Introduction
Infection and non-union are common complications of open fractures. Systemic antibiotics often cause adverse side effects in organs before sufficient levels can be achieved to eradicate bone infection. The local delivery of antibiotics is currently being explored as a means to circumvent systemic toxicity while minimizing infection, and to reach supratherapeutic levels to improve activity against biofilms. In addition, dual-delivery devices are being developed to deliver antibiotics while simultaneously delivering bone regeneration substances.

When choosing an antibiotic for a local delivery system, it is important to consider the significant diversity among antibiotics with regards to which pathogen they target, their mechanism of action, and the levels at which they become effective. An often overlooked factor that must be considered is the effect that they have on osteogenic cell viability. Although it is obvious that cell toxicity will affect bone regeneration, it may also be important to consider the osteogenic potential of surviving cells as well. Arguably this is as important of a consideration as is the cell toxicity, i.e., a cells survive but are not osteogenic may do little to aid in bone repair.

Materials and Methods
Human osteoblasts (Promocell, Heidelberg Germany) seeded at 12,500 cells/cm² in 24-well plates overnight, and then treated with the following antibiotics: Vancomycin (glycopeptide); Amikacin, Tobramycin, and Gentamicin (Aminoglycosides); Trimethoprim (Folate antagonist); Daptomycin (Lipopeptide); Meropenem and Imipenem (Carbapenems); Linezolid (Oxazolidinone); Cefazolin, Cefepime, and Cefotaxime (Cephalosporins); Azithromycin (Macrolide); Levofloxacin and Ciproflaxacin (Fluoroquinolones); Penicillin and Nafcillin (Penicillins); Colistin (Polymyxin); Doxycycline and Minocycline (Tetracyclines); and Rifampin (Antinutsercular) at 0, 10, 100, 200, 500, 1000, 2000, and 5000 µg/ml in osteogenic induction medium. Media was changed every 2-3 days.

Ten and 14 days after seeding, whole cell extracts were obtained using Cell Lytic™ M lysis buffer (Sigma). DNA content was determined as an index of cell number using the CyQuant® assay (Invitrogen, C7026). Alkaline phosphatase (ALP) was determined as an index of osteogenic potential using a colorimetric ALP assay kit (AnaSpec, 72146, Fremont.CA), and normalized to protein that was measured with the Bradford assay (Bio-Rad, Hercules, California). ALP per unit protein and DNA content comparisons within each antibiotic were made using a one-way measures analysis of variance (ANOVA). There were no statistical differences at doses between the 10 and 14 day samples so they were pooled. The data shown are mean ± SEM relative to control. Statistical significance was set at p < 0.05.

Results
Treatment with ≥ 200 µg/ml reduced cell number and ALP by ≥ 25% in 9 and 15 of the antibiotics, respectively (Figure 1). In general, alkaline phosphatase decreases were observed at doses lower than for cell number. Antibiotics within a class generally had similar effects in terms of their effect on cell number and osteogenic potential, with a few exceptions. Antibiotics that had the greatest decrement include Rifampin, Tetracyclines, Penicillins, Ciproflaxacin, Collistin, and Gentamicin. These antibiotics decreased both cell number and alkaline phosphatase activity at ≤ 100 µg/ml. Greater than 50% reductions in cell number and alkaline phosphatase activity were not achieved until a dose of ≥ 2000 µg/ml for the commonly used antibiotics Tobramycin and Vancomycin.

Discussion
This study is the first comprehensive evaluation of numerous antibiotics' effects on osteoblast viability and osteogenic potential for extended periods of treatment. This reference will facilitate clinicians and researchers to choose the optimal antibiotic for treatment of infection and maintenance of healthy host tissue.