INTRODUCTION: Infection associated with prosthetic implantation remains a serious problem. Infection can arise shortly after surgery due to contamination of the implant or it can arise from hematogenous sources at times long after the surgical procedure. Once infection is present, eradication is complex and often unsuccessful. Eradication depends on both systemic and local delivery of antibiotics, with local delivery usually reliant on controlled release systems, such as antibiotic-impregnated cement. We have taken the idea of local delivery of antibiotics one step further to synthesize an implant that has vancomycin covalently attached to its surface. We have hypothesized that this surface-bound vancomycin will be bactericidal against an establishing infection. In this abstract, we characterize the bactericidal activity of surface-immobilized vancomycin against S. aureus, the bacteria most frequently associated with peri-prosthetic infection.

METHODS: Modification of Ti alloy: Passivated Ti6Al4V (Ti) surfaces were aminopropylated with aminopropyltriethoxysilane followed by addition, via Fmoc coupling, of two aminoethoxyethylacetic acid linkers and vancomycin. Indirect immunofluorescence: Control and vancomycin-modified Ti wires (VANmTi) were washed 2X with phosphate buffered saline (PBS), blocked with 10% fetal bovine serum in distilled deionized water (dH2O; blocking buffer; all antibody dilutions are made in this buffer), 30 min, and incubated with mouse anti-vancomycin (1:300, US Biologicals) 4°C, 12h, followed by AlexaFlour 488-coupled donkey anti-mouse IgG (1:300, Molecular Probes), 1h, washed 5X with PBS, and visualized by confocal laser microscopy (Olympus Fluoview 300). Stability testing: Wires were incubated, 37°C, 7 days in (1) dH2O, (2) PBS, (3) Dulbecco’s Modified Eagle’s Medium (DMEM), or (4) in BBL trypticase soy broth (TSB, BD Biosciences) containing S. aureus subspecies aureus Rosenbach ATCC™ 29523 (C=1x10⁵ cfu/ml) under static conditions. For long-term stability, wires were incubated in PBS up to 45 days, and challenged with 1x10⁴ cfu/ml S. aureus, 37°C, 24h. After incubation, wires were washed 5X with PBS, or for wires incubated with bacteria, 2X in PBS with vortexing 1 min, 1% (v/v) Triton X 100 in dH2O, 15 min, and washed 5X in PBS, followed by staining. Bactericidal Activity: Weighed control and VANmTi were sterilized with 70% ethanol, 30 min, washed 5X with PBS, and incubated with 1x10⁴ cfu of S. aureus under static conditions. At 2, 5, 8, 12, and 30 h, six wires were removed, washed 5X with PBS, and three wires used for bacterial adhesion/viability and three used for total bacterial numbers. Bacterial Numbers: Adherent bacteria were suspended by sonication in 1 ml 0.3% Tween 80 in TSB, 5 min, followed by vortexing 5 min.⁵. Bacterial counts were determined by serial dilutions followed by triplicate plating on Todd-Hewitt agar. Total bacterial numbers were expressed as a function of pin weight. Bacterial Adhesion/Viability: After incubation with S. aureus, wires were washed 6X with PBS to remove non-adherent bacteria, followed by staining with the Live/Dead BacLight™ Viability Kit (Molecular Probes), to cause viable bacteria to fluoresce green. After labeling, wires were washed 3X with PBS and visualized with confocal laser microscopy.

RESULTS: We hypothesized that vancomycin covalently bound to Ti alloy wires would remain bactericidal over the long-term, allowing stable infection control at implant surfaces. We first ascertained the acid stability of VANmTi. Relative amounts of vancomycin were assessed onVANmTi or VANmTi that had been incubated for 7 days with dH2O, PBS, DMEM, or TSB containing an initial concentration of 10⁵ cfu S. aureus (Figure 1). Vancomycin staining appeared the same as control under all conditions. We next tested the ability of VANmTi to kill S. aureus as a function of time (Fig. 2). VANmTi was incubated with S. aureus for times to 30 h and effects on proliferation determined by staining for viability (2A) and by direct colony counting after plating (2B). The VANmTi showed little fluorescence suggesting minimal bacterial adhesion/proliferation whereas the unmodified rod showed a progressive increase in green fluorescence indicating bacterial colonization. Finally, we determined if VANmTi retained its activity for longer times. Control or VANmTi was incubated in PBS for times to 45 days, followed by a 24 h incubation with S. aureus. The ability to retard bacterial colonization was visualized using the Live/Dead assay (Fig. 3).

DISCUSSION: We have shown that antibiotics (1) can be covalently linked to a biocompatible implant surface, (2) maintain their bactericidal properties and prevent bacterial colonization, and (3) maintain their integrity and activity under simulated physiological conditions for an extended period. This modified surface holds great promise in the management of PPI, while being further expandable to many other bioactive agents and systems. Thus, our proposed modification in surface design serves as a starting point for the development of a new generation of implants that target biological activities to sites of physiological importance.

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