Differential Bacterial Expression on Silicon Nitride, PEEK, and Titanium Surfaces

Bryan J. McEntire[1], Erin N. Jones[1], Darin Ray[1], Ryan M. Bock[1], B. Sonny Bal[1,2], and Giuseppe Pezzotti[3]
[1]Amedica Corp., Salt Lake City, UT, [2]University of Missouri, Columbia, MO, [3]Ceramic Physic Laboratory, Kyoto Institute of Technology, Kyoto, Japan

Disclosures: Bryan J. McEntire (3A-Amedica Corporation), Erin N. Jones (3A-Amedica Corporation), Darin Ray (3A-Amedica Corporation), Ryan M. Bock (3A-Amedica Corporation), B. Sonny Bal (3A-Amedica Corporation), and Giuseppe Pezzotti (3B-Amedica Corporation).

INTRODUCTION: Infected implants are a serious concern in orthopaedic surgery, requiring aggressive treatment with significant morbidity. Considerable research has targeted the selection and development of biomaterials that may reduce or eliminate implant-related infections [1]. Bacterial biofilm adherence to biomaterial surfaces leads to chronic implant infection, often requiring implant excision. An *in vitro* test was developed to compare biofilm adherence to three biomaterials used in spinal fusion surgery – silicon nitride, PEEK, and titanium alloy – using one gram-positive and one gram-negative bacterial species.

METHODS: Biomaterials were: silicon nitride (Si_3N_4, *MC*$^{2®}$, Amedica Corporation, Salt Lake City, UT), polyether ether ketone (PEEK, ASTM D6262), and medical grade titanium alloy (Ti6Al4V, ASTM F136). Each sample (n=3) was shaped as a Ø12.7mm disc (coupon). All materials were ultrasonically cleaned and UV-sterilized prior to bacterial exposure. Each coupon was placed in a sterile well plate and inoculated with a 10^5 bacterial solution of *Escherichia coli* (ATCC® 14990™) or *Staphylococcus epidermidis* (ATCC® 25922™). The bacterial culture medium was phosphate buffered saline (PBS) containing 7% glucose and 10% human plasma. Media was designed to mimic physiologic fluids. Glucose was included as an energy source and human plasma was included as a source of proteins. Proteins are an essential part of the experiment because any implant that comes in contact with physiologic fluids is immediately coated with proteins, which influence bacterial attachment to surfaces [2-3]. Inoculated coupons in well plates were placed on a shaking incubator at 37°C and 120 rpm for 24 or 48 hours. The biofilm strength and density were exposed to shear forces, introduced by the shaking incubator and mimicking conditions of physiologic fluids *in vivo*. Biofilms formed under static *in vitro* conditions generally form weaker biofilms containing more nutrient channels, making them less dense, and more amenable to treatment with antibiotics, which is atypical of implant-related infections [1]. All 48 hour coupons underwent a media refresh at 24 hours to ensure sufficient nutrient availability for the duration of the study. At 24 and 48 hours, coupons were removed from the well plates and rinsed in PBS to remove planktonic bacteria. Coupons were placed in a centrifuge tube with fresh PBS and vortexed to remove adhered bacteria. The bacterial solutions obtained were then serially diluted and plated. Plates were incubated at 37°C for 24-48 hours, and the colonies were counted. Colony counts were multiplied by applicable dilution factors and divided by surface area to determine the average colony forming units per square millimeter (CFU/mm^2). A two-tailed, heteroscedastic student's T-test (95% confidence) was used to determine statistical significance.

RESULTS: For *S epidermidis* at 24 hours the number of bacteria growing on PEEK was approximately half an order of magnitude greater than on either silicon nitride or titanium. Further, increased numbers of bacteria grew on titanium at 24 hours compared to silicon nitride; however, that difference was not statistically significant. At 48 hours the mean bacterial growth on PEEK was approximately 2 and 3.5 orders of magnitude greater than titanium and silicon nitride, respectively. The differences in growth between titanium and silicon nitride were statistically significant, with silicon nitride growing fewer bacteria than titanium (p=0.0004). Only silicon nitride demonstrated less bacterial growth over time (p=0.0212; Figure 1(a)). For *E coli* at 24 hours bacterial growth was an order of magnitude greater on PEEK than on titanium and silicon nitride (p=0.0018 and p=0.0016, respectively). The difference between titanium and silicon nitride surfaces was also significant, with silicon nitride demonstrating fewer bacteria than titanium (p=0.0362). At 48 hours the trends remained the same between the materials. PEEK contained the most bacteria, followed by titanium, with silicon nitride demonstrating the fewest bacteria (Figure 1(b)). All differences in bacterial growth between respective biomaterials at 48 hours were significant (p<0.05).

DISCUSSION: Silicon nitride, PEEK, and titanium surfaces demonstrated significant differences in bacterial adhesion and proliferation for both gram-positive *S epidermidis* and gram-negative *E* coli, particularly at 48 hours post-inoculation. Silicon nitride showed the most favorable bacterial resistance for both species tested. Unlike *S epidermidis*, no significant increase in growth was observed for *E coli* on PEEK from 24 to 48 hours. This is likely due to the differences in the inherent biofilm forming abilities of the bacterial strains tested. *S epidermidis* is known for its ability to form very large and resilient biofilms, which is one reason why it is responsible for many orthopaedic implant-related infections [3]. *E coli* generally forms smaller or weaker biofilms than *S epidermidis*. The mechanism of the anti-bacterial behavior of silicon nitride is unclear; however, its suppressive effect on *S epidermidis* and *E coli*, when compared to PEEK and titanium, was demonstrated in these experiments. Future studies will be targeted at elucidating the mechanism whereby silicon nitride ceramic surfaces suppress bacterial growth.

SIGNIFICANCE: Implant-related infections are a serious clinical concern, requiring major surgery and prolonged antibiotics, with attendant morbidity and increased healthcare costs. Identifying biomaterial surfaces that resist biofilm adhesion and bacterial expression is a strategy toward addressing implant-related infections.

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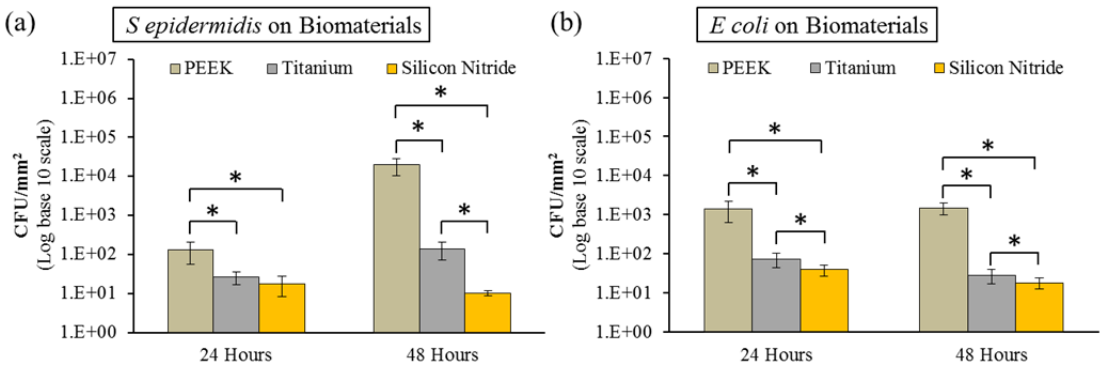

Figure 1- Mean bacterial growth on PEEK, titanium, and silicon nitride at 37°C for 24 and 48 hours for E coli (a) and S epidermidis (b). Error bars show standard deviation. Asterisks (*) indicate statistical significance (p<0.05) from students t-test (n=3).