Optimized Daptomycin Elution from Calcium Sulfate

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Statement of Purpose: Musculoskeletal infections introduce a major problem to wound healing. One treatment method is local antibiotic therapy targeting specific bacterial organisms. Antibiotics, such as daptomycin, delivery at concentrations above the minimum inhibitory concentration (MIC) for a prolonged time via a degradable implant are anticipated to efficiently eliminate local bacterial infections. The MIC is estimated to be ~0.50 µg/ml daptomycin for selected bacterial strains. Optimal delivery requirements are typically at a peak concentration of 100X MIC, or 50µg/ml, and 10 day maintenance concentration of 5X MIC, or 2.5µg/ml.[1] These requirements are not achievable using daptomycin loaded calcium sulfate ($CaSO_4$) alone, but are hypothesized to meet requirements after adding chitosan coating.

Methods: Initially a large array of 3 mm height x 3mm diameter $CaSO_4$ (Osteoset, Wright Medical Technology, Arlington, TN) implants were made with varying daptomycin (Cubicin, Cubist Pharmaceuticals, Lexington, MA) concentrations, potassium sulfate (K_2SO_4, used as a catalyst for casting) concentrations and chitosan (Primex, Iceland) coatings. Implants were cast by mixing $CaSO_4$ with various concentrations of K_2SO_4 aqueous solution (4:1 ratio), followed by mixing in various concentrations of daptomycin and allowing the putty to cast for 10 min in a silicone mold of desired geometry. Coatings were created by submersing the cast $CaSO_4$ pellet into a solution of 1 w/v% chitosan (71% degree of deacetylation) dissolved in 1 v/v % acetic acid, drying the coating, and repeating four times. The elution profile for each daptomycin loaded, coated and uncoated, implant variation (n=3) was determined by analyzing, through high pressure liquid chromatography, the concentration of eluate samples taken at 1, 2, 4, 6, 8, 10, 14, 21, and 28 day time points.[2] The optimized pellet variation was chosen by determining which used the least daptomycin to achieve the requirements. Differences between variations were determined by one-way ANOVA with Tukey's post hoc statistical analysis.

Results: Preliminary results indicated that chitosan coated $CaSO_4$ with 3% daptomycin and 2% K_2SO_4 met the peak concentration requirement of 54.31 ± 5.04 µg/ml at day two and maintenance concentration requirement by persisting at concentrations higher than 3.18 ± 0.37 µg/ml for 28 days. However, with material sourcing changes these results could not be reproduced in subsequent tests. Reevaluation of increased daptomycin percentages with the commercially available materials resulted in an optimal variation at 5% daptomycin and 3% K_2SO_4. This variation met the peak concentration requirement of 89.38 ± 4.66 µg/ml at day one and concentrations persisted at higher than 4.13 ± 0.65 µg/ml for 12 days. For a planned rabbit radius infection model,[3] the size of the implant was scaled up to fit the model's defect. An implant with dimensions of 10 mm height x 5 mm diameter was created and an elution study also preformed to verify that the MIC requirements were still met. This implant successfully met the MIC requirements with a peak concentration of 248.19 ± 47.56 µg/ml at day one and a maintenance concentration at higher than 43.45 ± 11.07 µg/ml for ten days which was significantly more efficient than the uncoated version of the same implant (Figure 1).

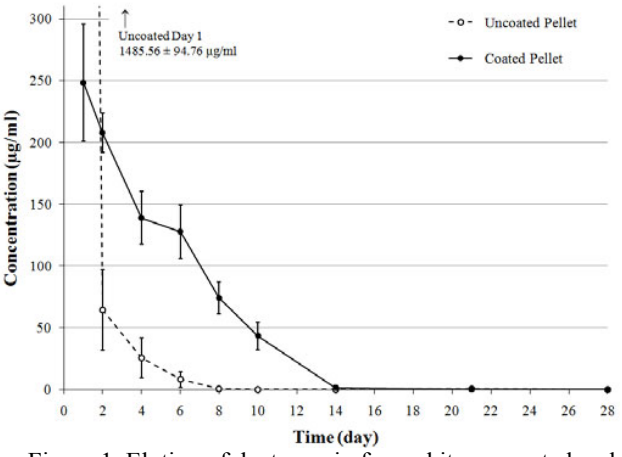

Figure 1. Elution of daptomycin from chitosan coated and uncoated, $CaSO_4$ pellet containing 5% daptomycin and 3% K_2SO_4 with dimensions of 10 mm height x 5 mm diameter. Statistical analysis confirmed significant differences ($p < 0.05$) between the coated and uncoated pellet.

Pellet Dimensions	Calcium Sulfate Formulation	Minimum Inhibitory Concentration Requirments: Peak Concentraion	Minimum Inhibitory Concentration Requirments: Maintenance Concentration
3 mm height x 3 mm diameter	3% Daptomycin, 2% K_2SO_4, Chitosan Coated	Day 2, at 54.31 ± 5.04 µg/ml	28 Days ≥ 3.18 ± 0.37 µg/ml
3 mm height x 3 mm diameter	5% Daptomycin, 3% K_2SO_4, Chitosan Coated	Day 1, at 89.38 ± 4.66 µg/ml	12 Days ≥ 4.13 ± 0.65 µg/ml
10 mm height x 5 mm diameter	5% Daptomycin, 3% K_2SO_4, Chitosan Coated	Day 1, at 248.19 ± 47.56 µg/ml	10 Days ≥ 43.45 ± 11.07 µg/ml

Table 1. Summary of the values of three significant $CaSO_4$ formulations meeting the MIC requirements.

Conclusions: This investigation confirms the hypothesis that chitosan coating can enhance the elution of daptomycin from $CaSO_4$ pellets. The coating maintains the antibiotic concentration at a higher level for an extended period of time. Utilizing chitosan coated $CaSO_4$ to optimize daptomycin's drug delivery should provide relevant information for future *in vivo* studies of musculoskeletal infections.

References: [1]Weiss BD. Antimicrob Agents Chemother. 2009;53:264-266. [2]Richelsoph KC. Clin Orhtop Relat Res. 2007;461:68-73. [3]Smeltzer MS. J Orthop Res. 1997;15:414-421.

Acknowledgements: Wright Medical Technology for donations of $CaSO_4$, Cubist Pharmaceuticals for donations of daptomycin. Funding by NIH R01A1069087-02