Robbins Professor of Sustainable Manufacturing

Annual Report – June 2019

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The Robbins Professor of Sustainable Manufacturing supports high risk and potentially high payoff research by Dr. Friedrich and his students in sustainable manufacturing. During the past year, work has focused on further developing inherently antibacterial orthopaedic implant surfaces and the breakthroughs to sustainably manufacture these new implants faster and less expensively than currently used processes.

The work reported here was funded by the Robbins Professorship during 2018-2019. We were granted US Patent 9,376,759 in June 2016 “Compositions, Methods and Devices for Generating Nanotubes on a Surface” to make the implant surfaces inherently antibacterial by integrating nanosilver particles on and inside the nanotubes in the same process that generates the nanotube surfaces thereby reducing cost, complexity, and hazardous materials.

- We have demonstrated that we can produce nanotube surfaces in as little as ten minutes, where in the past several hours were required.
- We have measured the shear strength of our nanotubes and they exceed the shear stress on him implant surfaces in normal physiological activity.
- We have investigated a simple modification to our standard nanotube fabrication process that leaves both calcium and phosphorus in the nanotubes. These elements are among the chemical building blocks of bone and should further enhance bone bonding with the nanotube surface.

Background

Our orthopaedic implant surface technology is based on a safe, low-cost 3-D electrochemical fabrication platform for etching nanotubes into the surfaces of titanium orthopaedic implants, including 3D powder printed and sintered implants, as shown in Figure 1.

To date we have not encountered any commercial titanium implant or bone screw that we cannot modify to have nanotubes, some in as little as 10 minutes. Our 3-D electrochemical etching process uses low cost and non-hazardous materials, requires minimal process equipment and maintenance, is environmentally safe, and requires less energy than current surface coating technologies that deposit materials at high temperatures and therefore have thermal mismatch resulting in a brittle surface.

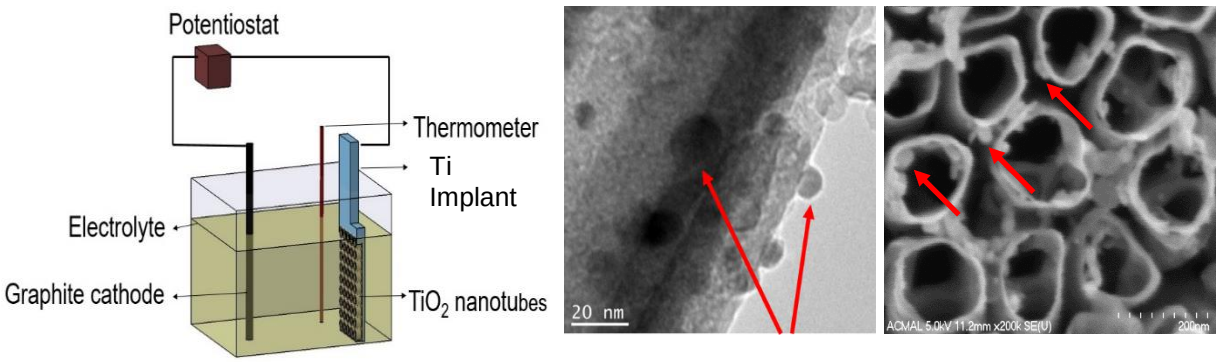

Fig. 1. Simple DC anodization with non-hazardous electrolyte etches surface leaving nanotubes, in as little as 10 minutes. Very small addition of silver compound (patented) produces nanosilver spheres decorating nanotubes in same process.

Antibacterial Efficacy of Nanotube Surfaces

Building on prior work by a state commercialization grant for testing implants in a rat model (as detailed in the 2016 annual report), we received a follow-on commercialization grant in 2017-2018 to assess the antibacterial characteristics of just nanotubes on implant surfaces, and also nanotubes that have antibacterial nanosilver. Industry is interested if nanotubes alone are effective in reducing infection because the material of the nanotubes is already FDA-approved.

We fabricated several diameters of nanotubes and tested the antibacterial effectiveness against plain titanium, and titanium with a thermal-plasma-sprayed (TPS) coating. TPS represents the "gold standard" of current implant surfaces as it presents high roughness allowing mechanical locking with regrown bone. We conducted cell culture studies and animal implantation studies using clinical methicillin-resistant Staphylococcus aureus (MRSA). MRSA is the most common bacteria resulting in orthopaedic surgical infection and treatment has a current burden of $1.6 Billion annually.

For the *in vitro* work, titanium coupons were inoculated with MRSA simulating the start of a post-operative infection on an implant. At various time points beginning with two hours (a clinically-critical time point), the coupons were sonicated to release MRSA from the surfaces. The nanotube surfaces released considerably more MRSA than did the plain titanium and the TPS surfaces. This indicated that the nanotubes did not provide a suitable surface for biofilm formation and adhesion, while the plain titanium and TPS apparently allowed the MRSA to form a strong biofilm.

Following the *in vitro* results, New Zealand White rabbits underwent a bilateral implantation of intramedullary tibial implants. One intramedullary canal was inoculated with clinically-derived MRSA at the time of implantation; one canal had sterilized culture media introduced (control). At

a 2-week endpoint, limbs were harvested for analysis which included implant sonication with the sonicant subsequently cultured to allow MRSA growth.

The TPS showed the lowest average released MRSA count, while plain nanotubes (TiNT) and nanotubes with nanosilver (TiNT+Ag) were the highest. As with the *in vitro* work, the cultured bacteria had been released from the extracted implant surface by sonication. This indicated that while implanted, the implants with the nanotubes did not provide a surface suitable for MRSA attachment and growth, and instead the plain titanium and TPS surfaces provided a good surface for MRSA attachment, so less was released by sonication.

In a follow-on therapeutic experiment encouraged by the prior results, rabbits underwent bilateral implantation, followed by 1 week of infection development, and then 1 week of antibacterial vancomycin treatment. At the endpoint, implants were sonicated with bacteria cultured from the sonicant. Nanotubes alone showed viable MRSA **at only 30%** that of TPS-coated levels, while TiNT+Ag implants showed viable MRSA **at only 5%** that of TPS-coated levels, as shown in Figure 2. In current infection treatment, systemic vancomycin is often not effective against an established MRSA infection. The initial reduction of MRSA and the disruption of a viable bacterial biofilm by the nanotubes may allow treatment by vancomycin to be more effective.

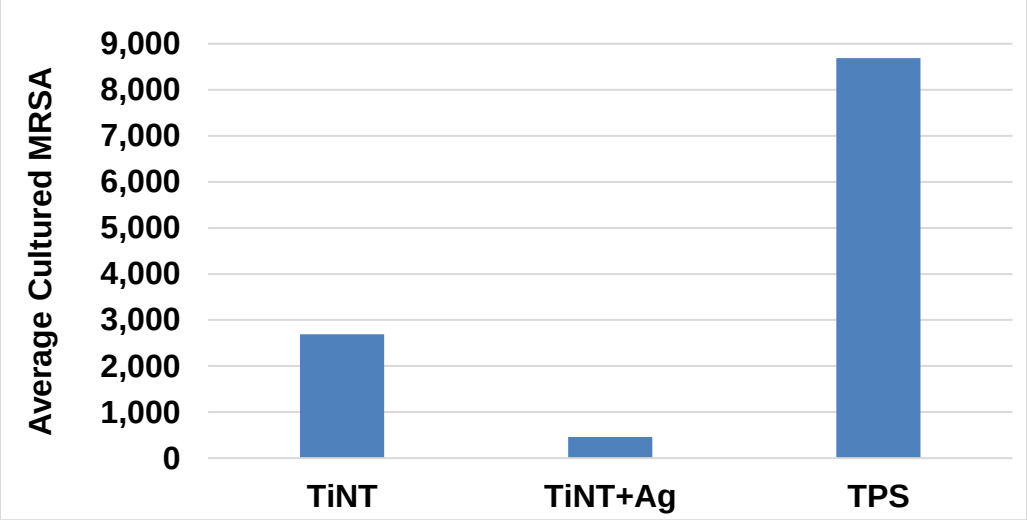

Fig. 2. Average cultured MRSA from implant sonicant after 1-week vancomycin treatment. The nanotubes alone, and with nanosilver, greatly aided the antibiotic treatment. The treatment may not have been effective with TPS due to the much larger number of bacteria initially present.

Shear Strength of Nanotubes

It is necessary to know that the shear strength of the nanotubes is sufficient to withstand normal physiological loads during walking, stair climbing, etc. Lap shear tests of the nanotubes were performed according to ASTM F1044, ("Standard Test Method for Shear Testing of Calcium Phosphate Coatings and Metallic Coatings" - Committee on Medical and Surgical Materials and Devices). The standard is for testing the bond shear strength of calcium-phosphorus hydroxyapatite coatings on implants. Because there is no standard test method for a nanotube surface, this appeared to be the most relevant medical standard. These shear tests simulated the primary mechanical loading between the implant stem and the femur cavity for a hip implant.

Each test used a titanium bar or foil with etched nanotubes on it, a bare titanium foil, and a thermally-cured adhesive that bonded the two. The adhesive was specified in the ASTM standard. The bonded pair was then pulled in shear using a tensile test machine with instrumented force readout. The general arrangement of the specimens in the shear test, and a typical failed surface, are shown in Figure 3.

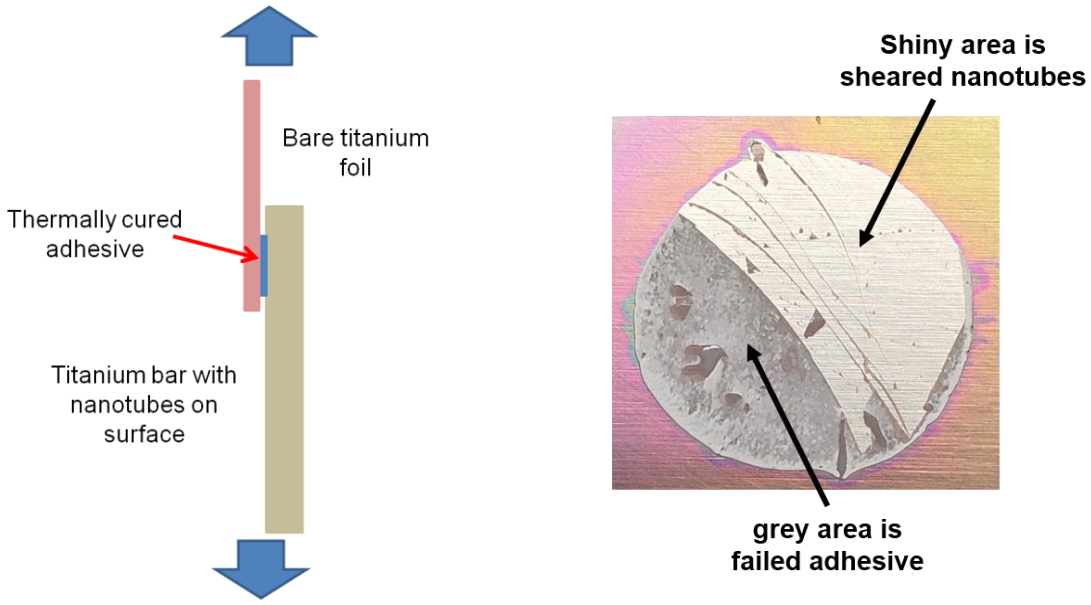

Fig. 3. (Left) General arrangement for conducting shear strength tests of nanotube surfaces. (Right) Typical surface after specimen test. The circular region is 0.6-inches diameter and required 1,523 pounds (6.78kN) of shear force to cause failure.

Preliminary tests showed there were three possible failure modes, each of which had to be quantified. The first failure mode was if the adhesive itself failed in shear but remained bonded to both surfaces. The second failure mode was the strength of adhesion between the adhesive and the plain titanium foil surface. The third failure mode was the shear strength of the nanotubes to

the underlying titanium (simulating the implant). Shear tests were conducted for the first two failure modes to determine the shear strength of the adhesive and its bond strength to titanium.

In these preliminary tests, it was found that the bond strength of the adhesive to the bare foil and the nanotube surface was insufficient to pull nanotubes from the surface. The strength specification of the adhesive by both the ASTM standard and the manufacturer was 5,000 psi (34.4MPa). With consultation with the adhesive manufacturer, we modified the adhesive curing process for better uniformity. The manufacturer stated that, to their knowledge, the adhesive had never before been used for such tests and were helpful with this process. We were able to get bond strength sufficient to pull nanotubes from the surfaces while maintaining good bond strength to the bare titanium.

After each test, the surfaces were analyzed with *ImageJ* software that helped determine the area of each of the three failure modes. From this analysis, the shear strength of the nanotubes of each sample was calculated. A typical pair of failed surfaces is shown in Figure 4. These surfaces are mirror-images of each other.

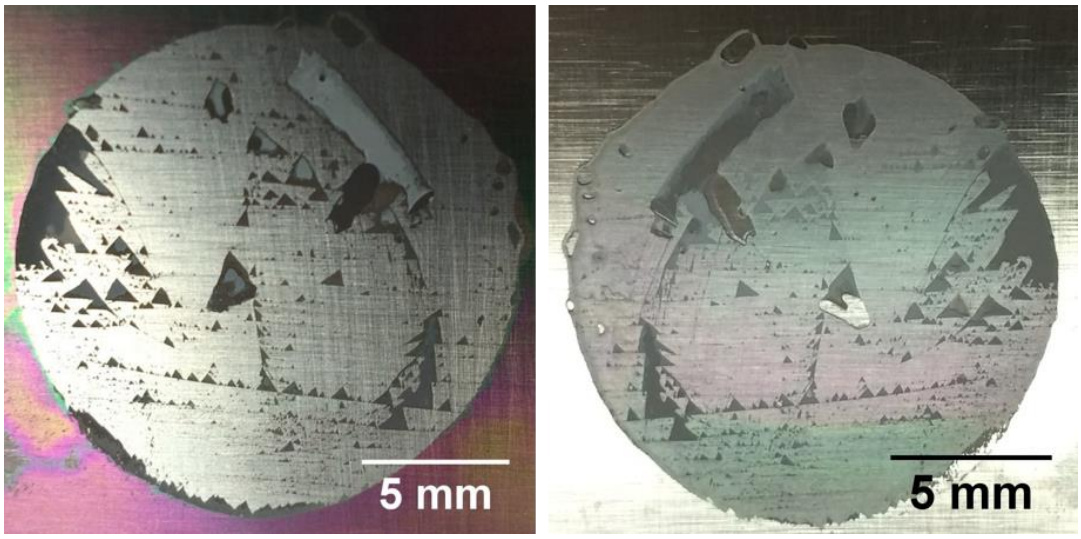

Fig. 4. Typical surfaces from nanotube shear tests. (Left) Pink-green area outside the central circular region are nanotubes not involved in the test. The circular bright area is the titanium after the nanotubes were sheared away. The dark areas are failed adhesive. (Right) Corresponding surface showing faint pink-green hue within circular region indicating bottom of nanotubes pulled from the shiny region at left.

The nanotubes diffract light, appearing with a pink-green hue. In the left portion of Figure 4, the large pink-green area surrounding the circular region are intact nanotubes on the foil area not involved in the shear test. The shiny portion of the circular region, where there was adhesive, is the bare titanium foil where nanotubes have been pulled from the surface. The darker regions are failed adhesive. In the right portion of the figure, a light pink-green hue can be seen within the

circular region. This hue is caused by the bottom of the pulled-out nanotubes from the left portion, adhering to the intact adhesive layer on the mating surface.

The analyses of the many tests showed the shear strength of the nanotubes to be as high as 9,500 psi (65MPa) with a typical shear strength of 8,000 psi (55MPa), and several surfaces with 6,000 psi (45MPa). The literature presents models predicting the shear stress in bonded orthopaedic hip stems using a cement adhesive. Assuming that TPS with nanotubes performs similar to a bonded hip stem, owing to the locking of the re-grown bone with the very rough TPS, a typical shear stress prediction for physiological loads is approximately 10MPa. The nanotube shear strengths in the tests were 4-6 times higher than the expected stress during normal use.

Presentations and Commercialization Efforts

Presentations were made and abstracts were published in the archival literature. Two of the presentations were to an international audience at the International Society for Technology in Arthroplasty in London. We have submitted an abstract on the shear strength work to the same conference in latter 2019. The work was also presented, via poster and conversations to mid-west venture capitalists at a recent *FastForward Medical Innovation* event at the University of Michigan. This event was sponsored by the Michigan Economic Development Corporation that also funded our prior collaborative work with Beaumont Health System. The bolded names below represent current or former Michigan Tech researchers and collaborators.

Poster

Craig Friedrich[1], Erin Baker[2], Paul Fortin[2], Matthew Sims[3], Daniel Justin[4] "MRSA Antibacterial Orthopaedic Implants",[1] Michigan Technological University, Houghton, MI, [2] Department of Orthopaedic Surgery and Research, Beaumont Health, Royal Oak, MI, [3] Department of Infectious Diseases, Beaumont Health, Royal Oak, MI, [4] Nanovation Partners, Orlando, FL.

Submitted Conference Abstract

Craig Friedrich, Shuo Wang, Adam Francis, Erin Baker, "Mechanical Integrity of MRSA Antibacterial Nanotube Surfaces", submitted to International Society for Technology in Arthroplasty 2019, Toronto, Canada.

Published Abstracts

D Justin, YS Nguyen, W Walsh, M Pelletier, **CR Friedrich, E Baker**, SH Jin, C Pratt, "Enhanced Bone Fixation of Total Knee Arthroplasty Tibial Tray Implants with TiO_2 Nanotubes", Journal Orthopaedic Proceedings, Volume 101, Issue SUPP 5, April 2019, Pg. 97, British Editorial Society of Bone & Joint Surgery.

CR Friedrich, E Baker, S Bhosle, D Justin, "In Vivo Anti-Bacterial Effectiveness of Nanotextured Titanium Implant Surfaces", Journal Orthopaedic Proceedings, Volume 101, Issue SUPP 4, April 2019, Pg. 43, British Editorial Society of Bone & Joint Surgery.

D Justin, **CR Friedrich**, **S Bhosle, E Baker**, SH Jin, C Pratt, "Enhanced Bone Fixation by Nano-Texturing via Titanium Oxide Nanotube Anodization", Journal Orthopaedic Proceedings, Volume 100, Issue SUPP 6, April 2018, Pg. 5, British Editorial Society of Bone & Joint Surgery.