Ultralow Fouling Surfaces Derived from Peptide Self Assembled Monolayers

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Statement of Purpose: Materials that resist nonspecific biomolecule and microorganism attachment have many potential biomedical applications including medical implants, drug delivery carriers, and biosensors. Previously, we have developed ultralow fouling peptide-based self-assembling monolayers (SAMs) derived from the design principle of alternating positively and negatively charged residues. These "zwitterionic-like" peptides exhibit high resistance to nonspecific protein adsorption (< 5 ng/cm^2 adsorbed protein), comparable to what is achieved by poly(ethylene glycol) (PEG-based materials.[1] Short peptides composed of the twenty naturally occurring amino acids offer a nearly infinite number of possible combinations that can result in varying surface properties. Peptide thin films are advantageous as biomaterials since their final metabolized products are natural amino acids.This work examines the low fouling properties of peptide SAMs composed entirely of amino acid residues, and specifically the influence of the anchor portion of the peptide sequence on nonspecific protein adsorption. Peptide SAMs were evaluated for protein adsorption via surface plasmon resonance (SPR) sensors. A cysteine residue anchor was shown to be inferior to the previously used thiol end group. However, a cysteine residue in conjunction with four proline residues was shown to result in a peptide SAM with ultralow fouling properties.

Methods: Peptides were ordered from Synthetic Biomolecules (San Diego, CA) at a purity of greater than 95%. Gold coated SPR chips were cleaned and then incubated with a phosphate buffered saline (PBS) aqueous solution (pH 7.4 and Ionic Strength of 150 mM) of 0.2 mg/mL peptide solution for 24 hours. Protein adsorption was determined by a custom-built four channel SPR sensor. Gold chips covered with peptide SAMs were rinsed with Millipore water, dried by filtered air, and mounted to the device. The temperature controller was set to 25 ± 0.01˚C. Protein adsorption was measured by flowing PBS buffer at 40 µL/min for 10 minutes, 1 mg/mL protein solutions of fibrinogen (from bovine plasma, Sigma) and lysozyme (from chicken egg white, Sigma) for 10 minutes, and PBS buffer again for 10 minutes. The wavelength shift between baselines before and after protein injection was used to quantify the total amount of protein adsorbed. A reference channel containing solely PBS buffer was flown for each chip and the baseline drift was subtracted from the final wavelength change. A 1 nm wavelength shift from 750 nm corresponds to 17 ng/cm^2 adsorbed proteins. The detection limit for the SPR sensor is 0.3 ng/cm^2.

Results: Rational design principles were applied via simulation and peptide self assembling knowledge to choose promising sequences. The low fouling segment of the peptide was kept consistent with previous work and was composed of the alternating positively and negatively charged sequence EKEKEKE attached to an anchor.

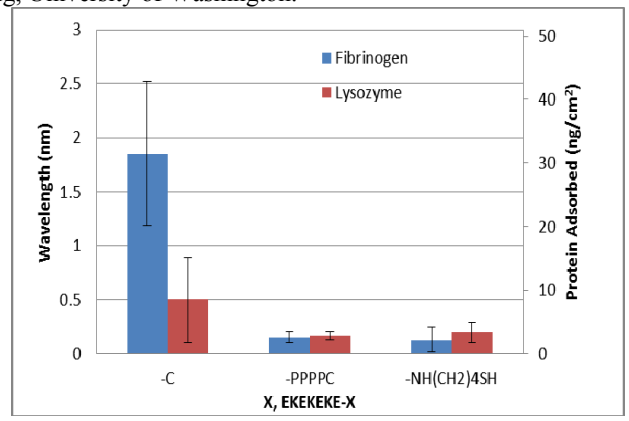

Figure 1. Adsorption of fibrinogen and lysozyme on peptide SAMs determined from a SPR sensor

The N-terminus was left as a free amine for the overall peptide charge to remain neutral. The C-terminus was amidated to cap the negative charge of the carboxylic acid which could potentially create a negative surface charge and impede packing. Cysteine was chosen as the anchor residue since it contains a thiol side group which binds strongly to gold. However, the replacement of the thiol anchor group in the peptide sequence EKEKEKE-$NH(CH_2)_4$-SH, with a cysteine residue containing a thiol side chain, EKEKEKEC-Am, resulted in the loss of ultralow fouling properties (ultralow <5 ng/cm^2 adsorbed protein). However, the inclusion of four proline residues in addition to the cysteine residue, EKEKEKEPPPPC-Am, restored the ultralow fouling properties as shown in Fig. 1. The proline residues are believed to allow a more rigid, extended structure that will allow improved packing and orientation of peptide chains on the surface.

Conclusions: The results of this experiment indicate that simply having any gold bonding group, such as a cysteine, is not sufficient to develop ultralow fouling peptide SAMs. Currently, further surface characterization methods such as atomic force microscopy (AFM) and x-ray photoelectron spectroscopy (XPS) are being employed to gain further insight into the organization of the peptide SAMs. In addition, simulations of peptide SAMs are being explored to obtain information regarding potential peptide conformations and interactions. Future work will involve the examination of other anchor groups such as the addition of hydrophobic residues. Furthermore, successful peptide SAM sequences can potentially be translated to a curved gold nanoparticle system, but will require more investigation. The fundamental understanding of the effect of the anchor group on the low fouling properties will lead to better design principles for peptide SAMs resulting in fully biodegradable, affordable, self assembling peptide systems.

References: [1](Chen S., Cao Z., Jiang S. Biomaterials. 2009;30:5892-5896)