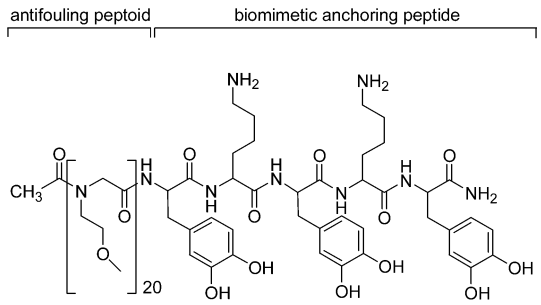
Protein, cell, and bacterial fouling of surfaces occurs spontaneously upon exposure of medical implants and diagnostic devices to physiologic fluids and tissues. In many cases biofouling is an adverse event that can impair function of medical devices. Examples of problematic biofouling include occlusion of cardiovascular implants by thrombus,[1] protein accumulation onto biosensor surfaces,[2] and bacterial colonization of contact lenses and indwelling catheters.[3] Patient infections and other complications from biofouling significantly increase the cost of healthcare delivery and can lead to compromised implant performance or even implant failure. Common strategies for inhibiting biofouling consist of grafting antifouling polymers or self-assembled monolayers onto surfaces.[4] A variety of synthetic polymers have been investigated as antifouling coatings and have met with variable success in in vitro and in vivo antifouling tests; however, none have yet proven ideal for long-term prevention of protein, cell, and bacterial fouling of surfaces.[5]

Here we report an entirely new class of synthetic antifouling macromolecules that are mimics of polypeptides. The polymers are specifically designed for robust, water-resistant anchorage to biomaterial surfaces and long-term resistance to fouling in the biological environment. The general design of our chimeric peptidomimetic polymers (Chart 1) consists of a short functional peptide domain for robust adsorption to surfaces, coupled to an *N*-substituted glycine (peptoid) oligomer of variable length that provides resistance to protein and cell fouling. Peptoids are nonnatural mimics of peptides that have a protein-like backbone, with side chain derivatization on the amide nitrogen instead of the α-carbon,[6] and are currently being explored as therapeutic protein/peptide mimics and for use in bioseparations.[7] The methoxyethyl side chain used in the present study was designed on the basis of current understanding of functional groups that provide fouling resistance to surfaces[8] and resembles the repeat unit of poly(ethylene glycol) (PEG), a widely studied antifouling polymer.

To achieve effective and long-lasting fouling resistance in a physiologic environment, the antifouling polymer must be robustly anchored and water-resistant. With this in mind, the composition of the anchoring peptide domain was chosen to mimic the adhesive proteins used by marine mussels to attach to underwater surfaces.[9] The adhesive properties of mussel adhesive proteins (MAPs) are believed to be due in part to the presence of L-3,4-dihydroxyphenylalanine (DOPA), an amino acid that is formed by posttranslational modification of tyrosine. Of the several blue mussel (*Mytilus edulis*) adhesive pad proteins identified by Waite and co-workers, Mefp-5 is located closest to the interface between the adhesive pad and substrate and has the highest DOPA content (~27%) of any isolated MAP, with over 75% of the DOPA residues immediately adjacent to basic residues such as lysine (Lys).[10] The anchoring peptide used in this study directly mimics Mefp-5, consisting of a 5-mer peptide of alternating DOPA and Lys residues.

Chart 1. Antifouling Peptidomimetic Polymer



PMP1

The peptidomimetic polymer (**PMP1**) was synthesized on solid-phase Rink amide resin by first synthesizing the adhesive peptide anchor with standard Fmoc strategy followed by synthesis of a 20-mer *N*-methoxyethyl glycine peptoid using the submonomer protocol.[11] The amine terminus was acetylated, and then the molecules were cleaved from the resin, purified by RP-HPLC, and analyzed by RP-HPLC and MALDI mass spectrometry.

PMP1 was found to be highly soluble in aqueous solutions and adsorbed strongly onto Ti surfaces by simple immersion of the substrate into the polymer solution. Unmodified and modified Ti surfaces were analyzed by time-of-flight secondary ion mass spectrometry (TOF-SIMS) and X-ray photoelectron spectroscopy (XPS) (see Supporting Information). The positive ion TOF-SIMS spectrum of **PMP1**-modified Ti revealed numerous fragments representing the presence of adsorbed **PMP1** including the methoxyethyl side chain of the peptoid. The negative ion spectrum contained peaks for 1-, 2-, and 3-mer peptoid fragments as well as fragments corresponding to Ti complexed with the catechol, DOPA, and peptoid residues. XPS analysis of the surfaces modified with **PMP1** revealed further evidence of adsorption onto the Ti surface. High-resolution spectra were used to calculate atomic composition, showing a significant increase in carbon and nitrogen and a significant decrease in titanium and oxygen upon adsorption of **PMP1** onto the Ti surface.

Protein adsorption experiments using optical waveguide light-mode spectroscopy (OWLS) revealed that modification of Ti surfaces with **PMP1** resulted in a dramatic reduction in protein adsorption (Figure 1). Unmodified Ti surfaces exposed to whole human serum for 20 min resulted in an adsorbed protein layer with a mass of approximately 435 ng/cm^2 after rinsing. In stark contrast, serum protein adsorption onto peptoid-modified waveguides under identical conditions was reduced to approximately 4 ng/cm^2. This amount of protein adsorption is similar to that adsorbed onto PEG coatings[12,13] and to oligoethylene glycol-terminated SAMs,[8] demonstrating the excellent protein resistance of the peptidomimetic polymer.

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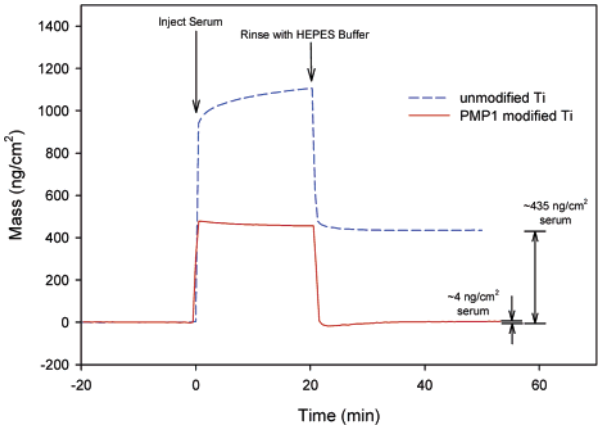

Figure 1. **PMP1**-modified surfaces exhibit short-term resistance to serum protein adsorption. Shown is the mass plot of serum protein adsorption on unmodified and **PMP1**-modified Ti.

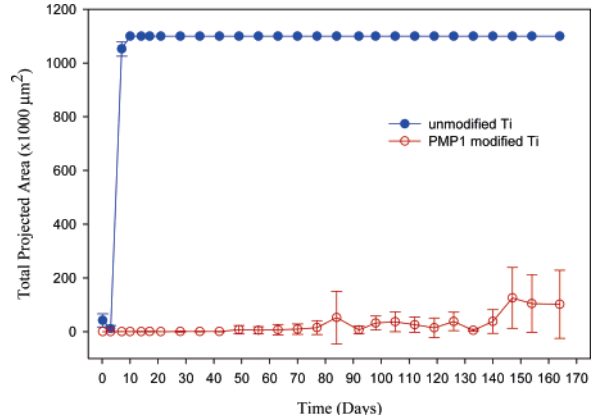

Figure 2. **PMP1**-modified Ti surfaces exhibit long-term resistance to 3T3 fibroblast cell attachment.

Finally, the ability of **PMP1**-modified surfaces to resist cell attachment over a long period of time was determined by culturing 3T3 fibroblast cells on unmodified and modified Ti surfaces in the presence of serum. Fresh cells were seeded twice weekly onto the Ti surfaces for several months, and cell attachment at various time points was quantified by fluorescence microscopy and image analysis. Although fibroblasts readily attached to unmodified Ti surfaces and were nearly confluent after several days (Figure 2), the **PMP1**-modified surfaces exhibited remarkably low levels of cell attachment for over 5 months. Since cell attachment to surfaces is typically mediated by adsorbed protein, we can infer from this result that serum protein adsorption also remained low throughout the course of the in vitro experiment.

The excellent protein resistance of **PMP1** and its ability to maintain fouling resistance for several months under frequent challenge with fresh serum and cells can be directly attributed to the chemical composition of the anchoring (peptide) and antifouling (peptoid) domains. The catechol side chains of DOPA are believed to form strong charge-transfer complexes to metal oxide surfaces,[12,14] and the cationic nature of the Lys residues contributes an electrostatic attraction to the negatively charged oxide surface,[15] forming a robust anchor for the antifouling (peptoid) portion of the polymer. The design of the peptoid side chain and backbone adheres to general principles put forth by others[8] for effective antifouling surfaces: presence of hydrogen bond acceptors, lack of hydrogen bond donors, neutral charge, and water solubility. Additional benefits of peptoids include protease resistance of the backbone,[16] precise control of molecular weight with high yield, and virtually unlimited compositional versatility obtained through variation of side chain composition in the form of both natural and nonnatural side chains.[11,17] These new synthetic peptide based antifouling polymers may provide long-term control of surface biofouling in the physiologic, marine, and industrial environments. In the future, the synthetic diversity of these peptidomimetic polymers could be exploited to better understand the fundamental relationship between fouling resistance and polymer chemical composition.

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Supporting Information Available: Complete ref 6, experimental details, analytical RP-HPLC, and MALDI-MS, TOF-SIMS and XPS data. This material is available free of charge via the Internet at http://pubs.acs.org.

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