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 samples 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.

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.

**Table 1. Peening Treatments**

<table>
<thead>
<tr>
<th>Process</th>
<th>Descriptions</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA</td>
<td>Micro bead peen only</td>
</tr>
<tr>
<td>BB</td>
<td>SCCW14 + Anodize</td>
</tr>
<tr>
<td>BC</td>
<td>SCCW14 + Micro bead</td>
</tr>
<tr>
<td>BD</td>
<td>SCCW14 + Micro bead + Anodize</td>
</tr>
<tr>
<td>BE</td>
<td>Z600 Zirshot + Microbead</td>
</tr>
</tbody>
</table>

**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% CO₂, 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.](image-url)
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

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.
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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