PEGylated-Peptide Coatings for the Inhibition of Pathogenic Biofilms on Titanium Metal

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Statement of Purpose: The lifetime, reliability, and performance of many medical implants are often hindered by bacterial adhesion and infection. It is estimated that over 50% of hospital-acquired infections are associated with implants and other indwelling medical devices. The importance of identifying a coating that prevents unfavorable bacterial interactions is evident considering the increasing utilization of these devices. Herein, we describe the design of a bacteriophobic PEGylated-peptide coating that assembles through adsorptive mechanisms onto material surfaces from a dilute aqueous solution. Specifically, we investigate the ability of these coatings to convert titanium (Ti), a ubiquitous implant metal, into a fouling-resistant surface. Treated surfaces demonstrate excellent resistance to fibronectin adsorption, as well as greatly reduced colonization and biofilm formation by *Staphylococcus aureus* cultures *in vitro*.

Methods: Ti-binding peptide sequences were identified using a combinatorial phage display process as previously described.[1] Peptides were synthesized using standard FMOC protocols and assessed for binding affinity using a modified ELISA. A high affinity sequence with minimal cross-reactivity to other materials was selected as a 'parent' sequence from which higher affinity peptides were derived through an amino acid substitution exercise. The best Ti-binding peptide (**TBP**) showed a 100-fold affinity improvement over the parent peptide ($K_A = 3.5 \times 10^8\ M^{-1}$). **TBP** was synthesized with a terminal PEG (Mw 3400) to afford a fouling resistant coating, **TBP-PEG**. Ti substrates were treated with peptide coatings (40µM in DPBS) and subject to contact angle, X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM) analysis. Real-time adsorption of **TBP** was studied using quartz crystal microbalance with dissipation monitoring (QCM-D). 40 µM peptide solutions were introduced into the system and the resultant frequency and dissipation shifts recorded. Fibronectin (FN) was subsequently introduced into the chambers to determine the ability of coatings to resist protein adsorption. Finally, bacteria adhesion assays were performed using a pathogenic strain of *Staphylococcus aureus*. Treated Ti surfaces were exposed to 10^6 CFUs/mL of a *S. aureus* suspension and assayed for adhesion and biofilm formation after 4h.

Results/Discussion: The various surface analyses indicate that simple immersion of Ti substrates in a dilute peptide solution results in the spontaneous formation of a thin and dense coating. Ti substrates exposed to **TBP** showed a large change in static water contact angles from 71.3° to 47.3° ($p<0.05$) while XPS analysis revealed a new strong nitrogen peak as compared to bare Ti, confirming the presence of an adhered peptide-based surface coating. Finally, AFM surface scans indicated the formation of a densely packed adlayer with an average peptide height of 0.5 nm. Ti incubated with unconjugated

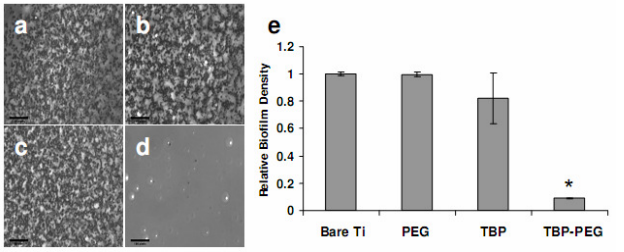

Figure 1: Assessment of biofilm formation. *S. aureus* adhesion on various coated Ti surfaces: (a) uncoated Ti, (b) PEG, (c) **TBP**, and (d) **TBP-PEG**. Scale bar = 35 µm. (e) Biofilm formation quantified using a crystal violet assay. Surfaces treated with **TBP-PEG** show significantly reduced biofilm density. (n=3; *P < 0.01).

PEG showed no change in surface properties, underscoring the importance of the **TBP** component for surface immobilization of molecules.
These findings were further confirmed by QCM-D. Exposure of Ti substrates to peptides resulted in immediate adsorption, as evidenced by a rapid decrease in the frequency signal. QCM-D was also used to compare FN binding on coated and uncoated surfaces. The resulting frequency shifts show that the Ti surface initially exposed to **TBP-PEG** showed no change in frequency signal upon FN addition, proving inhibition of FN adsorption. All other surfaces showed significant non-specific adsorption of FN. More importantly, Ti substrates treated with **TBP-PEG** showed significantly reduced *S. aureus* attachment and colonization after a 4h incubation while control surfaces were completely overrun with bacteria. (Figure 1) These initial studies strongly support the utilization of these coatings for the prevention of implant-associated infections in the clinic.

Conclusions: In summary, we have designed and characterized peptide coatings with high affinity and specificity for titanium metal. In particular, we demonstrate the ability of PEGylated-peptides to greatly reduce *S. aureus* adhesion and biofilm formation on treated surfaces. These PEGylated coatings show promise in terms of resolving two major problems in device applications - (i) non-specific protein adsorption and (ii) bacterial infections.[2] In addition, the modular nature of these coatings allows the immobilization of other functionalities (e.g., growth factors, drugs etc.) on virtually any target substrate. Continued research in this area will foster the development of next-generation bio-inspired medical implants and devices.[3-5]

References:

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