Supplementary Methods

Directed Assembly of PEGylated-Peptide Coatings for Infection-resistant Titanium Metal

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Isolation of Parent Titanium-Binding Peptides

Metal-binding sequences were identified using phage display libraries developed in-house from phage type M13. Fourteen different peptide phage libraries displaying random peptide sequences on their pIII coat proteins were screened for sequences that bind strongly to titanium (Ti). Ti-6Al-4V beads approximately 4 mm in diameter (Dynamet, Washington, PA) were prepared for selections by washing extensively with 70% ethanol, 40% nitric acid, distilled water, 70% ethanol, and acetone to remove any surface contaminants. Clean beads were then characterized by SEM and XPS. For the panning procedure, one Ti bead was placed into each well of a 96-well polypropylene plate. Non-specific binding sites on the beads and the polypropylene were blocked by incubating with 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS) for 1 h at RT with gentle shaking. Wells were then washed 5 times with PBS-Tween 20. Each library was diluted in PBS-T and added at a concentration of

10^{10} pfu/mL in a total volume of 100 µL. Following a 3 h incubation at RT and shaking at 50 rpm, unbound phage were removed by 5 washes of PBS-T. To recover and amplify the phage bound to the Ti beads, the phage-bead complexes were added to E. coli DH5αF' cells and incubated overnight at 37 °C in an incubator/shaker at 210 rpm. Phage supernatant was harvested after spinning at 8500 g for 10 minutes. The amplified phage population then served as input for the next round of selection. Three such rounds of screening were completed.

On-Phage Binding Measurements

Freshly amplified phage particles were added to vials each containing Ti-6Al-4V beads and allowed to bind for 1 hour at RT with gentle shaking. Beads were washed five times in PBS-T to remove unbound phage, then incubated with 1:5000 HRP-anti-M13 antibody (Amersham-Pharmacia; Piscataway, NJ) in PBS-T for 1 h. Vials were washed five times with PBS-T buffer before the addition of the chromogenic substrate ABTS [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)]. Following a 15 minute incubation at RT, supernatants were transferred to a 96-well ELISA plate (Costar, Corning, NY) and the absorbance signal indicative of bound peptide was measured at 405 nm using a plate reader (Beckman Coulter, Fullerton, CA).

Peptide Synthesis

Peptides were commercially synthesized by solid-phase peptide synthesis techniques. The resultant peptides were purified to at least 95% purity and analyzed using HPLC and mass spectroscopy. Biotinylated peptides for affinity constant calculations were synthesized with a C-terminal biotin attached through the epsilon amide of a lysine residue.

Binding Affinity and Specificity ELISAs

Binding affinities were calculated using a modified ELISA assay. Grade 200 titanium beads (Abbott, washed with acetone) were blocked with 1% BSA for 1 hour, then exposed to sequential 1/3 dilutions (starting from 10 μM) of appropriate biotin-terminated peptides in PBS in multi-well plates and incubated for 1 hour at RT with 500 rpm shaking. After 3 washes in PBS-T, 200 μL of 1:1000 streptavidin-AP (Promega) in TBP + 1% BSA was added to each well and again incubated for 30 min at RT. After 3 washes in PBS-T to remove unbound streptavidin, the chromogenic substrate p-Nitrophenyl Phosphate (PNPP) was added to each well. Upon color development, supernatants were transferred to the corresponding well of a clear plate (containing 10 μL NaOH 1M to stop the reaction) and the absorbance signal indicative of bound peptide was determined at 405 nm using a plate reader in endpoint mode. A plot of absorbance versus log peptide concentration yielded a sigmoidal binding curve. The apparent dissociation constant (or "relative K_d") was extracted from the peptide concentration corresponding to 50% of the maximal absorbance signal. The apparent dissociation constant is typically within a factor of 2 to dissociation constants determined using a direct assay. Starting with the initial peptides identified by phage display on titanium, a series of additional experiments, including focused library screening and amino acid substitution studies, were performed to define the amino acids critical for binding and improve the affinity of the peptides on Ti. To investigate the cross-reactivity of **4**, ELISAs were repeated on a variety of different beads/coupons, including oxinium, stainless steel and polystyrene using a similar protocol.

Preparation of Titanium Substrates

Due to the difficulty in evaporating alloys because of differing vapor pressures of the components, commercially pure Ti (c.p. Ti; 99.998% purity) films evaporated on glass substrates were substituted as a model for subsequent studies. Clean glass substrates were coated with 20 nm – 100 nm of c.p. Ti using electron beam evaporation (Sharon Vacuum, Brockton, MA). X-ray photoelectron spectroscopy (XPS) analysis shows a similar surface composition (Ti2p/O1s = 0.313 vs. 0.302) for the Ti-6Al-4V and c.p. Ti substrates, which suggests that coatings are likely to be exposed to similar TiO_2 surfaces regardless of the underlying Ti composition.

Contact Angle Measurements

Clean 22 x 22 mm glass coverslips were coated with 100 nm of c.p. Ti and cleaned by sonication in ethanol and ultrapure H_2O, and dried under argon gas. Clean substrates were incubated in solutions (26 μM in DPBS) of **4**, **5**, PEG (Mw 3400), or buffer alone for 2 hours at RT then extensively rinsed with DPBS and ultrapure H_2O. Modified and unmodified Ti substrates were loaded onto the stage of a Kruss DSA 100 contact angle goniometer. Drops of distilled water were placed on the specimens, and the resulting image was captured and analyzed using the proprietary drop shape analysis software. Reported static angles were calculated by using a tangential fitting method and by averaging the angles from both the left and right sides of the droplet. (Figure S1) At least 9 static angle measurements were obtained per experimental condition, and all analyses were performed at the same temperature and humidity.

XPS Analysis

Clean 9 x 9 mm glass coverslips were coated with 100 nm of c.p. Ti and cleaned by sonicating for 10 minutes in ethanol, 10 minutes in ultrapure H_2O, and dried under argon gas. Clean substrates were incubated in 26 μM peptide solution in DPBS for 2 hours at room temperature. Modified substrates were extensively rinsed with DPBS and ultrapure H_2O and dried under argon gas. XPS spectra were collected on a Kratos AXIS Ultra Imaging X-ray Photoelectron Spectrometer (Kratos Analytical Inc., New York, USA). Modified and unmodified samples were mounted on standard sample studs using double-sided adhesive tape. All binding energies were calibrated using the Ti 2p peak (458.7 eV). Samples were first analyzed using a broad survey scan (160.0 eV pass energy), then a medium resolution scan (40.0 eV pass energy) at a 20-degree glancing angle. The higher resolution data was then used to calculate the surface atomic compositions for each sample (Table S1). Curve fitting of the C1s peaks was used to determine the relative compositions of the different types of carbon bonds (Table S2).

AFM Measurements

0.0026 μM and 26 μM peptide solutions were prepared in 10mM PBS. 1 mL of each solution or a control PBS solution was added over freshly cleaved 12 mm mica discs in sterile 24-well tissue culture plates and incubated for 2 hours at room temperature. Discs were rinsed three times with PBS and three times with ultrapure water and then dried with argon gas before imaging. Modified mica substrates were examined using a Nanoscope IIIa AFM (Digital Instruments, Santa Barbara, CA) in tapping mode. Samples were imaged in air using a silicon

nitride cantilever (NSC15, MikroMasch, OR) at a scan rate of 1-2 Hz. Topographic data was obtained at two scan sizes – 1 μm and 5 μm. Images were flattened using a first or second order fit to correct for piezo bow and/or sample tilt during analysis.

Buffer Solutions

Two groups of buffer solutions with a range of pH and NaCl concentrations were prepared: One group of 150mM phosphate buffer at pH 3, 7.4, and 11, and the other with added NaCl to make 50, 200, 500 μM at pH 7.4. Buffer solutions were prepared by dissolving NaH_2PO_4 (Sigma, St. Louis, MO) in distilled water and the pH was adjusted by adding either 3M HCl or 3 M NaOH. Polymers were dissolved in aqueous buffer solutions at a concentration of 26 μM unless otherwise stated.

QCM-D Measurements

5 MHz, AT-cut quartz crystals (Q-Sense AB; Gothenburg, Sweden) were coated on one side with 150 nm of c.p. Ti. Immediately prior to use, the crystals were cleaned by sonication in a 2% SDS solution, rinsed with ultrapure water, then exposed for 30 min in a UV/ozone chamber. Polystyrene, gold, stainless steel and silicon dioxide coated quartz crystals were also purchased from Q-Sense for specificity experiments. All measurements were performed in a temperature-stabilized chamber (Q-Sense E4 system) at a working temperature of 23 °C to avoid drifts in f and D. Up to 4 separate measurements were performed in parallel. For each measurement, PBS buffer was first loaded into the liquid cell until stable baselines were obtained. 26 μM peptide solutions in the same buffer were then introduced into the liquid cell to replace the PBS buffer. For the protein resistance measurements, a fibronectin solution (4

μg/mL; Sigma) was subsequently introduced into the same chambers, followed by a buffer wash to remove weakly bound protein. Surfaces were challenged under both a short-term (1.5 hr) and long-term (5 hr) exposure to Fn. Changes in *f* and *D* were monitored and recorded in real-time using proprietary Q-Sense software (Qsoft). All raw data was subsequently analyzed using the QTools software package. Frequency and dissipation curves were fitted to a Voigt viscoelastic model to yield relevant mass, thickness and kinetic information. The adsorbed mass (Δm) can be obtained from the frequency change (Δ*f*) according to the Sauerbrey equation:

$$\Delta m = -\frac{C}{n}\Delta f$$

where *C* is the mass-sensitivity constant *C* (=17.7 ng cm^2 Hz^{-1} at *f*=5 MHz) and *n* is the overtone number.

In vitro Bacteria Adhesion

Glass coverslips and slides coated with 20 nm c.p. Ti were used as model substrates for the bacteria adhesion assays. The thinness of the Ti film allowed for substantial light transmission and enabled the tracking of bacteria adhesion via phase contrast microscopy. Bacteria adhesion assays were performed as previously described,[1,2] using a pathogenic strain of *Staphylococcus aureus* (MZ100). 26 μM solutions of peptide coatings **4** and **5** were applied in 0.5 mL aliquots to glass coverslips alongside PBS buffer alone, and incubated at room temperature overnight. Coverslips were rinsed three times in buffer then exposed to 1 mL of a S. *aureus* suspension (10^6 CFUs/mL). This suspension was derived from a 1:50 dilution of an overnight culture grown in fresh TSB-lite (66% TSB) plus 0.2% glucose. After 4 or 16 hours of incubation with bacteria in a closed, humidified plastic container, coverslips were rinsed

five times with buffer, then visualized using a Leica DM IRBE microscope (Leica Microsystems, Wetzlar, Germany).

Biofilm Assay

To assess biofilm formation, 1-well press-to-seal silicone isolators (2 x 20 mm, Sigma-Aldrich) were used to create wells on Ti-coated glass slides. 450 µL of 26 µM coating solution was then applied to triplicate wells and incubated for at least 2h at room temperature. The wells were washed twice with PBS, then an overnight culture of *S. aureus* strain MZ100 grown in TSB-lite at 37°C was diluted to ~5x10^7 CFUs/mL in TSB-lite plus 0.2% glucose, and 450 µL of the diluted culture was added to each well. The slides were incubated in a humidified chamber at 37°C for 4 hrs then stained with crystal violet, as reported, to visualize the biofilm. The crystal violet was solubilized using 30% glacial acetic acid for 15 minutes, and then 100 µL aliquots of solubilized crystal violet was moved to 96-well microtiter plates. Relative biofilm formation was assayed by reading optical density at 550 nm using a Vmax kinetic microplate reader (Molecular Devices, Sunnyvale, CA).

S.I. References:

1. Caiazza, N. C. & O'Toole, G. A. Alpha-toxin is required for biofilm formation by Staphylococcus aureus. *J. Bacteriol.* **185**, 3214-3217 (2003).
2. Shanks, R. M., Sargent, J. L., Martinez, R. M., Graber, M. L. & O'Toole, G. A. Catheter lock solutions influence staphylococcal biofilm formation on abiotic surfaces. *Nephrol Dial Transplant* **21**, 2247-55 (2006).

Supplementary Tables

Table S1: Surface atomic composition of various Ti substrates as determined by X-ray photoelectron spectroscopy (XPS).

Samples	%Ti	%O	%C	%N
Uncoated Ti	14.04	49.58	35.25	1.13
PEG	13.87	47.32	37.81	1
4	9.12	35.74	43.74	11.4
5	10.86	40.25	41.6	7.29

Table S2: Percent composition of each type of carbon bonds on modified Ti substrates.

Coating	Composition of bonds (%)		
	C-C/C-H	C-O	C=O
4	52.3	27.0	20.7
5	42.6	38.5	16.6

Supplementary Figures

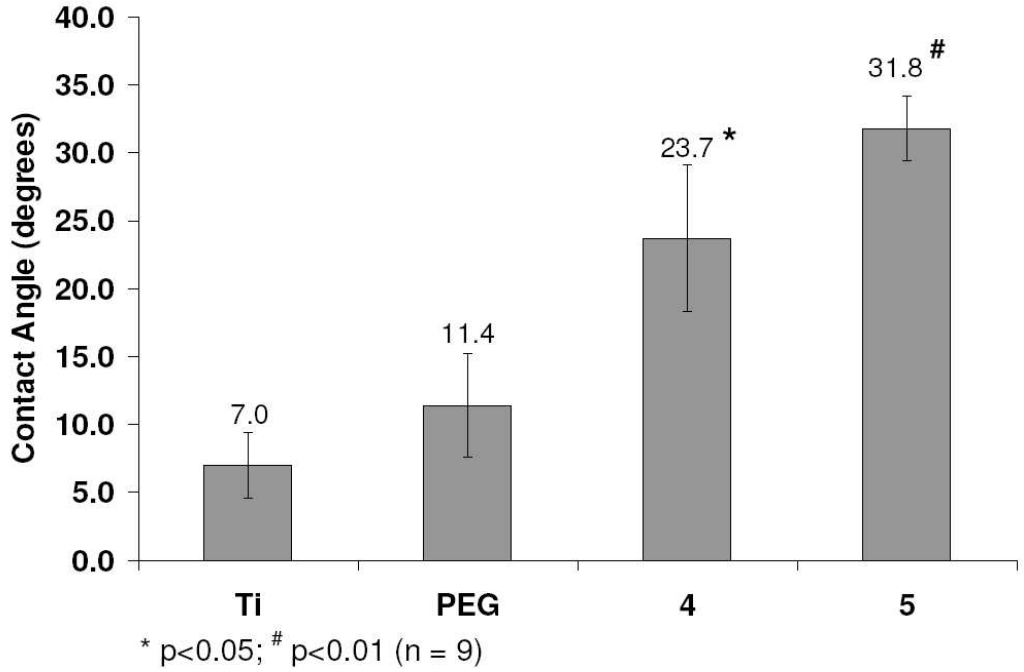

Figure S1: Water contact angle measurements on treated Ti surfaces. Surfaces coated with **4** and **5** showed contact angles significantly different than control surfaces, confirming modification with immersion in a dilute aqueous solution.

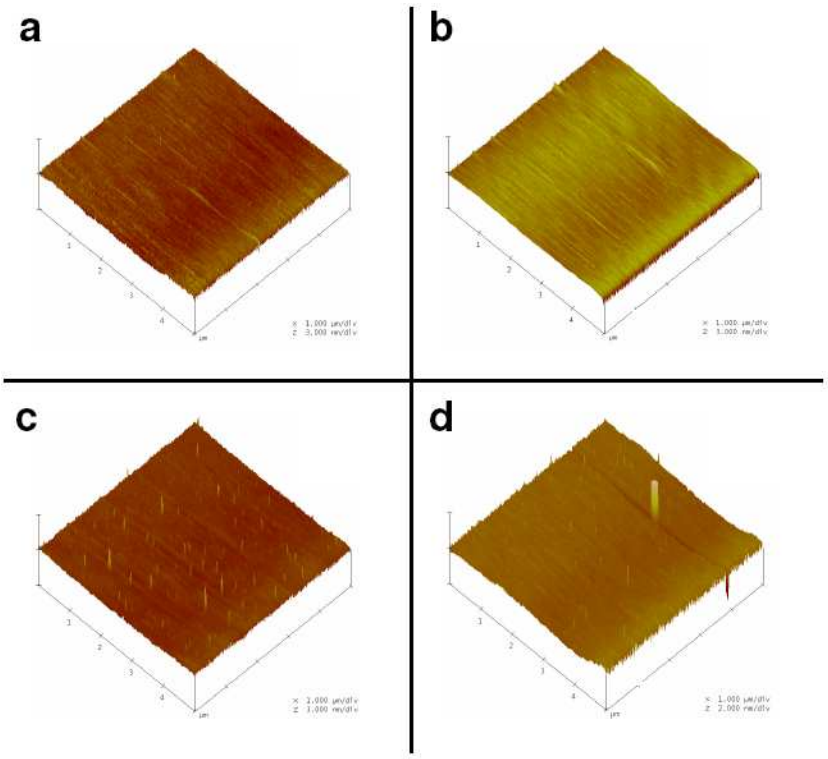

Figure S2: AFM surface profile of coated mica surfaces. **a**, uncoated mica shows a smooth uniform surface. **b**, PEG-treated substrate also shows a smooth surface, indicating no adsorption. **c**, **4**-coated substrate shows small surface structures, indicating adsorption. **d**, the PEGylated-peptide, **5**, shows similar adsorption on mica. All coating solutions were made up to a 4μM concentration.

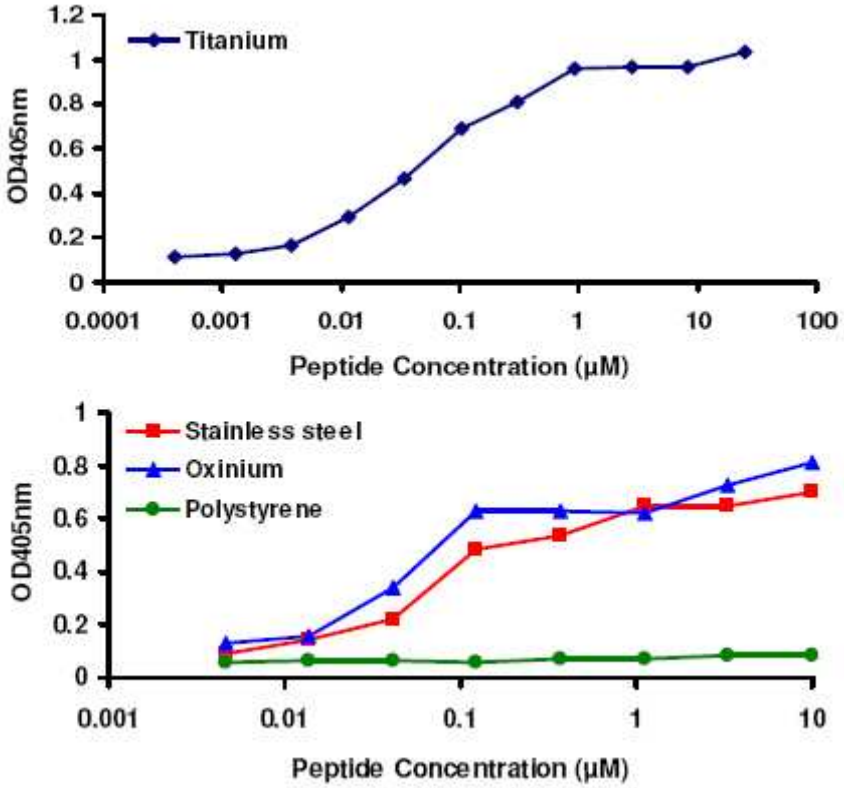

Figure S3: Binding curves for peptide **4** on (top) titanium, (bottom) stainless steel, oxinium and polystyrene. Significant adsorption is observed on stainless steel and oxinium, with dissociation constants of < 80 nM (as compared to 35 nM with Ti), but not on polystyrene.