INTRODUCTION
Alternative methods for the treatment of bone infections due to trauma, musculoskeletal diseases or orthopaedic implants are an emerging area of study. Localized delivery of antibiotics provides a broader scope of treatment options for both the patient and surgeon. One advantage of administering locally instead of orally or intravenously is that a beneficial dosage of drug can be achieved while avoiding high serum concentrations that can be harmful to the patient. An optimum delivery system would prolong the release of therapeutic levels of antibiotic while being resorbable. Calcium sulfate has been studied as a carrier for antibiotics in many previous investigations and is recognized as an effective bone graft substitute. Chitosan-coated calcium sulfate pellets offer a potential method to achieve both extended localized delivery of antibiotics while acting as a bone graft. Chitosan is a linear polysaccharide derived from chitin that has been shown to be biocompatible and antimicrobial. Chitosan coatings provide a permeation barrier that may decrease degradation and extend therapeutic drug release. In order to create a more desirable elution profile, a crosslinking agent can be used to modify the chitosan layer. Genipin is a naturally occurring non-toxic crosslinking agent that may reduce drug elution from the calcium sulfate carrier. The combination of a crosslinked chitosan coated calcium sulfate delivery system loaded with antibiotics, specifically gentamicin, tobramycin, and daptomycin may provide an enhanced treatment method than currently available. The objective of this study is to determine the efficacy of the delivery system by elution and zone of inhibition studies.

MATERIALS AND METHODS
Chitosan and crosslinked chitosan fabrication
Plain chitosan
- A 2.5 (w/v)% chitosan solution was prepared by dissolving 2.5 g of 87.4% deacetylated (DDA) chitosan (Vanson) in 97.5 ml of a 1 (w/v)% acetic acid solution and stirred for 6 hours
Crosslinked chitosan with genipin
- A 0.5 (w/v)% genipin solution was prepared by mixing 15 mg of genipin (Wako) with 3 ml of deionized (DI) water. This solution was then mixed with a 2.5 (w/v)% chitosan solution and stirred for 6 hours.

Pellet fabrication
Gentamicin and Tobramycin 4 (w/v)%
- Pellets were prepared by dissolving 2.6 g of antibiotic with 12.5 ml of DI water, and then mixed with 50 g of alpha hemihydrate calcium sulfate. The calcium sulfate solution was then cast into a silicone mold to create cylindrical pellets (4.8mm dia, 3.3mm h).
Daptomycin 4 (w/v)%
- Pellets were prepared by dissolving 100 g of alpha hemihydrate calcium sulfate with 31 ml of 4 (w/v)% K$_2$SO$_4$ solution. After two minutes of stirring, 5.5 g of lyophilized daptomycin powder was added in order to make 4 (w/v)% pellets. (Cast the same as above)

Coating methodology
Each set of pellets tested were submersed into the chitosan or crosslinked chitosan solution and then removed and dried at 37°C for 1 hour. This process was repeated for multiple layers.

Efficacy studies
Elution study
- Elution was characterized by placing groups of eight pellets in 20 ml phosphate buffered saline (PBS) at 37°C. Samples of PBS were removed at various time points and measured for the antibiotic concentration (gentamicin and tobramycin only) using a florescence polarization immunoassay technique (TDxFLX system, Abbott Laboratories, Abbott Park, IL).
- HPLC was used to determine the release rates of daptomycin. Adapted from Dvorchick, J Clin Pharm 45(1): 48-56, 2005.

Zone of Inhibition study
- A zone of inhibition study was performed using the modified Kirby-Bauer Method to determine the levels of S. Epidermis activity inhibited by antibiotic release in the sample eluents. http://w3.whosea.org/bct/book28/11.htm

RESULTS
Elution study
- Gentamicin and Tobramycin - Figure 1 shows the elution profile of gentamicin. The gentamicin concentrations have been normalized to the initial mass of the pellets (μg/ml/g). Peak concentrations of 860 to 490 μg/ml/g occurred at day 1 and dropped to a steady release range by day 5 (24 to 0.1 μg/ml/g) and continued through day 28. The tobramycin release profile is very similar to that of the gentamicin profile illustrated above.
- Daptomycin – Figure 2 shows the elution profile of daptomycin. The daptomycin concentration measured from the crosslinked chitosan at day 1 was 3000 ppm to 657 ppm at day 5 whereas the concentrations measured from the non-coated samples were 2340 ppm at day 1 to 50 ppm at day 5.

Zone of Inhibition study
Gentamicin, Tobramycin, and Daptomycin – Results of the zone of inhibition study were measured at days 1, 3, 7, 14, and 21. The gentamicin and tobramycin samples ranged from 1 mm at day 1 to 0.6 mm at day 21. The daptomycin group ranged from 0.8 mm at day 1 to 0.5 mm at day 21. The ZOI data showed that the eluents of the crosslinked chitosan pellets was improved by up to 9% with a plain chitosan layer and up to 16% further enhanced by crosslinking the chitosan layer. The elution profile was improved by up to 9% with a plain chitosan layer and up to 16% with a crosslinked chitosan layer from days 3-21. The results from the zone of inhibition study further confirmed the biologic activity of antibiotic over time.

DISCUSSION
The elution test results confirm that the chitosan coating successfully acted as a permeation barrier for antibiotic release over the time period tested. A more desirable release profile was achieved and this result was further enhanced by crosslinking the chitosan layer. The elution profile was improved by up to 9% with a plain chitosan layer and up to 16% with a crosslinked chitosan layer from days 3-21. The results from the zone of inhibition study further confirmed the biologic activity of antibiotic over time.

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