Local Delivery of D-Amino Acids Reduces Bacterial Burden in Contaminated Rat Segmental Defects

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Statement of Purpose: The healing of open bone fractures is often complicated by infection which causes delayed union or amputation [1]. Even though current treatments include wound debridement and the administration of antibiotics, new methods are required to target bacterial persistence in biofilms. Recently, D-Amino Acids (D-AAs) have been identified as molecules with minimal toxicity that can inhibit biofilm formation and promote biofilm disassembly [2]. In order to evaluate whether the delivery of D-AAs in an infected wound could be used as a strategy to reduce bacterial burden in vivo, the current study aimed to a) identify a combination of D-AAs effective in preventing/dispersing biofilms from clinical bacterial strains, b) incorporate the D-AAs into a porous scaffold, and c) evaluate the performance of the scaffold in a contaminated rat bone defect model.

Methods: 24 clinical isolates of bacterial strains were sampled from a repository collected from patients admitted for treatment not related to research at the San Antonio Military Medical Center (SAMMC; Ft. Sam Houston, TX, USA) from 2004–2011. The bacterial strains were cultured with D-AAs (Sigma Aldrich , St. Louis, MI) added to the media. Crystal violet staining was used to quantify the biofilm biomass after D-AA treatment. MIC and MBIC of antibiotics in the absence and presence of D-AA were determined following CLSI performance standards [3] and using the Calgary Biofilm device [4], respectively. A mixture of lead D-AA candidates was incorporated into a polyurethane foam as solid particles (PUR-DAA). D-AA release profiles from the foam were measured using HPLC. The efficacy of PUR-DAA foams against biofilms was tested *in vitro* and *in vivo*. Cylindrical foam samples containing D-Trp:D-Met:D-Pro (1:1:1 mass) were implanted in a contaminated critical size defect in Sprague-Dawley rat femurs [5] and the bacterial counts in the host bone, hardware, and scaffold were quantified after 2 weeks. Two S. Aureus strains were used to contaminate the defect: Xenogen 36 (weak biofilm former- Caliper Life Science, Hopkinton, MA) and UAMS-1 (strong biofilm former-received from Kai Leung at ISR). Two initial bacterial loads were tested: 10^2 and 10^5 CFUs.

Results: Biofilm biomass remaining after treating preformed biofilms with D-AAs at different concentrations was used to identify 4 lead D-AAs effective in dispersing biofilms produced by a broad spectrum of clinical strains at concentrations ≥5mM: D-Phen, D-Met, D-Trp, and D-Pro. These D-AAs also inhibited new biofilm formation, and did not affect bacterial growth. When used as a mixture, the minimum effective D-AA dose to disperse biofilms decreased 5-fold, which has also been observed by other groups [2] and suggests that D-AAs act synergistically to prevent and disperse biofilms while the specific D-AAs might be strain-dependent. The MIC of antibiotics on planktonic bacteria was not affected by the addition of D-AA. However, the efficacy of select antimicrobial agents against biofilms was enhanced with the addition of the D-AA mixture as indicated by the reduction in the MBIC. The D-AA concentrations used were not cytotoxic for fibroblasts and osteoclast as suggested by in vitro viabilities higher than 70% after D-AA treatment. Polyurethane scaffolds with 0, 0.1,1,5,10 wt% D-AA mixture were 88-91% porous with a pore diameter of about 370μm. The release kinetics of D-Pro, D-Met, and D-Trp was characterized by an initial burst followed by a sustained release for 14 days (Fig. 1A). *In vitro* culture showed that the D-AA mixture reduced the bacterial load on the foam ~5 orders of magnitude compared to the empty scaffold (Fig. 1B). When tested in the contaminated defect in vivo, the release of D-AA mixture from the foam had a minimal effect on the defects contaminated with the low biofilm-producing XEN (Fig. 1C). However, the number of contaminated bone samples (Fig. 1D) and the bacterial load on the host bone of defects contaminated with 10^2 CFUs of the heavy biofilm-producing UAMS-1 significantly decreased compared to the empty scaffold.

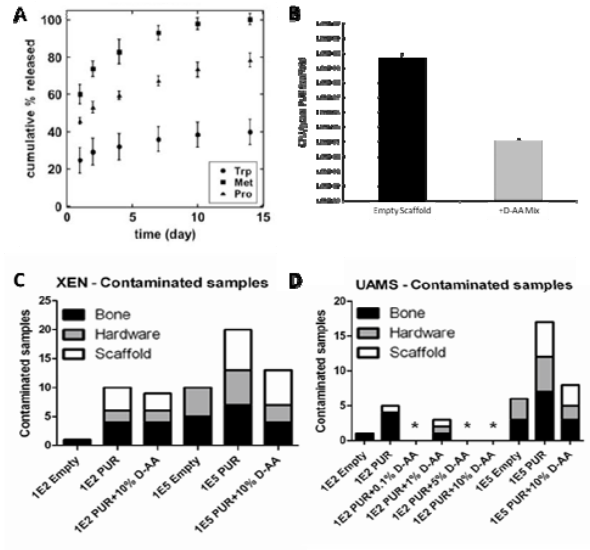

Figure 1. *In vitro* (A,B) and *in vivo* (C,D) characterization of the PUR-DAA scaffold. *Significantly less contaminated bone samples compared to the empty scaffold ($p<0.05$)

Conclusions: The above results suggest that the delivery of D-AA from polyurethane foams promotes improved healing outcomes in a contaminated bone defect. Although more studies are needed to optimize D-AA dose, the local delivery of a combination of D-AAs is a promising therapeutic strategy to reduce the bacterial burden in contaminated wounds.

References: [1]Harris, et.al. J Orthop Trauma. 2009; 23:1-6 [2]Kolodkin-Gal. et.al. Science. 2010; 328:627-629 [3]CLSI – M100-S22, [4]Ceri et.al. J Clin Microbiol. 1999:37(6):1771-1776, [5] Li,et.al.J Control Release. 2010; 145(3):221-230