Evaluation of a Gentamicin-Loaded Hydroxyapatite/Chitin Bone Cement

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Statement of Purpose: In orthopaedic surgery, infections can lead to failed devices, revision surgeries and extended healing times. Antibiotic loaded bone cements have been shown to clinically reduce 1.6-6% infections after total hip arthoplasty or more when used in addition to systemic antibiotics[1]. However, the clinically available bone cements such as polymethyl methacrylate (PMMA) and calcium sulfate are not ideal because of high curing temperature or fast resorption rates. In this study we investigated a novel hydroxyapatite – chitosan based scaffold as an antibiotic loaded bone cement. We hypothesized that this material can deliver a broad-spectrum antibiotic, gentamicin, while maintaining good mechanical and biocompatibility properties.

Methods: *Preparation of injectable bone cement* - Aseptically, the solid and liquid components of the bone cement in US patent number US 2011/0136935 A1 were mixed for 1 minute, loaded into a syringe and injected into a stainless steel mold and cured at 37°C ON. Gentamicin sulfate powder was added to the cement prior to curing in amounts of 0%, 0.5%, 1.1% and 1.6% wt%. *Mechanical Testing* - The compressive strength of the gentamicin loaded cements (GLC) was determined with an INSTRON 3345, loaded with 1kN at a rate of 20 mm/min until fracture. Stiffness was determined as the slope of the linear fit detected during the test. *Gentamicin Elution and Activity* - GLC in 100mL of sterile PBS (37°C) were sampled every 24hrs for 8 days. Antibiotic concentrations were determined by HPLC. Antibiotic activity against *S. aureus* (strain ATCC 29213) was determined with a zone of inhibition test every 24hrs for one week. *Mesenchymal Stem Cell Viability* - GLC were tested for biocompatibility with mesenchymal stem cells over a 4-day period. Daily samples were analyzed with an MTT assay. *Statistical Analysis* - Kruskal-Wallis, was used to determine differences in time point and gentamicin levels for the elution, zone of inhibition and viability data. Only differences in gentamicin levels were analyzed for the mechanical data. Significance was determined at $p<0.05$ and differences within groups were analyzed with the post hoc Mann-Whitney test.

Results: *Mechanical Testing* - There was no significant difference in the Young's Modulus and stiffness for the GLC groups. Only a difference between 0% and 1.6% GLC was found for compressive strength ($p=0.004$). *Gentamicin Elution and Activity* – The elution of gentamicin from the cylinders generally followed a trend with increasing loading percentages, 1.6% > 1.1% > 0.5% (Figure 1). The cumulative Gentamicin concentration and percent recovery in parentheses after 5 days was: 8.14 µg/mL (33.5%), 11.95 µg/mL (25.35%) and 36.89 µg/mL (51.64%) for 0.5%, 1.1% and 1.6% groups respectively. The release of gentamicin during the study was sufficient to inhibit *S.aureus*. The bacterial inhibition followed the same trend as the elution profile, the greater the loading the greater the inhibition. The zone of inhibition for 0.5%, 1.1% GLC was consistent over the 8 days, but 1.6% GLC showed increasing in inhibition through day 6. *Mesenchymal Stem Cell Viability* - The viability of MSC over 4 days in the presence of 0%, 0.5%, 1.1% and 1.6% GLC were not statistically different from each other ($p>0.1$). All samples showed approximately 50% viability in cells compared to cells grown on tissue culture plastic.

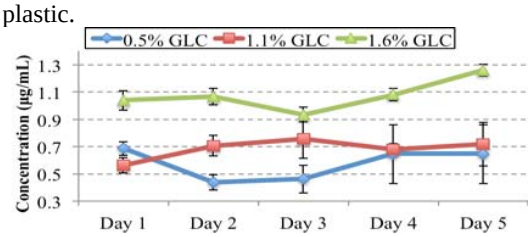

Figure 1: Gentamicin elution. n=3

Conclusions: The GLC groups were able to sustain constant release of antibiotic over 5 days. There was a higher percent recovery of gentamicin from the 1.6% GLC (~50%) as compared to the other groups, because of the higher loading and dissolution of antibiotic. All GLC groups showed a stable and extended release of gentamicin, instead of a burst release from other antibiotic loaded bone cement systems[2]. The sustained release shows that this cement is a suitable delivery vehicle for a prophylaxis agent. The compressive strength of the cement studied (7-11MPa) did not reach that the ISO 5833 standard of compressive strengths for bone cements (70MPa)[3], however, it is within the range of cancellous bone. Statistical analysis showed that gentamicin did affect the compressive strength at the 1.5% level, however, none of the samples were near the ISO standard and thus cannot be used in load bearing situations. The inhibition of *S.aureus, a* common bacterial in prosthetic infections[4], for 7 days gives reassurance that this cement will provide sufficient initial prophylaxis as necessary in prosthetic joint surgeries. The GLC groups were found to be biocompatible with MSC over a 4-day study and no statistical differences were found between antibiotic loaded and unloaded control groups. There was however, only 50% viability for all groups that received the cement compared to cells on tissue culture plastic alone. This could be due to the cement cylinders physically rolling on the cells during the transport of the plates, which dislodged, crushed and killed the cells. Future studies will investigate this cement in an infected animal model to determine bone regeneration in the presence of an infection with and without gentamicin loaded into the cement.

References: 1. Passuti N. Joint Bone Spine. 2003;70(169–74). 2 Habraken WJEM. Adv. Drug Delivery Rev. 2007;59(234-248). 3. Pelletier MH. J Arthroplasty. 2009;24(454-60). 4. Brock HS. J Arthroplasty. 2010;25(990-7).

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