Implant failure due to infection remains a significant problem in joint replacement. Current approaches to combat infection focus on developing microbicidal implant coatings that are of short duration. We have proposed a novel method of directly attaching antibiotics to implant surfaces through a covalent bond that produces a microbicidal and stable surface. We have asked how this surface compares to antibiotics in solution in terms of innate activity as well as activity after serum coverage and in the face of repeated bacterial challenges.

**METHODS: Modification of TiAl6V14 (Ti) surfaces were aminopropylated with aminopropyltriethoxysilane followed by addition, via Fmoc coupling, of two aminoethoxyethylacetic acid linkers and vancomycin (Ti-VANC).** Indirect Immunofluorescence. For vancomycin, rods were incubated with mouse anti-vancomycin IgG (1:300, US Biologicals) followed by AlexaFluor 488-coupled donkey anti-mouse IgG (1:300, Molecular Probes). For fibroblastic detection, samples were incubated with PBS for 24 h, fixed with 4% paraformaldehyde, and incubated with rabbit anti-bovine fibronectin IgG (1:500, Invitrogen) followed by donkey anti-rabbit IgG (1:500, Invitrogen). All samples were visualized by confocal microscopy (Olympus Fluoview 300). Bacterial Adhesion/ Viability. After 24 h incubation with *S. aureus* or *S. epidermidis* in tryptase soy broth (TSB), wires were washed 6 times 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. Repeat challenges: Ti and Ti-VANC rods were incubated with *S. aureus* and *S. epidermidis* for 24 h, followed by surface stripping with 1% Triton-X-100 and 10% SDS in PBS with gentle shaking for 24 h, as sterilization in 70% EtOH for 15 min, and challenged again with bacteria for another 24 h. Resistance Development Testing: Ti and Ti-VANC rods were incubated with bacteria for up to 1 month with media changed every 3-4 days. To examine washed vancomycin treated with PBS to remove non-adherent bacteria, sonicated for 5 min to remove adherent bacteria. The adherent bacteria were plated on vancomycin resistance screening agar (BBL). tissue biocompatibility: MC3T3 pre-osteoblasts were cultured on Ti and Ti-VANC. To evaluate cell proliferation, MTT (Molecular Probes) and PicoGreen DNA quantification (Molecular Probes) was used. For toxicity evaluation, LDH measurements (LDH Kit, Sigma) were determined.

**RESULTS:** Ti-VANC is Microbicidal: Our previous studies show Ti-VANC to be potently microbicidal while maintaining surface stability and antibiotic presence despite extensive incubations in various physiological buffers. To test if implant coverage by blood and tissue during surgical insertion could affect the microbicidal surface, we have incubated the rods in FBS, and tested for fibroblastic coverage on the surface (Fig. 1i). Vancomycin is readily detected despite serum coverage with no diminution compared to dH2O (Fig. 1ii). The serum-coated antibiotic surface maintains its effectiveness and continues to inhibit bacterial colonization (Fig. 1iii).

**Vancomycin Modified Rods:** Superior to Antibiotic Solutions: Peri-operative IV antibiotics are standard during orthopaedic surgery. However, we have found that bacteria are able to populate implant surfaces despite presence of antibiotics in solution (Fig. 2). Control rods incubated with rod quantity of vancomycin show extensive population by bacteria, expected considering the sub-MIC concentration (Fig. 2C). However, 4x MIC vancomycin allowed bacterial coverage of control rods (Fig. 2B), suggesting the limited effectiveness of antibiotic solutions in the presence of a foreign material. Adherent bacteria demonstrate resistance to the antibiotic possibly by encapsulation with a glycocalyx matrix. Only high antibiotic concentrations managed to eliminate bacterial colonization of the implant (Fig. 2D). In contrast, the vancomycin-modified rods prevented surface colonization under all conditions with superior effectiveness. Ti-VANC Rods Resist Repeat Challenges: Despite repeated bacterial challenges as might happen clinically during a hematogenous bacterial infection, the Ti-VANC rods showed inhibition of bacterial colonization (Fig. 3) even after 5 challenges, while control surfaces showed extensive bacterial colonization. Ti-VANC Rods Prevent Resistance Development: At 2 weeks continuous incubation with bacteria, the vancomycin-modified rods did not show presence of resistant organisms growing on screening media. Extended times are currently being tested. Biocompatibility: Ti-VANC surfaces allowed surface attachment and proliferation of musculoskeletal cell lines with minimum toxicity. No considerable differences were observed compared to Ti (Fig. 3ii).

**DISCUSSION:** We have described a novel surface modification on Ti implants that render them bactericidal and effective in simulated physiological conditions. Such a surface is superior to systemic antibiotic treatments without the associated toxicity of high dose local antibiotic delivery systems. This proposed modification in surface design may serve 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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**BIOCOMPATIBLE ANTIBIOTIC TI SURFACE SHOWS MULTIFACETED MICROBICIDAL EFFECTS**

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