Bone Allograft coupled with Doxycycline Provides Protection Against Bacterial Colonization and Biofilm

Constantinos Ketonis MD/PhD, Isabelle Mortalena DDS, Javad Parvizi MD, Christopher Adams, Ph.D., John Abraham, MD, Noreen Hickok PhD

Background:

Bone allograft continues to be used extensively in orthopaedics to help restore bony defects and to provide structural stability. Infection of this initially foreign implant remains the most devastating complication, with a reported incidence of up to 37% in the setting of massive bone allograft implantation following tumor resections. We propose that infection could be decreased by covalently-modifying allografts with antibiotics to inhibit bacterial colonization and biofilm formation; this modification would far outlast current technologies.

Questions Purposes:

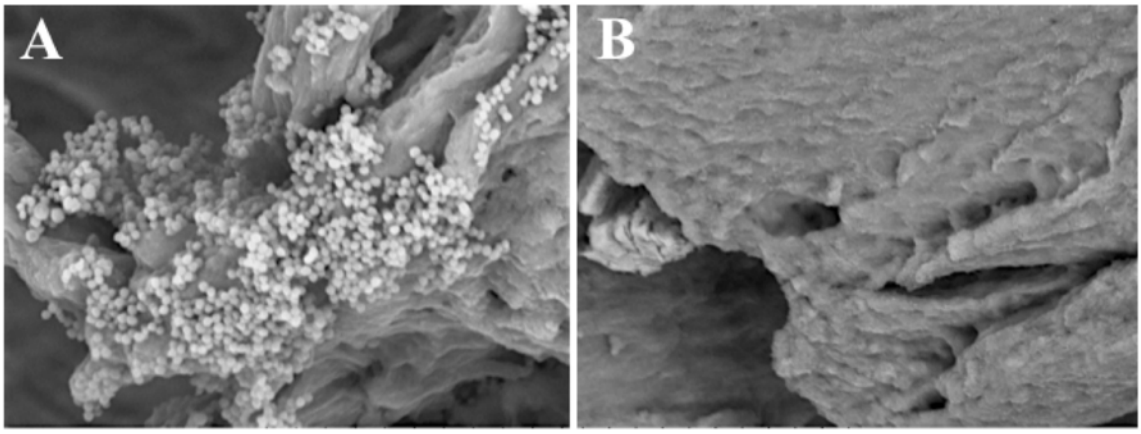
1. We first asked whether it is possible to attach antibiotics on the surface of bone allografts, in a permanent and uniform fashion without impacting the natural topographical features of the graft.
2. We then tested if modified allograft resisted bacterial (both Gram-positive and Gram-negative) colonization and biofilm formation as demonstrated by qualitative (imaging) and quantitative (bacterial counts) methods.
3. Finally, we asked if this modification has any toxic effects on mammalian cells as manifested by alterated morphology or cytoskeletal architecture.

Materials & Methods:

Morselized cancellous or cortical human bone allograft, was sequentially coupled with two Fmoc-AEEA linkers and doxycycline (DOX). Scanning Electron Microscopy (SEM) was used to assess topography during the stages of synthesis, and immunofluorescence was used to confirm doxycycline coverage and uniformity. Before assessing allograft antibacterial activity, non-attached antibiotic was allowed to elute from the bone surface for over 2 weeks. Antibacterial activity of the modified allograft (DOX-graft) was determined by incubation with *Staphylococcus aureus* (SA) and Escherichia coli (*E. coli).* Adherent bacteria were visualized by direct immunofluorescence or SEM visualization or number determined by sonication and plating. To assess biocompatibility, an osteoblastic cell line was cultured on DOX-graft for 48 hours followed by visualization of actin organization (rhodamine-phalloidin) and nuclear morphology (propidium iodide).

Results:
DOX was uniformly and stably attached to allograft without altering the bone's natural surface topography. After elution of adsorbed antibiotic, the DOX-graft was not colonized by SA or *E. coli*. By immunofluorescence, few bacteria were apparent on the surface of the DOX-graft and SEM imaging did not yield evidence of biofilm formation. DOX-grafts consistently decreased bacterial burden by more than 95% as compared to naïve allografts that were heavily colonized with SA or *E. coli* encased in a glycocalyx matrix consistent with biofilm. Importantly, these antibacterial bone grafts remained biocompatible. Following immunostaining, cell morphology and cytoskeletal architecture appeared similar to cells grown on naïve bone grafts suggesting little to no cytotoxicity.

Discussion: We have previously developed antimicrobial bone grafts by covalent attachment of vancomycin to their surface. We now demonstrate the successful attachment of doxycycline, furthering the applications of this technology. Doxycycline is active against Gram-negative and Gram-positive bacteria. Thus, these DOX-grafts could serve as new tools against polymicrobial infections often found in musculoskeletal tumor patients undergoing chemotherapy/ radiation therapy after resection of large bony masses, immunocompromised individuals, or *E.coli* osteomyelitis as seen in IV drug users and young children. Our proposed surface modification serves as a starting point for the development of a new generation of infection-resistant grafts that could potentially protect the allograft from the time of implantation through its vascularization and incorporation. Such constructs hold a promise in eliminating bacterial colonization associated with transplanted orthopaedic tissues.



Scanning Electron Microscopy (SEM) of S. aureus inoculated allograft morsels: **(A)** Small spherical structures that aggregate in grape-like clusters characteristic of SA are seen on control naive bone. **(B)** Bacteria are notably absent from the DOX-allograft, where the underlying surface topography of the bone morsel can be distinguished.

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