Evaluating the Bacterial Biofilm Inhibition of a Novel Silorane-based Biomaterial for Orthopedic Applications

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Statement of Purpose: Poly(methyl methacrylate) (PMMA)-based bone cements are the industry standard in orthopedics for implant anchoring and post-surgical void filling. PMMA cement has been shown to be a prime surface for bacterial attachment and subsequent biofilm development. Biofilm formation has been implicated as a primary contributor to bacterial resistance and difficulty in treating orthopedic-related infections.[1,2] While efforts have been focused on the design of biofilm resistant implants, little attention has been given towards addressing bacterial adhesion to bone cements, aside from antimicrobial incorporation, which can lead to bacterial resistance. The need for non-antibiotic measures to prevent bacterial biofilm formation has led to the development of a novel silorane-based biomaterial (SBB) for use as an orthopedic cement. Originally used in dental applications, our SBB has been reformulated and shown to be non-toxic, have weight-bearing strength, and undergo significantly less shrinkage during polymerization than PMMA. This study aims to measure bacterial attachment and subsequent biofilm formation on the surfaces of both PMMA and SBB cements. We hypothesized that SBB would be less conducive to both initial bacterial attachment and further biofilm maturation over 72 hours.

Methods: Commercially available PMMA (Depuy SmartSet MV, Depuy, Warsaw, IN) was used for a control comparison, while the SBB was synthesized and prepared by our collaborators in the UMKC Chemistry Department. Disc-shaped samples were prepared by placing the polymerizing cements into polytetrafluoroethylene (PTFE) molds of two sizes for both static and dynamic testing. *Staphylococcus aureus* (SA, ATCC 29213) was used for all biofilm testing. Static testing was performed by placing the cement discs at the bottom of 24-well plates and inoculating with a $1.0x10^6$ CFU/mL aliquot of SA, with a new inoculum every 24 hours. Dynamic testing was performed using a CDC Biofilm Reactor™, which employed a sheer force via stirring. Samples were removed after 24, 48, and 72 hours.

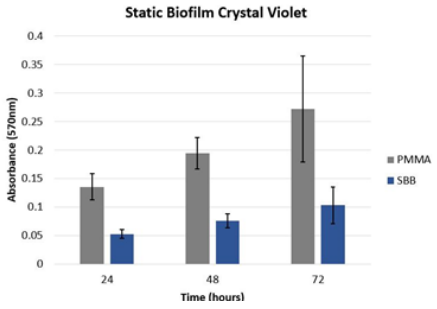

Figure 1. Crystal Violet-stained biofilm mass on each cement type over the course of 72 hours in static growth conditions. n=8

Crystal Violet staining was used to quantify attached bacteria and biofilm mass after rinsing the surface with PBS. Stained biomass was removed from the cement surface by vortexing, followed by sonication, and a final vortex into PBS. Unstained static and dynamic specimens from each cement type were collected and qualitatively evaluated using Scanning Electron Microscopy (SEM).

Results: The average absorbance for the specimens tested in the static assay is displayed in Figure 1. PMMA samples had significantly more attached bacteria both initially and overtime than did SBB samples under the same conditions ($p<0.05$). The absorbance of biofilm from SBB did not significantly increase over the course of 72 hours ($p<0.05$). Under dynamic growth conditions, this trend continued with PMMA showing significantly more bacterial attachment than SBB ($p<0.05$). SEM images of both PMMA and SBB cements after 48 hours of dynamic biofilm growth are shown in figure 2. Qualitatively, PMMA appears covered in bacteria, with massive colonies assembling to begin biofilm formation. On the other hand, bacterial colonization on SBB appears sparse, and no large aggregations could be identified.

Conclusions: This study demonstrated the characteristic of the SBB cement to be significantly less conducive to bacterial colonization and biofilm development than PMMA cement. Such an innate ability to reduce biofilm formation without the use or incorporation of antibiotics may be essential in the future battle against infection around implants. Surface roughness, porosity, tension, or surface chemistry may be contributing factors to the differences observed. These surface property differences may play a large role in bacterial attachment, growth, and biofilm formation especially in a dynamic testing environment where a sheer force is present. Surface roughness is visually different as the PMMA cement is composed of small PMMA beads sintered together with a monomer, which leads to a rougher surface than SBB. Our study showed that the novel SBB cement provided substantially more bacterial inhibition than PMMA.

References: [1]Song F. J Dent Res. 2015;94:1027-34. [2]Inzana J. Biomater. 2016;81:58-71.

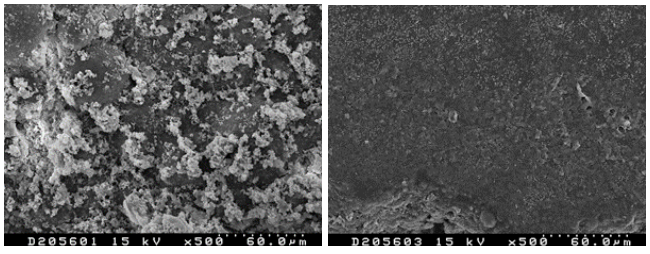

Figure 2. SEM images of bacterial attachment and biofilm formation on the surfaces of both PMMA (left) and SBB (right) cements after 48 hours in dynamic growth conditions.