Characterization of a Metal-binding Interfacial Peptide Using AFM and QCM-D

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Statement of Purpose: Unwanted host responses or poor integration at the tissue-implant interface often lead to implant failure or rejection, resulting in sub-optimal medical outcomes. The ability to modulate the biological and chemical reactions at this interface will have considerable impact on implant performance in a variety of applications. Towards this goal, we have synthesized and developed biomimetic interfacial peptides capable of recognizing specific biomaterials. In this study, peptide sequences with specific affinity to titanium (Ti) were identified using a combinatorial phage display screening process and modified with a terminal biotin group to afford functionality. Atomic force microscopy (AFM) and quartz crystal microbalance with dissipation (QCM-D) techniques were employed to investigate the physical characteristics of these peptides and to quantify their affinity, kinetics and overall performance on titanium.

Methods: Ti-binding peptide sequences were identified using a phage display process as previously described.[1] Using standard FMOC protocols, peptides with corresponding sequences were synthesized with a C-terminal biotin. One such peptide with a high Ti affinity, SHKHGGHKHGSSGK-biotin (Ti-biotin, K_A = 5 x 10^6 M^{-1}), was selected for further characterization. For AFM studies, 0.1 mg/mL Ti-biotin was diluted in 10 mM PBS (pH 7.4) to obtain concentrations down to 0.1 ng/mL. Freshly cleaved mica (Ted Pella Inc., CA) was used as a model hydrophilic substrate. Mica discs were incubated with Ti-biotin for 2 hours at RT. Streptavidin-alkaline phosphatase (Strep-AP, 10 µg/mL) was then added to investigate biotin functionality. Substrates were washed with buffer and Milli-Q water and dried with N_2 gas. Samples were then imaged in air using a Nanoscope IIIa AFM (Digital Instruments, Santa Barbara, CA) under tapping mode. Provided software was used to extract quantitative data, such as surface profiles, from images.

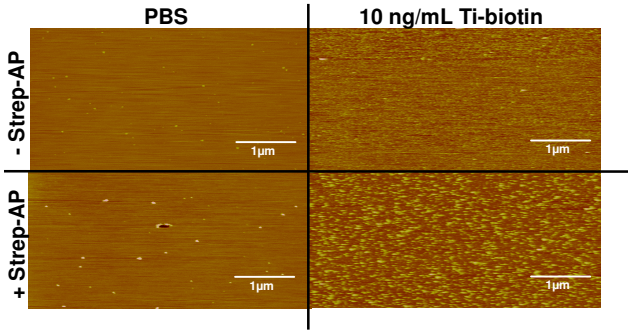

Figure 1: AFM images of mica surfaces incubated for 2 hours in PBS or 10 ng/mL Ti-biotin solution, with and without the subsequent addition of 10 µg/mL Strep-AP.

For QCM-D studies, gold-coated quartz crystals (Q-sense AB, Gothenburg, Sweden) with a fundamental frequency of 5 MHz were coated with 200 nm of Ti. Crystals were cleaned with UV/Ozone and 2% SDS before loading into the Q-sense E4 system. A stable baseline in buffer was obtained before introduction of the peptide solution. Following a buffer wash to remove unbound peptides, 10 µg/mL Strep-AP was introduced to the system. A buffer control was run in parallel using a second measurement chamber. Frequency (*f*) and dissipation (D) values measured at the seventh overtone were used for analysis.

Results/Discussion: AFM images on mica (Fig. 1) reveal Ti-biotin adsorbed as small globular macromolecules. The cross-sectional profile indicates a mean peptide height of 0.5 nm. A dense, continuous peptide layer was observed at a concentrations 0.1 µg/mL and above. Upon addition of Strep-AP, a second layer of larger globular proteins (mean height ~ 2.5 nm) was observed over the initial peptide film. As shown in Figure 2, Ti-biotin binding leads to a Δf of -8 Hz, corresponding to an adsorbed mass of 150 ng/cm^2 using the Sauerbrey relation.[2] Binding of a large protein like Strep-AP results in a big decrease in *f* and increase in D due to the formation of a thicker, more dissipative film. A Langmuir isotherm was used to deduce adsorption kinetics. K_{on} and k_{off} were 6.14 x 10^3 $M^{-1}s^{-1}$ and 8.04 x 10^{-4} s^{-1} respectively. K_A was estimated as 5.43 x 10^6 M^{-1}, similar to ELISA results. The results reveal a rapid adsorption (t < 1 min) to form a thin, dense peptide film that retains a biotin-streptavidin binding capability.

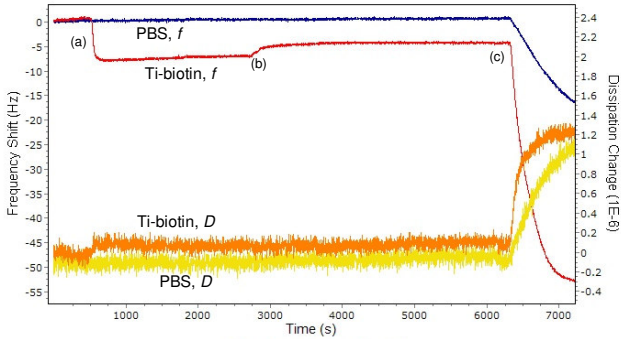

Figure 2: Real-time *f* and D shifts following (a) Ti-biotin (10 µg/mL) binding, (b) buffer wash to remove loosely bound peptide, and (c) Strep-AP (10 µg/mL) addition.

Conclusions: We have developed a novel class of titanium-binding peptides with high affinity and functionality as established by QCM-D and AFM. These peptides present a non-covalent but robust method of surface modification. The biotin group can be functionalized with specific ligands and other biological mediators, imparting the ability to control the invoked response at the tissue-implant interface. Furthermore, the phage display platform allows identification of unique peptides sequences for a variety of biological and synthetic materials, affording a powerful tool for the improvement of medical implant performance. We would like to thank the NIH for their financial support.

References:

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