Prevention of Periprosthetic Joint Infection Through Electrophoretic Deposition of Gentamicin into Titanium Nanotubes: An In Vivo Investigation

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INTRODUCTION: Periprosthetic joint infection (PJI) is the leading cause of failure of modern joint replacements. Treatment success for PJI can be less than 50%, with a 5-year mortality over 25%. PJI prevention and treatment measures include systemic antibiotics. Systemic antibiotics are hindered by low local bioavailability at the surgical site and possible off-target effects. In this study, we created TiO_2 nanotubes (TNTs) on the surface of titanium (Ti) implants with subsequential loading with gentamicin and chitosan, acting as a control release agent, by exploiting electrophoretic deposition (EPD). We hypothesized femoral implants with TNTs loaded with gentamicin and chitosan would localize antibiotic to the implant and surgical site and prevent PJI in our mouse model.

METHODS: Medical grade Ti (Ti-6Al-4V ELI) wires underwent TNT surface modification (**Figure 1a**) by two-step anodization as previously decribed[1]. EPD was then used to load gentamicin and chitosan onto the Ti wire with surface TNTs (**Figure 1b**) as previously performed[1]. Control Ti wires contained TNTs with EPD of chitosan only. Wires were coated to a diameter of 0.65 mm and cut to a length of 7 mm for use as implants in the mouse PJI model. Use of 12-week-old male C57BL/6J mice were approved by the IACUC. Mice underwent a right medial parapatellar arthrotomy and received a right femoral intramedullary implant followed by inoculation at the surgical site with 1×10^3 CFUs of bioluminescent Xen36 *Staphylococcus aureus* (*S. aureus*). Mice were randomly divided into two implant groups: 1) Gentamicin + Chitosan Group = Ti implants with TNTs loaded with gentamicin + chitosan (*n*=7; experimental group); 2) Chitosan Group = Ti implants with TNTs loaded with chitosan (*n*=7; control group). Outcomes included: 1) Relative *S. aureus* abundance by bioluminescence imaging (day 1, 3, 5, 7, 10, and 14 post-surgery and infection)(*n*=7 per group); 2) Quantification of *S. aureus* amount at the implant and surrounding tissue by colony forming unit (CFU) analysis (day 14) (*n*=6 per group); 3) Scanning electron microscopy (SEM) for implant bacterial biofilm (day 14) (*n*=1 per group); 4) Radiographic signs of PJI (periosteal reaction scores; peri-implant bone loss scores; and combined values for both scores as a combined radiographic PJI score) using X-ray (day 14)(*n*=7 per group); scoring was performed as previously described[2]. Two-way ANOVA with Bonferroni's multiple comparisons test was performed for longitudinal data. With the remaining data, tests for normality were performed with a Shapiro-Wilk test. Normally distributed data was assessed with an unpaired t-test, and non-normally distributed data was assessed with a Mann-Whitney U test. A $p<0.05$ was considered significant. Data represented as mean $\pm$ SD. Data analysis performed using GraphPad Prism 9.5.0 statistical software.

RESULTS: Over 14 days assessment following implant placement and inoculation with *S. aureus*, the Gentamicin + Chitosan Group had no evidence of infection based on *i)* no increased Xen36 *S. aureus* bioluminescence signal at the surgical site (day 1, 3, 5, 7 ,10, and 14) (**Figure 1c and d**) and *ii)* no CFUs present at the implant and surrounding tissue at day 14 (**Figure 1e**). All control mice (Chitosan Group) had increased bioluminescence signal, above baseline, at all time-points over 14 days (**Figure 1c and d**) and presence of CFUs at the implant or surrounding tissue at day 14 on CFU analysis (**Figure 1e**). On bioluminescence imaging, peak Xen36 *S. aureus* signal in the Chitosan Group occurred at day 3, which was significantly different than the Gentamicin + Chitosan Group ($p=0.02$)(**Figure 1d**). On X-ray, at day 14, periosteal reaction was increased in the Chitosan Group ($p<0.001$), and there was a trend toward increased peri-implant bone loss in the Chitosan Group (**Figure 2a and b**). Correspondingly, the Chitosan Group had elevated combined radiographic PJI scores at day 14 ($p=0.003$)(**Figure 2b**). On SEM analysis at day 14, no evidence of bacteria or biofilm was found at the implant surface in the Chitosan + Gentamicin Group; in contrast, bacteria and biofilm were abundantly present at the implant surface in the Chitosan Group (**Figure 2c**).

DISCUSSION: Ti femoral implants modified with surface TNTs and coated with gentamicin and chitosan through EPD prevented evidence of PJI in all mice through 14 days. In comparison, in this mouse PJI model, all control implant mice demonstrated evidence of PJI over 14 days. Surface modification with TNTs have multiple benefits for orthopedic implants, including increased surface area for antimicrobial coatings as well as enhanced osseointegration, which we will investigate further. Use of EPD provides an efficient method to coat Ti implants with antimicrobial agents, such as gentamicin, and/or slow-release vehicles, such as chitosan, and implant coating provides and effective method to localize and high concentration of antibiotics to the implant and surgical site.

SIGNIFICANCE/CLINICAL RELEVANCE: Implants with TNTs and EPD of gentamicin were highly effective in the mouse PJI model. Further investigations are warranted to develop this technology for primary and revision arthroplasty to prevent or treat PJI, which is of high clinical priority.

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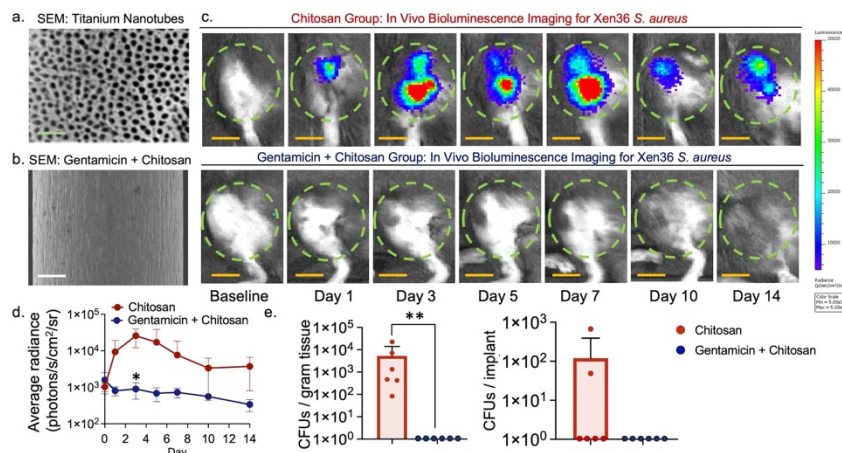
Figure 1: (a) SEM of the titanium nanotubes (TNTs) generated at the implant surface. (b) SEM of implants with TNTs coated with gentamicin and chitosan through electrophoretic deposition (EPD). (c) Bioluminescence imaging for Xen36 S. aureus at the surgical/inoculation site. Green dashed circles = region of interest for quantification of the bioluminescent signal. (d) Quantification of Xen36 S. aureus bioluminescent signal. (e) CFU analysis at the peri-implant tissue (CFUs/gram tissue) and implant (CFUs/implant). Data represented by mean $\pm$ SD; *p<0.05; **p<0.01; ***p<0.001. Green scale bar = 500 nm; white scale bar = 100 μm; orange scale bar = 4 mm.
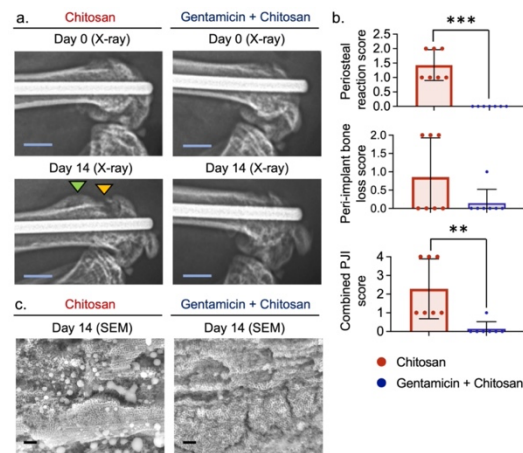
Figure 2: a) X-ray of the femoral implant and distal femur. Green triangle = evidence of periosteal reaction; orange triangle = evidence of peri-implant bone loss. (b) X-ray radiographic scoring for periosteal reaction, peri-implant bone loss, and combined PJI score. (c) SEM analysis of implants for bacterial biofilm. Data represented by mean $\pm$ SD; *p<0.05; **p<0.01; ***p<0.001. Blue scale bar = 1.5 mm; black scale bar = 1.5 μm.