INTRODUCTION:
Currently, the standard of care for osteomyelitis cases is prolonged systemic antibiotic therapy and implantation of antibiotic carrier beads. However, this method of treatment requires a secondary surgery to remove the beads after the infection has cleared. The opportunity exists to produce an antibiotic-infused bone void filler that would inhibit bacterial colonization of the device and promote bone regeneration while eliminating the need for a secondary surgery. In this study, we examined the feasibility of loading a broad spectrum antibiotic into a commercially available xenograft bone void filler.

METHODS:
BioCleanse®-sterilized bovine cancellous chips that underwent final package sterilization using 1.8 megardas of gamma irradiation were used in each experiment.

Release Kinetics: Quantification of the antibiotic was performed using a C18 reverse phase column and an Agilent 1200 Series High Performance Liquid Chromatography (HPLC) with an Ultraviolet detector. One gram of bovine cancellous bone loaded with various concentrations of antibiotic was added to 5 ml of 1X phosphate buffered saline (PBS) and incubated for 24 h at 37°C. At the designated time points, 1 mL of the solution was removed and analyzed via HPLC. The remaining 1X PBS solution was removed, replaced with fresh 1X PBS, vortexed, and incubated for an additional 24 h at 37°C. All release kinetic studies were performed in quadruplicate (n=4).

Bacteria Zone of Inhibition Study: A biofilm of Staphylococcus aureus was formed on Mueller-Hinton agar plates. A single Microbiologics lyfodisk was placed in the center of the agar plate and 100 µl of elution solution was added to the lyfodisk. The agar plates were then incubated for 16 h at 37°C at which time the distance between the center of the lyfodisk and the bacteria was measured.

Bacteria Colonization Study: Four grams of bovine bone were added to 20 mL of 10^4 CFU/mL of staphylococcus aureus concentration in 37°C Mueller-Hinton broth and allowed to incubate at 37°C for 1 h while shaking at 45 rpm. After 1 h, 250 mg ± 12.5 mg of bone were removed, added to 1 mL of 37°C sterile 1X PBS and vortexed for 30 sec ± 1 sec. One hundred microliters of the resultant solution was diluted with additional PBS and streaked on Mueller-Hinton agar plates then incubated at 37°C for 16 h. The colonies present on each agar plate were counted and recorded. The remaining Mueller-Hinton broth was removed, replaced with fresh pre-warmed broth, vortexed and incubated for an additional 24 h at 37°C. This was repeated on a daily basis for up to 14 days. The study was performed in triplicate.

Osteoblast Alkaline Phosphatase (AP) Activity/Proliferation: Rat calvarial osteoblasts were plated in triplicate at a density of 20,000 cells per well in 1 mL of basal media (DMEM, 1% FBS and antibiotic/antimycotic). The 24 well plates were incubated for 24 hours at 37°C with 5.0% CO₂. The media was then aspirated and each well washed with 1 mL of PBS. One milliliter of basal media lacking antibiotic/antimycotic but containing either 100 µg/ml, 250 µg/ml or 500 µg/ml of the antibiotic was added to the wells. The plates were incubated at 37°C/5.0% CO₂ for four days. Cell proliferation was also investigated. The effect of various drug concentrations on osteoblast cell numbers can be found in figure 2. There was no observable decrease in alkaline phosphatase produced by the same cells in response to the various concentrations of antibiotic.

DISCUSSION:
This study demonstrates that the antibiotic-infused bovine cancellous bone inhibits bacterial colonization on the device prior to implantation. In addition, the levels of antibiotic released from these grafts remains above the minimal inhibitory concentration (MIC) against Staphylococcus aureus (1 µg/mL) for at least fourteen days (figure 1). The ability of the antibiotic loaded graft to inhibit bacterial colonization was also determined with a 14-day study. The antibiotic loaded bone was exposed to a concentration of 10² CFU/mL staphylococcus aureus. At 24 h intervals, an aliquot of 250 mg ± 12.5 mg of bone was removed from the broth, vortexed in PBS and the resultant solution was plated. The number of colonies on each plate was recorded for each time point. The results show complete inhibition of bacteria growth on the antibiotic loaded graft by the first hour. The non-antibiotic loaded samples did not inhibit bacterial colonization throughout the entire 14 days and maintained a colony count of 10⁷ CFU/mL throughout the experiment.

The effect of various concentrations of the antibiotic on cellular proliferation was also investigated. The effect of various drug concentrations on osteoblast cell numbers can be found in figure 2. There was no observable decrease in alkaline phosphatase produced by the same cells in response to the various concentrations of antibiotic.