Release and Activity of Adenosine Incorporated Into Calcium Sulfate

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Statement of Purpose: Each year, approximately six million bone fractures occur in the United States, many with accompanying bone loss [1]. Among these fractures, up to 10% suffer from delayed union or nonunion [2]. Current treatment for bone fractures or non-union of bone can include expensive growth factors or an additional painful surgical procedure, such as autografts or bone graft substitutes to facilitate bone healing. Adenosine has been shown to increase the proliferation rate of fibroblasts and osteoblast precursor cells [3]. Adenosine could be a viable option as a therapeutic to improve bone healing rates and may be a viable alternative to growth factors by improving proliferation of osteoblasts and osteoblast precursor cells. Calcium sulfate ($CaSO_4$) has been used as a biomaterial bone graft substitute and local drug delivery system, with advantages of complete resorption and osteoconductivity [4]. In this study, the elution profile and biological activity of adenosine incorporated into $CaSO_4$ was characterized.

Methods: Adenosine-loaded $CaSO_4$ pellets were made by combining $CaSO_4$ (unaccelerated type V hemihydrate donated by Wright Medical Technologies, Inc.) with water in a 1.67:1 ratio and 0%, 5%, or 10% (by weight) free base adenosine powder (MP Biomedicals). $CaSO_4$ pellets 5mm in diameter and 3mm in height were created by casting into a mold and allowing the pellets to set (convert to dihydrate form) overnight. Three $CaSO_4$ pellets from each group (0%, 5%, 10% adenosine) were placed in aliquots containing 10mL of alpha-MEM. The aliquots were subjected to constant rocking in a 37°C incubator and samples were taken each day for 7 days, with complete media refreshment each day (n=4). Adenosine concentration was determined using High Pressure Liquid Chromatography (HPLC) using 0.01 M potassium phosphate in 10% methanol at pH 4 as a mobile phase. Adenosine peaks at 7.5 minutes were characterized at 256 nm with a C18 reverse phase column, and normalized to adenosine standards. Murine osteoblast precursor cells (MC3T3-E1) were seeded in 32 wells of a 96-well plate at approximately 1 x 10^5 cells/cm^2. After overnight attachment in a 37°C, 5% CO_2 incubator, the alpha-MEM media was exchanged for 75uL of alpha-MEM containing 20% fetal bovine serum and 200mg/L Normocin. The plate was allowed to equilibrate in the CO_2 incubator for an hour before 75uL of each eluate sample were added to separate wells. Eluate samples were centrifuged before drawing the 75uL to prevent particulate from $CaSO_4$ degradation from being transferred to the wells. A control group was exposed to 150uL of 10% FBS, and 100mg/L Normocin. Based on results of initial elution, Day 1 and Day 2 eluates were tested to evaluate activity of adenosine released (n=4 in each group) and left for 30 hours. Cell viability was determined using Cell Titer Glo (Promega) assays.

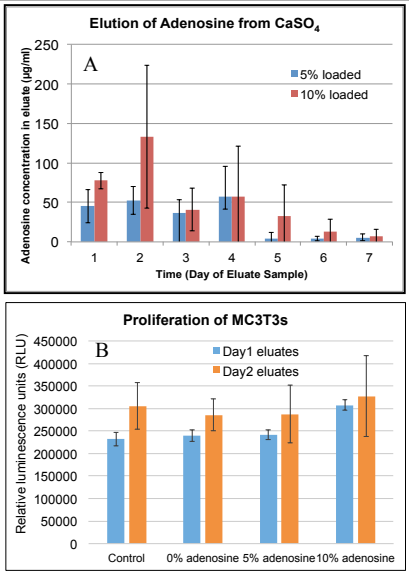

Figure 1. Graphs of A.) concentration of adenosine in eluate over the course of 7 days, and B.) proliferation of MC3T3 cells after 30 hours of exposure to day 1 or 2 eluates. Data is mean ± standard deviation. No significant differences in proliferation were detected at the 5% significance level using one-way ANOVA.

Results: Addition of adenosine did not affect setting properties or increase friability of the $CaSO_4$ dihydrate pellets. Elution followed a burst release pattern with the highest burst during the second day and concentrations trailing off after day 4 or 5. Pellets loaded with 10% adenosine released more adenosine and released over an extended course of time versus the 5% loading. Although no statistically significant increases in cell proliferation were detected at the 5% significance level (p=0.015), a trend of increased proliferation as loading with increasing eluted adenosine was found. A large variation between sample concentrations may have led to variability in proliferation results.

Conclusions: $CaSO_4$ releases adenosine at concentrations that may have activity in promoting proliferation of osteoblasts. The pattern of release found in this preliminary study can be used to modify the concentration of adenosine loaded in order to maintain biologically active concentrations over longer periods of time. This preliminary study suggests that active adenosine is released from $CaSO_4$. Adenosine's role in encouraging proliferation and osteogenic differentiation of mesenchymal stem cells is also being evaluated. Future studies are planned to evaluate higher loading levels in the $CaSO_4$ pellets to increase elution and biological activity. Promotion of bone growth by adenosine-loaded $CaSO_4$ *in vivo* is also planned in future evaluations.

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