Release of Bioactive Molecules from a Moldable Calcium Sulfate Bone Graft Substitute

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Introduction

Large bony defects that can result from high energy trauma cannot heal spontaneously and often become infected. The current clinical method of treating these types of wounds is debridement followed by an autologous bone graft and systemic antibiotics. Calcium sulfate (CS) has a long history of clinical use as a biomaterial, and with the addition of viscous biopolymers, it can become a moldable bone grafting material capable of conforming to any irregular defect. Furthermore, addition of antibiotics and osteogenic molecules allows the composite to treat the infection before stimulating the growth of new bone.

In the present work, a moldable CS bone filler capable of delivering bioactive molecules was developed for the treatment of large, infected bony defects.

Methods

Bone filler samples we re prepared using pre-dried cores and a moldable outer shell in order to provide a physical barrier for the delayed release of simvastatin while releasing antibiotics. Cores containing 90 wt% CS, 5 wt% hyaluronan (HY), and 5 wt% simvastatin acid were dried at 40°C for at least 24 hours prior to the beginning of the release studies. Shells containing 87 wt% CS, 10 wt% HY, 1.5 wt% gentamicin, and 1.5 wt% vancomycin were molded around the pre-dried cores before being encased in dialysis tubing (8000 Da cutoff) and placed in 8ml of phosphate-buffered saline (PBS). The solution was collected and changed every day and supernatants were analyzed using UV-vis to determine antibiotic concentration over time.

Kirby-Bauer (KB) tests were performed following a British Society for Antimicrobial Chemotherapy (BSAC) standardized disc susceptibility method. *Staphylococcus aureus* were plated on blood-agar plates at a density according to the 0.5 McFarland standard. Filter paper discs approximately 7.1mm in diameter were soaked with 5µl of supernatant, placed on the agar plates, and incubated at 37°C for 24 hours prior to measuring the zones of inhibition.

Results and Discussion

The moldable bone grafting composite was designed to conform to any irregular defect, treat a bacterial infection, and promote new bone growth using osteogenic molecules**.** Delivering antibiotics at concentrations well above the MIC for a prolonged period of time is critical in clearing an infected wound site and allowing more effective bone regrowth. The concentration of vancomycin released over time can be seen in Figure 1 alongside the total cumulative released from the samples. Although the amount of antibiotic decreased linearly over the first six days, it remained constant around 20 µg/ml through at least the first ten days of the study. The MIC and MBC of vancomycin on *S. aureus* are 2 and 8µg/ml, respectively.

Zones of inhibition from the KB assay can be seen in Figure 2. A possible reason for the decrease in inhibition of the bacteria at later time points when the concentration is around 20µg/ml, which is above the MIC, is that the physical amount of antibiotic present in the filter paper is so low. Each filter paper contained 5µl of supernatant and ≤0.5µg of vancomycin.

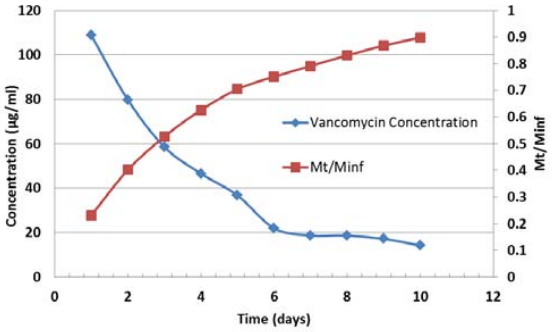

Figure 1: Concentration and cumulative release of vancomycin from bone filler composites over time. (Data are means, n=2)

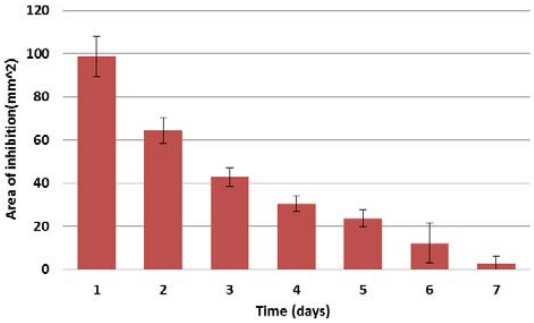

Figure 2: Zones of inhibition from Kirby-Bauer assays of bone filler supernatants. (Data are mean ± SD, n=6)

Conclusion

To create a moldable bone filler material that is capable of treating infection before stimulating new bone formation, a moldable antibiotic loaded shell was wrapped around a pre-dried osteogenic core. The concentration of vancomycin was maintained at >MIC levels for the 10 days of the study, and the antibiotic was effective in inhibiting bacteria growth in the KB assay for at least 7 days.

Acknowledgement

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