Effect of Electrical Stimulation on Staphylococcus Aureus Growth on Anodized Nanotubular Titanium

Kim M. Kummer, Batur Ercan, Erik N. Taylor, Thomas J. Webster
Division of Engineering, Brown University, Providence, RI, 02912

Statement of Purpose: Currently 1.5-2.5% of all hip and knee arthroplasties suffer from infection, which results in a revision surgery and in severe cases, amputation [1]. Previous research has proposed that through the process of anodization, the alteration of nanotopography of titanium, bone forming cell (OB) proliferation and function can be improved [2]. Additionally, electrical stimulation imposes a bacteriostatic or bactericidal effect on microbes that commonly colonize the wound during the healing process [3]. Furthermore, electrical stimulation has been successfully used in orthopedics to heal bone non-unions and fractures. In this study, the electrical stimulation approach was combined with anodized nanotubular titanium to investigate their anti-bacterial properties. To investigate this, *Staphyloccocus aureus* (*S. Aureus)* adhesion and proliferation was characterized on anodized nanotubular titanium using clinically relevant electrical stimulation.

Methods: 99.2% pure titanium foils were anodized using 20V DC current for 6 minutes with a 1.5% HF electrolyte and a platinum cathode. *S. Aureus* (ATCC 25923) was cultured using Luria broth (LB) consisting of 10 g tryptone, 5 g yeast extract, and 5 g NaCl/L ddH_2O at pH7 under standard cell conditions using 200 rpm agitation. For all experiments, bacteria were seeded on the substrates at a density of $1x10^7$ bacteria/mL (as estimated by the McFaland scale). The proliferation experiments were performed for up to 3 days under standard cell conditions and constant shaking at 200 rpm. For the electrical stimulation experiments, bipolar pulses were used each day for 1 hour with a pulse duration of 0.4ms, frequency of 20 Hz and voltage of 15V (4.2 A/m^2). A BacLight Live/Dead solution was used to visualize adherent bacteria. To assess bacteria numbers, their optical densities were measured using a spectrophotometer at 562nm. Numerical data was analyzed using standard analysis of variance (ANOVA) techniques.

Results: The results show that through anodization of titanium, a nanotubular oxide film was successfully created (Figure 1).

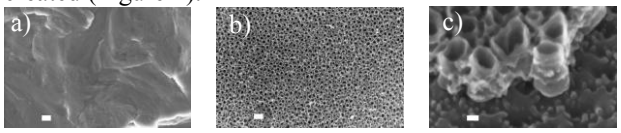

Figure 1: SEM images showing the surface features of a) conventional, b) anodized nanotubular and c) cross-section of the anodized nanotubular titanium. Scale bars are a) and b) 200nm, c) 30 nm.

When 1-hour *S. Aureus* adhesion experiments were conducted, it was observed that anodized nanotubular titanium showed higher colony counts than conventional titanium samples (Figure 2). However, when culturing for 4 hours, no difference was observed between the colony counts (Figure 3). Moreover, preliminary results indicated that there was no difference in *S. Aureus* optical densities when cultured for up to 3 days (Figure 4). Most importantly, when the samples were electrically stimulated, a decrease in bacteria OD was observed on all samples, showing evidence for the anti-bacterial effects of electrical stimulation. Perhaps the presence of fluoride or the evidence of enhanced nanoroughness (data not shown) can also be one of the reasons for the observed decrease in bacterial cell growth.

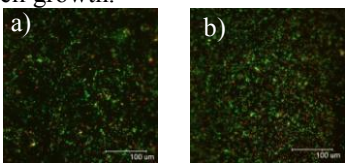

Figure 2: Fluorescent microscope images of *S. Aureus* after 1 hour adhesion on a) conventional and b) anodized nanotubular titanium. Scale bars are 100µm.

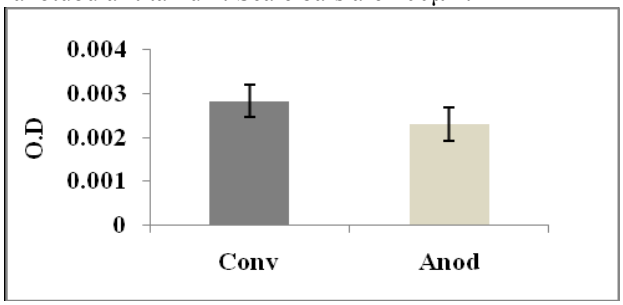

Figure 3: 4-hour *S. Aureus* adhesion on conventional (Conv) and anodized (Anod) nanotubular titanium. Values are mean ± SEM, n=3.

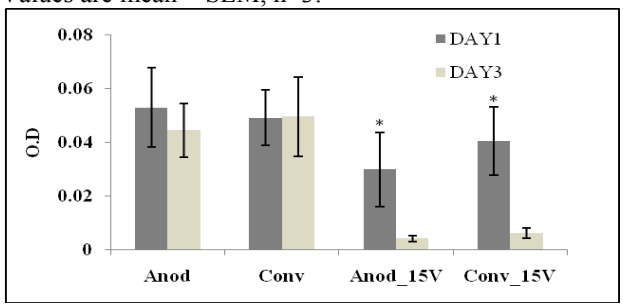

Figure 4: a) *S. Aureus* growth at day 1 and 3. Values are mean ± SEM, n=2. ∗p<0.05 compared to their own 1 day counterparts.

Conclusions: The combined effect of electrical stimulation and creation of a nanotubular oxide film was shown to decrease *S. Aureus* proliferation compared to non stimulated conventional titanium and, thus, should be further studied for orthopedic applications.

Acknowledgements: Authors would like to thank Herman Foundation for funding.

References:

[1] Lentino J. , Clin Infect Dis, 2003, 36, (9), 1157-1161
[2] Ercan B., Inter. J. of Nanomed., 2008, 3, (4), 477-485
[3] Kloth C. L., Inter. J. of Lower Ex. Wounds, 2005, 4, (23), 23-44