Development and Evaluation of a Biofilm Dispersing Scaffold

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Background/Purpose: Open fractures are universally contaminated, leading to bone and hardware infections and decreased osseous union. Biofilm formation is a central event contributing to the development of chronic infections; moreover, it has been linked to nonosseous union in up to as many as 67% of patients with negative cultures. Thus, antibiofilm agents have recently gained considerable interest as therapeutics for contaminated wounds. This study investigates the spectrum of activity of biofilm dispersal activity of D-amino acids (D-AAs) on clinical isolates of *Staphylococcus aureus* and toxicity to human cells in vitro. We also investigated whether local delivery of D-AAs from biodegradable polyurethane scaffolds with D-AAs could reduce infection in a contaminated segmental defect model.

Methods: Human fibroblasts and osteoblasts were exposed to individual D-AAs in vitro to assess toxicity. The ability of D-AAs, individually or as a mixture, to disrupt and prevent biofilm formation in a collection of clinical wound isolates of *S. aureus* and *Pseudomonas aeruginosa* was evaluated using the conventional 96-well plate microtiter model. D-AAs most effective at preventing biofilm formation (D-Met, D-Pro, and D-Trp) were then embedded into polyurethane scaffolds (PUR) in a 1:1:1 ratio. Porosity of the scaffolds and the release kinetics of the D-AAs were determined using scanning electron microscopy and high performance liquid chromatography, respectively. The embedded PURs were then tested in vivo against two strains of *S. aureus*, Xen36 (low-biofilm producer), and UAMS-1 (high-biofilm producer recovered from an osteomyelitis patient) in a previously characterized contaminated critical-size rat femur defect that utilized systemic, postoperative antibiotics.

Results: D-AAs were observed to have minimal toxicity on the viability of human osteoblasts and fibroblasts. D-Phe, D-Met, D-Trp, and D-Pro were found to be the most effective at dispersing and preventing biofilm formation. Combining D-Pro, D-Met, and D-Trp enhanced these effects and adding cefazolin to the D-AA decreased the MBEC (minimal biofilm eradication concentration) of the *S. aureus* by 16-fold. D-AAs varied in their release from PURs with 60% of D-Met but only 25% of D-Trp being released on day 1. All D-AAs had close to 100% release by 30 days. The D-AA-embedded PUR significantly ($P < 0.05$) reduced the microbial burden within the contaminated, critical-size defects when compared to empty PUR scaffolds with the biofilm forming the UAMS-1 *S. aureus.* There was no reduction seen when the wound was contaminated with the low-biofilm forming Xen-36 *S. aureus*.

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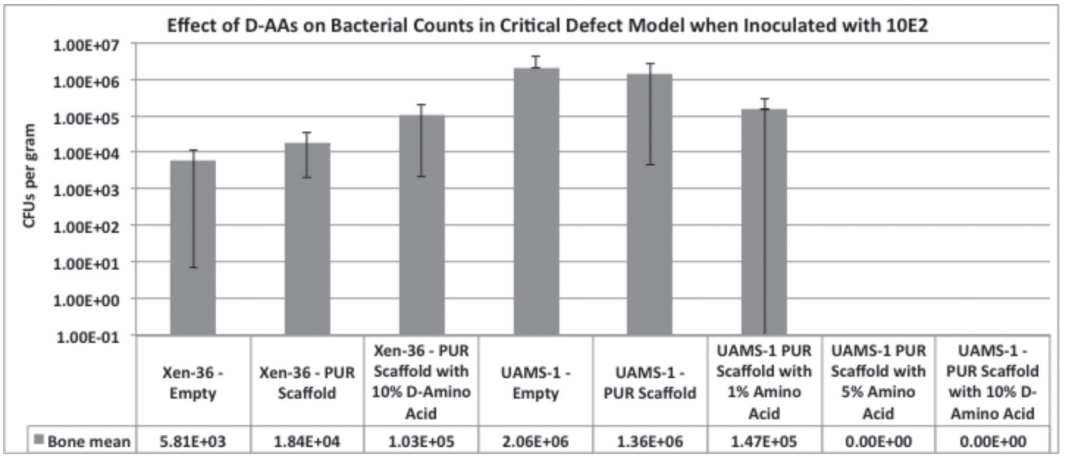

Conclusion: The vast majority of chronic infections are caused by biofilm-forming bacteria. Our results suggest that D-AAs have a broad spectrum and that local delivery of D-AAs reduces the biofilm and can enhance the activity of antibiotics against biofilms.

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Intraoperative Dip-coating Inhibits Biofilms and Supports Bone Healing During Infection

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Background/Purpose: Today, implant-associated infections contribute to increased patient morbidity and cost. Adhesion of serum proteins to the implant and low vascularity in the area of trauma create an ideal environment for bacterial adherence. Within a biofilm, bacteria synthesize an extracellular matrix that protects them from the host's immune response and systemic antimicrobials. Importantly, bacterial colonization onto substrates appears to be one critical step in biofilm formation. Consequently, we have focused our attention on surface modifications that inhibit the adherence of bacteria to implants and thereby prevent the root cause of orthopaedic infections. We hypothesized that coating orthopaedic fracture plates with certain hydrophobic polycations could favorably influence bone healing in a large animal fracture infection model.

Methods: 12 mature female sheep were enrolled in a prospective study using a previously validated long bone infection model. A unilateral middiaphyseal transverse tibial osteotomy was performed and reduced using a narrow 4.5-mm 8- or 9-hole stainless steel 316L locking compression plate (LCP). After soft-tissue closure, 10^6 CFUs (colony-forming units) of *Staphylococcus aureus* ATCC25923 were inoculated via a temporary catheter. Six sheep received coated implants (treatment cohort) and the remaining six animals received non-coated implants (control cohort). Implants were dip-coated intraoperatively. Radiographs, obtained immediately postoperative and at the conclusion of the study (30 days postoperative), were scored by three blinded reviewers for presence of septic osteomyelitis and callus morphology. The left hind limb was harvested and aseptically prepared for implant retrieval. A sterile culture was taken before implant removal. Tibias underwent micro-CT for qualitative 3-dimensional reconstructions. The osteotomy region was harvested and processed for histology and sections were scored by a blinded veterinary pathologist. Plate pieces were processed for scanning electron microscopy (SEM) and viewed for evidence of bacterial colonization. Statistical analyses were carried out on scores from radiographic, histologic, and explant evaluations. A paired *t* test was used to form preliminary associations and a statistical significance of $P < 0.05$ was used for all tests.

Results: All animals completed the study. Radiographic evaluation revealed significantly greater healing and bony remodeling consistent with normal "fracture healing" in treatment animals compared to controls ($P < 0.05$). Gross evaluation revealed the osteotomy sites in control animals to be grossly unstable with evidence of infection ($P < 0.05$). Micro-CT and histological evaluation corroborated radiographic and macroscopic data with lower scores in treatment when compared to controls ($p<0.05$) consistent with normal bone healing. SEM visualization of explanted LCPs displayed abundant biofilm formation covering >95% of the plate surface in control plates compared with no bacterial growth on coated implants.

Conclusion: Advantages of this surface modification are (1) the ease with which the coating can be applied intraoperative to any geometry implant and (2) death of the bacteria

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by mechanical disruption of the cell wall, which is less likely to create multidrug resistant bacteria. Conferring protection from pathogenic bacteria to an orthopaedic implant of industrial size and geometry in vivo is promising for reducing implant-associated infections in the orthopaedic patient.

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Evaluation of an Absorbable Gentamicin-Eluting Plate Sleeve in an Ovine Fracture Healing Model

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Background/Purpose: Lower extremity fractures have been associated with surgical site infection (SSI) and osteomyelitis. Implants can serve as substrates for bacterial adhesion and formation of bacterial biofilm, increasing the risk of surgical site infections. The recent development of an antibacterial plate sleeve (APS) allowing for controlled local delivery of gentamicin, shows great promise in mitigating SSIs. The purpose of this study was to evaluate the effect of the APS on fracture healing using a large animal fracture healing model.

Methods: 48 skeletally mature ewes underwent a unilateral mid-diaphyseal tibial osteotomy repaired with a locking plate. The tubular polymer plate sleeve was fitted over the plate for the treatment cohorts (1 APS or 4 APS) while control cohorts (Cx) received no sleeve. Sheep were euthanized at 4 and 12 weeks postoperatively. Outcome measures included: blood counts, serum chemistry, gentamicin plasma concentration, lameness scoring, and radiography for the in vivo phase. Explanted tibiae were analyzed using micro-CT, histopathology, and a semiquantitative scoring to evaluate bone healing at the osteotomy site.

Results: Surgical procedures were without complications and all animals had uneventful anesthetic recoveries. One sheep sustained a catastrophic failure of the repair and was eliminated from the study. No abnormal findings were noted for clinical pathology, serum chemistry, and gross necropsy observations in any of the study cohorts. Both treatment groups showed a peak serum concentration of gentamicin at 1 to 4 hours, with detectable gentamicin plasma concentration up to 10 days at 10.50 ± 6.98 ng/mL. The highest concentration measured was a maximum plasma gentamicin concentration of 781 ng/mL. There were no significant differences in radiographic scores between 1 APS, 4 APS, or Cx cohort at 12 weeks; there was a significant difference between 1 APS, 4 APS, and Cx for the 4-week cohort ($P < 0.05$). There was no significant effect of treatment (1 APS, 4 APS) on lameness scores and all clinical observations were unremarkable. Macroscopic evaluation of the tibial osteotomy sites, including the soft-tissue envelope, was unremarkable. Micro-CT analysis corroborated normal bone healing and there were no statistical differences found among the 3 treatment groups (1 APS, 4 APS, or no sleeve). All osteotomy scores at all sites (cranial, caudal, lateral) were significantly increased with time and significantly affected by time, confirming the progression of healing between 4 and 12 weeks. The osteotomy scores were also significantly lower in group 1 APS than in group Cx at 12 weeks only, but was not significantly different between group 4 APS and group Cx. Histopathology evaluation of the soft tissues surrounding the plate and screws showed no treatment-dependent variations except for the presence of the polymer sleeve and an associated low-grade chronic foreign body response. Polymer remnants of the APS implant were observed at 4 weeks postoperative but bioresorption was mostly completed by 12 weeks.

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Conclusion: In summary, clinical observations, digital radiography, and multiple additional ex vivo analytical methods indicated that the APS technology applied to commercially available fracture hardware in this preclinical large animal model is safe.

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