The Antimicrobial Resistance of Oxide and Non-Oxide Ceramics

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Introduction: Prosthetic joint infections (PJI) in the USA are predicted to more than double in the current decade, eventually reaching ~60,000 cases and costing the US healthcare system ~$1.7 billion.[1] These increases are driven by an older population seeking to maintain an active lifestyle, coupled with the growing antibiotic resistance of common nosocomial bacterial strains.[2] A recent statistical review of total hip arthroplasty (THA) patients from the Medicare population associated a reduced risk of PJI with the use of alumina-based ceramics.[3] However, this review was insufficient to assign causality and therefore the authors stated that their findings "support further research into the association between ceramic bearings and complication avoidance in primary THA." Consequently, the current study was designed to examine the bacteriostatic behavior of oxide and non-oxide bioceramics for use in THA devices. It was hypothesized that significant differences in antimicrobial behavior would be evident between these two types of ceramics.

Materials and Methods: The oxide and non-oxide ceramics selected for bacteriostatic testing included zirconia-toughened alumina, ZTA (BIOLOX®*delta*, CeramTec), and silicon nitride, Si_3N_4 (*MC*$^{2®}$, Amedica Corporation), respectively. Disc samples (∅12.7 x 1 mm) were machined from pristine ZTA femoral heads; whereas dimensionally identical Si_3N_4 discs were directly fabricated.[4] Both sets of discs were polished to surface finishes of $R_a \leq 5$ nm. Polyether-etherketone, PEEK (Optima® Invibio), samples of the same geometry were also included in the test matrix as negative controls. All samples were UV sterilized prior to testing. *Staphylococcus epidermidis*, *S. epi.* (14990®ATCC™), was cultured in a brain heart infusion (BHI) agar medium at an initial concentration of 1.8 x 10^{10} CFU/ml and subsequently diluted to 1 x 10^8 CFU/ml using phosphate-buffered saline solution. A 100 ml aliquot was then transferred to separate Petri dishes, each containing BHI agar and a single ZTA, Si_3N_4, and PEEK disc (n=4 of each material). Specimens were incubated at 37°C under aerobic conditions for 8, 12, 24, 36, and 48 h. The bacteria were stained with 4',6-diamidino-2-phenylindole (DAPI) and Sytox®Green (ThermoFisher Scientific) for DNA imaging and for cell viability, respectively. Imaging was performed by confocal fluorescence microscopy (BZ-X700; Keyence) using a 488 nm Krypton/Argon laser source. Differences in cell viability were compared with the unpaired Student's *t*-test ($p \leq 0.05$).

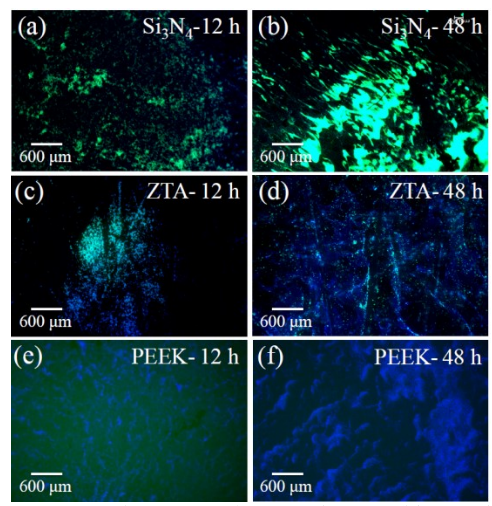

Figure 1. Fluorescence images of DAPI (blue) and Sytox® (green) stained Si_3N_4, ZTA, and PEEK biomaterials at 12 and 48 h, indicating concentrations of live and dead *S. epi.* bacteria, respectively.

Results: Fluorescence images of *S. epi.* on the three biomaterials at 12 and 48 h are shown in Fig.1(a)~(f). The bacteria exposed to Si_3N_4 predominately stained green, demonstrating a greater fraction of dead bacterial cells with exposure time. The PEEK substrates stained blue, indicating increased cell viability with contact time. Intermediate results were observed for the ZTA, with a combination of both dead and proliferating cells. Fig. 2 presents the statistical results of these tests using quantitative image analyses. The data reveal an exponential increase in live bacteria for both PEEK and ZTA. Conversely, the Si_3N_4 showed an initially mild increase in live CFUs up to 24 h followed by a significant decrease at 48 h.

Discussion: The current data demonstrate substantial differences in the inherent bacteriostatic capabilities of oxide and non-oxide bioceramics. While ZTA proved to be more antimicrobial resistant than PEEK, it was significantly less effective than Si_3N_4. A prior study demonstrated that the release of nitric oxide (NO) from the surface of Si_3N_4 and its conversion to peroxynitrite is at least partially responsible for its bacterial lytic effects;[5] whereas no similar or alternative chemical mechanisms are available for either PEEK or ZTA.

Significance/Clinical Relevance: The use of biomaterials that innately resist biofilm formation is of increasing importance due to the predicted greater number of THA procedures and the growing virulence of hospital acquired infections. The active bacteriostatic effectiveness of Si_3N_4 represents a significant advancement over the passive microbial resistance of Al_2O_3 and ZTA.

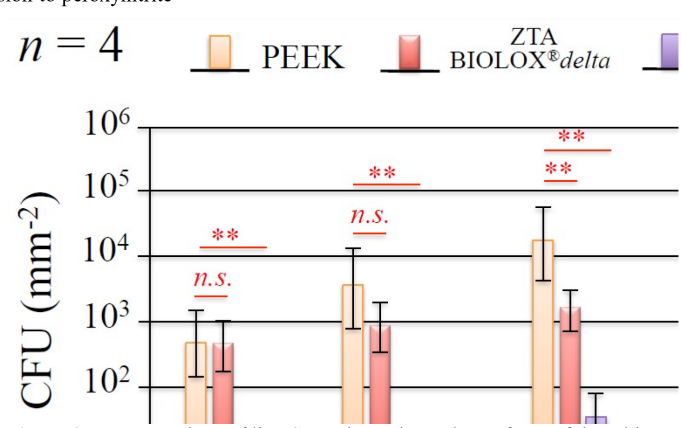

Figure 2. Concentrations of live *S. epi.* bacteria on the surfaces of three biomaterials, PEEK, ZTA, and Si_3N_4, as a function of incubation time.

References: [1]S. M. Kurtz, *et al.*, *J. Arthroplasty*, **23** [7] 984-91 (2008); [2]T. Frieden, *Brochure, US Cent. Dis. Control Prev.*, 114, (2013); [3]S. M. Kurtz *et al.*, *J. Arthroplasty*, **32**, 743-49 (2016); [4]B. J. McEntire *et al.*, *Bioceram. Dev. Appl.*, **6** [1] 1000093 (2016); [5] G. Pezzotti *et al.*, *Langmuir*, **32** [12] 3024–3035 (2016).