Decreased Bacteria Functions on Shot Peened Titanium

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Abstract
Currently implanted titanium and titanium-based alloys do not possess antibacterial properties. The objective of the present in vitro study was to modify currently implanted titanium by shot peening to implement nanoscale surface features that can reduce bacteria function without the aid of pharmaceutical agents. Results demonstrated significantly lower functions of bacteria on shot peened titanium. In this manner, aside from the well documented mechanical properties, this study provided evidence that infection can be reduced on shot peened titanium and, thus, should be further explored for orthopedic applications.

Keywords Implant Peening, bacterial infection, titanium implants, Staphylococcus aureus, *Staphylococcus epidermidis,*

Introduction
Infection of bone and medical devices, such as those caused by *Staphylococcus aureus (S. aureus)* and *Staphylococcus epidermidis (S. epidermidis)*, are frequently chronic and painful to patients. Implant failure from infection is often mediated by matrix-enclosed surface associated bacteria (called biofilms), which are resistant to antibiotic treatment. Therefore, most believe that an infected prosthetic device must be physically removed before the infection can be resolved [1]. Recent studies estimate the infection burden on all revision surgeries is about 15% for all total knee and total hip arthroplasties [2, 3].
Most bacteria found in prosthetic infections are Gram-positive and, furthermore, are commonly composed of *S. aureus* and *S. epidermidis* [4-6]. *S. aureus* biofilms have been found in about one-fourth of all prosthetic infections [4-6] and one-half of all reported cases of osteomyelitis [7]. In particular, methicillin resistant *S. aureus* (MRSA) has caused great concern for the spread of antibiotic resistant bacteria. A recent report estimates that the number of MRSA infections in hospitals has doubled nationwide, from approximately 127,000 in 1999 to 278,000 in 2005 [8]. *S. epidermidis* on the other hand is found ubiquitously on the human skin (for example, the patient's skin, a likely source of contamination) as part of the normal bacterial flora [9]. *S. epidermidis* species are cited as being the most frequently isolated organism in prosthetic infection, found in about one-third (32-36%) of all infections [4, 5].
For the above reasons, the objective of this study was to transform currently implanted titanium by shot peening and determine in vitro bacteria functions on such materials.

Experimental Methods
Materials:
Test Samples: .500" dia. x .375 long Grade 5 Titanium (6Al – 4V)
Condition: flat surface ground and polished to 6-8 Ra µinch
Stainless Steel Cut Wire SCCW14 [0.35mm] at 0.127mmA (0.005in A) intensity.
Sinto Fine Particle media SBM100 (50-300 µm) at 0.127mmA (0.005in A) intensity
Zirshot is ceramic bead size 100µm at 0.127mmA (0.005in A) intensity
Anodize: Type #2
Equipment: Clemco model VIP Direct Pressure blast cabinet

Titanium sample which are shown in Figure 1 were mounted into a holder shown in Figure 2. and exposed on one end to various peening treatments as indicated in Table 1. using the machine arrangement shown in Figure 3. The peening intensity was maintained at a 0.127mmA (0.005in A) intensity and 100% coverage for all types of media used.

Figure 1. Titanium samples

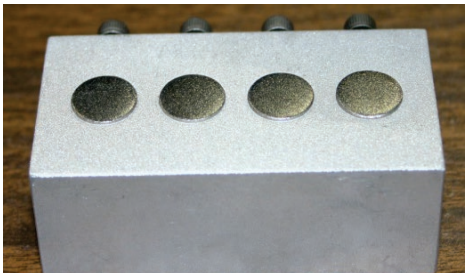

Figure 2. Holder for Titanium samples

The samples were placed into special holding fixtures shown in Figure 2. for the various peening treatments listed in Table 1. The nozzle alignment and standoff distance is shown in Figure 3. The table was rotated at a speed of 3.5 RPM for eight revolutions to provide 100% coverage for each test. Samples were then anodized to type #2 treatment and then sent to Brown University for culture tests.

Figure 3. Machine set-up showing nozzle standoff distance

Table 1. Peening Treatments

Process	Descriptions
BA	Micro bead peen only
BB	SCCW14 + Anodize
BC	SCCW14 + Micro bead
BD	SCCW14 + Micro bead + Anodize
BE	Z600 Zirshot + Microbead

Bacteria Studied

Bacteria used were *S. aureus* (#25923), *S. epidermidis* (#35984), and *P. aeruginosa* (#260) obtained commercially from the American Type Culture Collection. The bacteria were cultured in Tryptic Soy Broth (TSB; MP Biomedical), incubated (37 °C, 5% CO_2, humidified

environment), and agitated 24 hours. The second passage was diluted at a ratio of 1:200 into fresh TSB and incubated for 24 hours, then mixed 15% glycerol (Sigma) and frozen at −80 °C. Frozen stock was streaked onto sterile TSB agar plates (used to determine culture purity), grown for 24 hours, then used to inoculate TSB with a sterile 10 µL (Sigma) loop and grown for 18 hours. At that point, bacteria were diluted at a ratio of 1:100 in PBS and seeded onto the materials of interest to the study and were cultured for up to 7 days. After the prescribed time period, the bacteria functions were assessed using the crystal violet staining method and a Spectromax 340PC (Molecular Devices). Experiments were completed in triplicate and repeated at least three times. Statistical differences were assessed using the Student t-tests.

Results and Discussion

Results of the present study demonstrated, for the first time, significantly less bacteria (all of the bacteria studied here) on BD shot peened samples compared to titanium controls for up to 7 days in culture (Figures 4 – 6). This was without the use of antibiotics. It is speculated that due to the unique nanometer surface features shot peening creates on titanium, surface energy changed which altered initial protein interactions to inhibit bacteria attachment. Since less bacteria adhered at early time points, at longer time points, differences between bacteria functions were more pronounced.

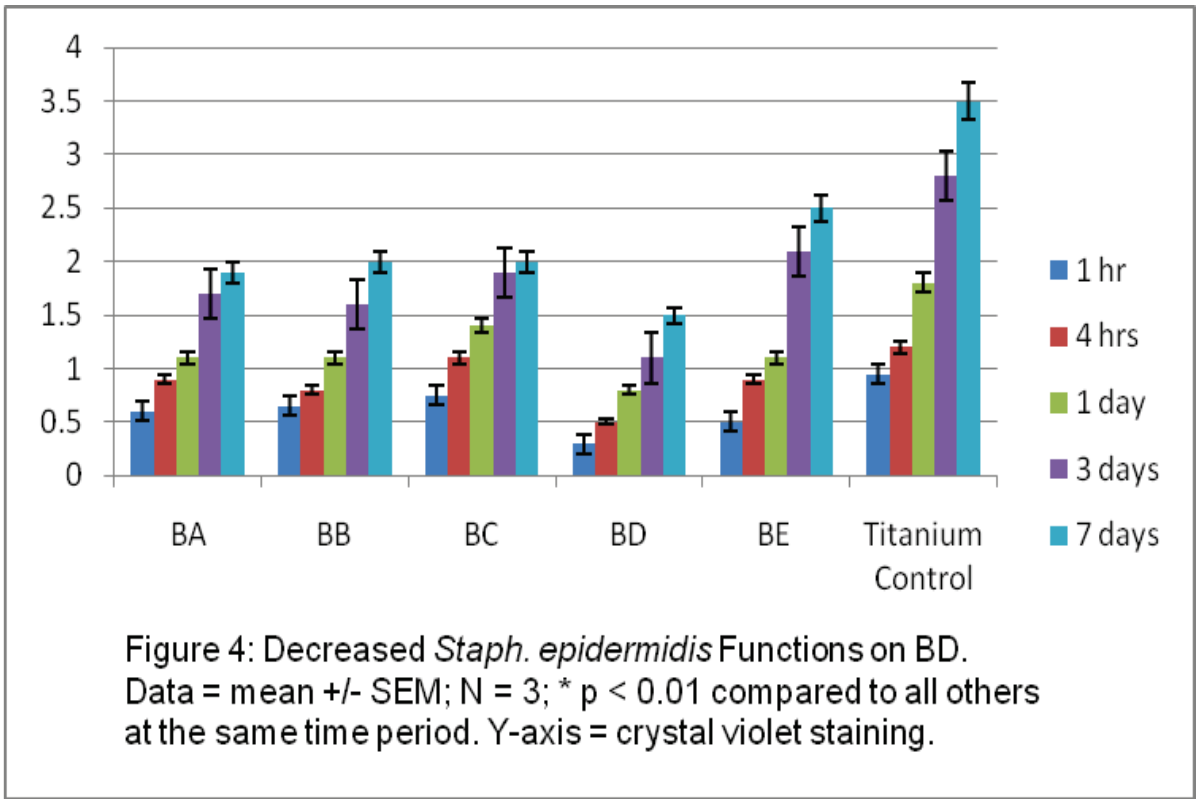

Figure 4: Decreased *Staph. epidermidis* Functions on BD. Data = mean +/- SEM; N = 3; * $p < 0.01$ compared to all others at the same time period. Y-axis = crystal violet staining.

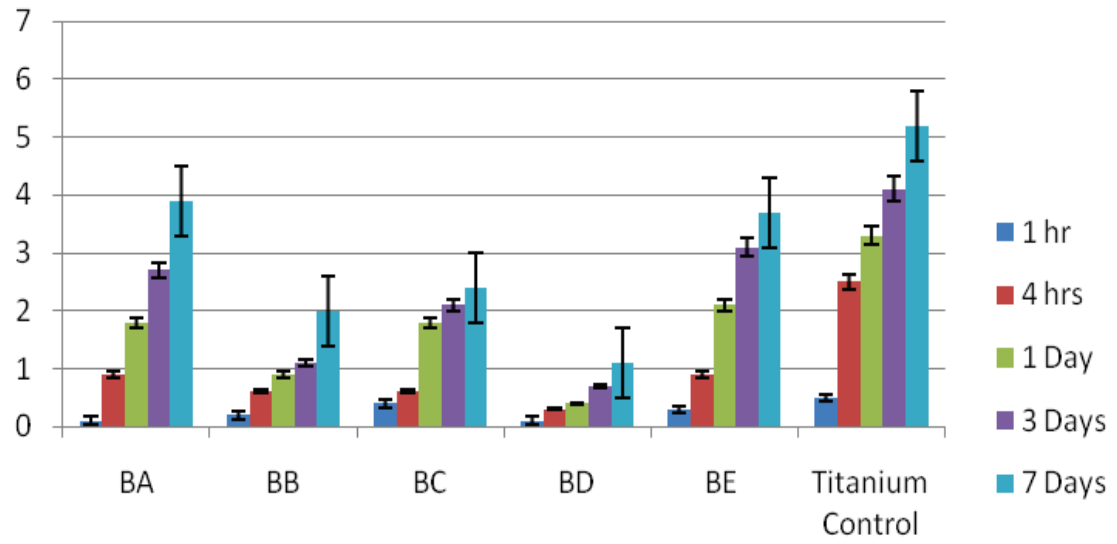

Figure 5: Decreased *Staph. aureus* Functions on BD.
Data = mean +/- SEM; N = 3; * $p < 0.01$ compared to all others at the same time period. Y-axis = crystal violet staining.

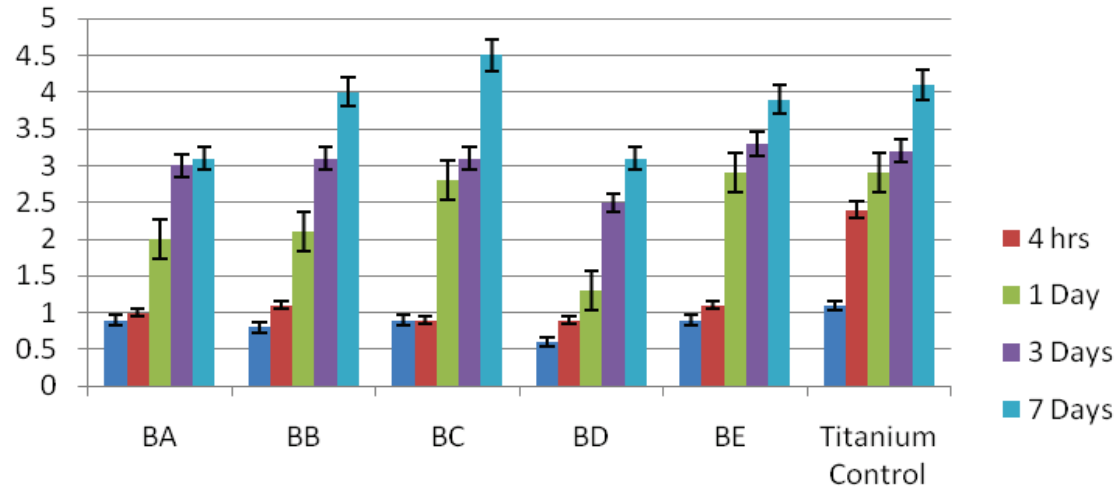

Figure 6: Decreased *Pseudo. aeruginosa* Functions on BD.
Data = mean +/- SEM; N = 3; * $p < 0.01$ compared to all others at the same time period. Y-axis = crystal violet staining.

Conclusions

Due to the current high rate of orthopedic implant infections, the objective of the present in vitro study was to modify currently implanted titanium by shot peening to implement nanoscale surface features that can reduce bacteria function without the aid of pharmaceutical agents. Results demonstrated significantly lower functions of all bacteria of

interest to the present study on shot peened titanium. In this manner, aside from the well documented mechanical properties, this study provided evidence that infection can be reduced on shot peened titanium and, thus, should be further explored for orthopedic applications.

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References

[1] Hall-Stoodley, L., J.W. Costerton, and P. Stoodley, *Bacterial biofilms: from the Natural environment to infectious diseases.* Nat Rev Micro, 2004. 2(2): p. 95-108.
[2] Kurtz, S.M., et al., *Infection burden for hip and knee arthroplasty in the United States.* Journal of Arthroplasty, 2008. 23(7): p. 984-991.
[3] Bozic, K.J., et al., *The Epidemiology of Revision Total Hip Arthroplasty in the United States.* J Bone Joint Surg Am, 2009. 91(1): p. 128-133.
[4] Phillips, J.E., et al., *The incidence of deep prosthetic infections in specialist orthopaedic hospital - A 15-year prospective survey.* Journal of Bone and Joint Surgery-British Volume, 2006. 88B(7): p. 943-948.
[5] Sharma, D., et al., *Microbiology of infected arthroplasty: implications for empiric peri-operative antibiotics.* J Orthop Surg (Hong Kong), 2008. 16(3): p. 339-42.
[6] Hamilton, H. and J. Jamieson, *Deep infection in total hip arthroplasty.* Canadian Journal of Surgery, 2008. 51(2): p. 111-117.
[7] Acosta, F.L., et al., *Diagnosis and management of adult pyogenic osteomyelitis of the ervical spine.* Neurosurgical FOCUS, 2004. 17(6): p. 1-9.
[8] Klein, E., D.L. Smith, and R. Laxminarayan, *Hospitalizations and deaths caused by methicillin-resistant Staphylococcus aureus, United States, 1999-2005.* Emerging Infectious Diseases, 2007. 13(12): p. 1840-1846.
[9] Otto, M., *Staphylococcus epidermidis - the 'accidental' pathogen.* Nature Reviews Microbiology, 2009. 7(8): p. 555-567.