Particle Size and pH Effects of Borate Bioactive Glass and Poly(Methyl Methacrylate) Bone Cement Composite on Bacterial Growth

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Statement of Purpose: Poly(Methyl Methacrylate) (PMMA) is the most common bone cement used in total joint replacements. While its mechanical strength is advantageous to this application, its affinity to harbor bacteria is one reason bacterial infection is a primary cause of total joint replacement failures. Borate bioactive glass, 13-93B3, is a readily biodegradable material that has been shown to promote osteogenesis, angiogenesis, anti-inflammatory responses, and antibacterial activity. It has been postulated that bioactive glass has an antibacterial effect due to several different mechanisms including increased pH, physical damage to the bacterial cell wall, ionic concentrations in the local environment, and osmotic pressure. Additionally, different bacteria may have varying levels of susceptibility to different antibacterial mechanisms. In order to better identify the factors contributing to antibacterial effectiveness of bioactive glass, various sizes of borate bioactive glass particles were incorporated into PMMA bone cement and were compared to a pH-neutral bioactive glass, likewise incorporated into cement, after exposure to an orthopedic-relevant bacterium, *staphylococcus aureus* (SA). We hypothesized smaller glass particles would be better at preventing bacteria growth and additionally, the borate bioactive glass would show a stronger antibacterial effect than the pH-neutral bioactive glass.

Methods: Two types of bioactive glass were obtained from Missouri University of Science and Technology (Rolla, MO). Borate bioactive glass, 13-93B3 (BG), has a composition of mol %: 6 Na_2O, 7.9 K_2O, 7.7 MgO, 22.1 CaO, 54.6 B_2O_3, 1.7 P_2O_5, and pH-Neutral bioactive glass (BG-N) is composed of mol%: 16 Na_2O, 24 CaO, 40 B_2O_3, 20 P_2O_5. The powder portion of PMMA bone cement, SmartSet MV (Depuy, Blackpool, UK) was mixed separately with the bioactive glasses so the glass comprised of 30% (wt/wt). Discs of size 6x12mm of the glass-incorporated PMMA bone cement were formed and UV-sterilized. BG samples contained 3 different groups, each with different average glass particle size incorporated: 5µm, 33µm, and 100µm (BG5, BG33, BG100, respectively). BG-N sample group were made with an average sized glass particle of 3µm. A control sample group was made with PMMA only (PCON). Eight discs from every group were placed in an inoculum of 10^6 CFU/mL of bioluminescent SA strain, Xen29 (PerkinElmer). The top surfaces of the discs were imaged after 48hrs using in-vivo imaging system (IVIS) to observe the presence of live (luminescent) bacteria. Each disc was then sonicated in Mueller-Hinton Broth (MHB) which was re-plated and taken to a plate reader to evaluate the total amount of bacteria attached to the discs via luminescence. Independent two-sample t-tests were used to assess differences in the number of live bacteria adhered to the surface of the discs. Additionally, a standard pH meter was used to measure the pH of PCON, BG5, and BG-N after sitting in MHB for 48hrs.

Results: Borate bioactive glass-incorporated PMMA cement reduced the adhesion of live bacteria on the surface of the pellets (Figure 1). There was statistical significance between BG5, BG33, and BG100 compared to PCON ($p<.001$). When comparing the different glass particles there was a statistically significant difference between the BG5 and the two other larger particle samples ($p<.05$). The pH neutral glass group (BG-N) however, showed a statistically significant increase in bacterial adhesion on the surface of the discs when compared to PCON and BG5 discs ($p<.001$, Figure 2). The average pH after 48 hours of the MHB solutions containing the PCON, BG5, and BG-N were 7.14, 8.46, and 6.6, respectively (n=3).

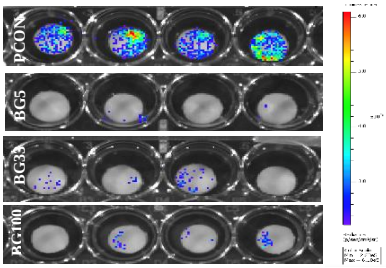

Figure 1. Radiance of live Xen 29 bacteria on surface of discs post 48-hour incubation. (n=8)

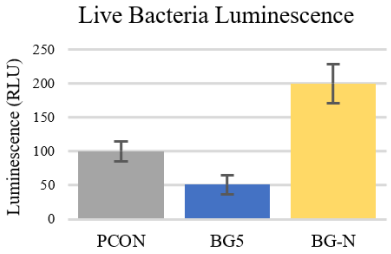

Figure 2. Luminescent bacteria detected of disc groups. Error bars represent 95% confidence interval. (n=10)

Conclusions: Glass particle size does not seem to drastically alter the antibacterial property of bioactive glass, but a smaller size had a slightly greater affect. A change in pH of the local environment is likely a primary mechanism for antibacterial effect shown in borate bioactive glasses against SA. Further studies plan to better understand the long-term antibacterial effect of a bioactive glass incorporated PMMA cement composite as well as elucidating other mechanisms for additional bacterial strands. Additionally, we plan to identify any potential biocompatibility issues of this composite with mammalian cells.