Infected Femoral Segmental Defect Model: Effects of Nanosilver in Re-Establishing BMP-2 Osteoinductivity in Infected Wounds

INTRODUCTION:
Bone graft materials are placed in a variety of skeletal defects to promote bony union. Infection of bone graft devices are devastating complications that require multiple debridement surgeries, systemic antibiotic treatment, and may result in osseous non-union. Besides significant medical costs, there are also high costs from lost productivity and function. It is therefore critical to develop a systematic approach to study and treat bone graft infections. The aim of this study is to establish an infected segmental defect model to simulate acute bacterial infections in the setting of a critical-sized, segmental bone loss, and to use this model to test BMP2 efficacy with antibacterial treatment. Staphylococcus aureus (S. aureus) was used in this model because it is the bacterial pathogen responsible for ~80% of all cases of human osteomyelitis. Silver in nano particle size (nanosilver; AgNANO), rather than an antibiotic, was used in this study because antibiotics such as silver are broad spectrum, potentially low cytotoxicity agents that non-selectively target any bacterial cell activities and are thus less likely to promote bacterial resistance. We hypothesize that S. aureus can effectively create an acute osteomyelitis model without use of sclerosing agents and that nanosilver can restore BMP2 efficacy in osteomyelitic bone defects.

METHODS:
Acute infection model. In order to establish an acute infection model, a 6 mm critical sized femoral segmental defect (FSD) was created in 3 month old male Sprague Dawley rats. All surgical procedures were approved by the UCLA Chancellor’s Animal Research Committee. A polyethylene plate (23 x 4 x 4 mm dimension) was fixed on each femur using six 0.9 mm diameter threaded Kirschner wires. 10\(^3\), 10\(^4\), 10\(^5\), or 10\(^6\) standard S. aureus (strain SA113) or vancomycin- and methicillin-resistant S. aureus (Mu50) inoculated on Gelfoam were implanted into the defect prior to closure. After two weeks, the wounds were opened and degree of infection through bacterial culture and staining for bacterial residue were performed, as well as assessment of hardware fixation stability. Nanosilver cytotoxicity and bactericidal testing. Bacteria were inoculated onto nanosilver-loaded poly (lactic-co-glycolic acid) (PLGA) scaffolds of the exact dimension to be implanted for reconstructing the FSD. Cytotoxicity testing was performed on 3D PLGA scaffold rather than under 2D culture conditions because 3D conditions more closely simulate in vivo conditions and can improve cellular survival with cytotoxic agents. Bactericidal testing was also performed on the PLGA scaffold to better assess whether nanosilver coating effectively prevented infected nidus formation in the PLGA. Cytotoxicity testing was performed by seeding 5,000 passage 18 MC3T3-E1 cells onto 0%, 0.5%, 1%, 1.5%, and 2% AgNANO-PLGA cylinders in 96-well plates containing α-minimal essential medium and maintenance medium. Viable cell density and proliferation on days 2, 4 and 6 were assayed using the MTT Cell Proliferation Assay Kit. Bactericidal testing of nanosilver was performed using bacterial microplate proliferation assays, Nanosilver and BMP2 efficacy in vivo. Using our developed acute infected FSD model infected with 10\(^7\) S. aureus Mu50, nanosilver PLGA scaffolds + BMP2 or PLGA only scaffolds were implanted into the defects. High resolution faxitron imaging were performed at week 0, 2, 4, 6, 8, 10, and 12. Femurs were harvested at 12 weeks. Histomorphometric assessment including microCT imaging and histological staining to evaluate bone formation were performed.

RESULTS:
Acute infection model. We observed that 10\(^7\) S. aureus Mu50 resulted in abundant pus and was the highest inoculum possible without hardware fixation loss, excessive osteolysis, or animal mortality. S. aureus Mu50 was superior, as the SA113 strain inconsistently produced infection at similar inoculum doses. Nanosilver cytotoxicity and bactericidal testing. Nanosilver exhibited strong antibacterial properties in vitro and in vivo. Nanosilver coupled PLGA scaffolds did not inhibit adherence, proliferation, alkaline phosphatase activity, or mineralization of MC3T3-E1 pre-osteoblasts compared to uncoupled PLGA scaffold controls. Nanosilver in vitro assays showed that 0.1% AgNANO-PLGA delayed 10\(^8\) CFU S. aureus SA113 growth, while 0.5%, 1.0%, 1.5%, and 2% AgNANO-PLGA 2.0% inhibited 10\(^8\) and 10\(^6\) CFU S. aureus SA113 growth completely. Furthermore, 2% AgNANO was the most effective bactericidal dose, consistently killing both 10\(^8\) and 10\(^6\) CFU of the more virulent S. aureus Mu50, while lower doses were only variably bactericidal. Nanosilver and BMP2 efficacy in vivo. Nanosilver did not affect the in vivo osteoinductivity of BMP2. 20-40% of the animals implanted with 2% AgNANO-BMP2-PLGA group healed by weeks 8 and ~60% of the animals healed by 10 weeks as assessed by high resolution imaging by 12 weeks (Fig 1). A mineralized bony bridge connecting the two defect ends was clearly identified by both Masson’s trichrome staining and osteocalcin (OCN) immunohistochemistry staining (Fig 1). High intensity OCN signals signify active bone formation in the defect area. In contrast, 0% and 1% AgNANO-BMP2-PLGA groups exhibited no healing. Furthermore, no S. aureus Mu50 survival was evident in the contaminated femurs implanted with 2% AgNANO-BMP2-PLGA bone grafts after 12 weeks. By eliminating bacteria in the defect, 2% AgNANO-BMP2-PLGA grafts promoted significantly more bone formation compared to the control group.

CONCLUSION(S):
In this study, we established a consistent acute FSD infection model using Mu50 S. aureus without the use of sclerosing agents. Our results using this model indicate that nanosilver of defined particle size is bactericidal without discernable negative effects in vitro or in vivo on osteoblast toxicity or BMP2 osteoinductivity, making it an ideal antibacterial for bone regeneration in infected wounds. These results show that it is possible to integrate robust bactericidal and osteoinductive components in one scaffold. This approach shows great promise in shifting the clinical osteomyelitic treatment paradigm from staged debridement and reconstructive surgeries to a single-stage surgery allowing debridement and immediate reconstruction.

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