Combined DAIR and PhotothermAA Gel Decreases Implant Biofilm Burden and Soft Tissue Infection in a Rabbit Model of PJI, a Pilot Study

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INTRODUCTION: In periprosthetic joint infection (PJI), bacteria are often protected by a biofilm matrix which may compromise effective treatment. PhotothermAA gel combines two anti-biofilm approaches, D-amino acids and hyperthermia via laser heated gold nanoparticles. *In vitro*, this innovative treatment strategy has proven to completely eradicate 2-week old biofilm. *In vivo* results have shown that it significantly decreases implant biofilm coverage immediately after treatment. The purpose of this study was to test whether the combined use of PhotothermAA gel and debridement, antibiotics, and implant retention (DAIR) decreases implant biofilm burden and soft tissue infection for a prolonged time in a knee PJI model.

METHODS:

New Zealand white rabbits were fitted with a titanium knee implant and inoculated with 5 x 10^6 colony forming units (CFU) *Staphylococcus aureus*. At two weeks, rabbits were randomized into three treatment groups – sham (n=2), DAIR (n=2), or DAIR + PhotothermAA gel (n=3). For DAIR, rabbits underwent debridement and irrigation with normal saline, with cefazolin treatment for two weeks postoperative. For DAIR + PhotothermAA gel treatment, rabbits underwent the same DAIR treatment plus PhotothermAA gel for two hours, laser heated, and washed out with 50mL saline. Tissue and implant were collected two weeks after treatment for cultures (n=4/animal) and biofilm coverage, respectively. For biofilm coverage, implants were prepared for scanning electron microscopy (SEM). Twenty standardized images were taken of the implant surface at 1,500x and percent coverage was calculated using the Trainable Weka Segmentation plugin in Fiji. Tissues were sonicated and incubated for one week then plated overnight for cultures and CFU.

RESULTS: Implants isolated two weeks after treatment with PhotothermAA gel + DAIR had significantly less biofilm coverage compared to sham or DAIR ($p < 0.0001$; Figure 1A and 1B). Periprosthetic tissue and synovial fluid contained less culturable bacteria compared to sham or DAIR ($p = 0.117$; Figure 1C) and reduced CFU ($p < 0.0001$, Figure 1D).

DISCUSSION AND CONCLUSION: A decrease in both implant biofilm coverage and bacterial burden were apparent after treatment with PhotothermAA gel + DAIR when analyzed two weeks after treatment. One limitation worth noting is that biofilm thickness was not accounted for in this study, although growth differences were visibly apparent between images of sham vs. DAIR groups while percent biofilm coverage was not significantly different. PhotothermAA gel and laser treatment decreases biofilm coverage and bacterial burden in a rabbit model of PJI in this pilot study and warrants further testing.

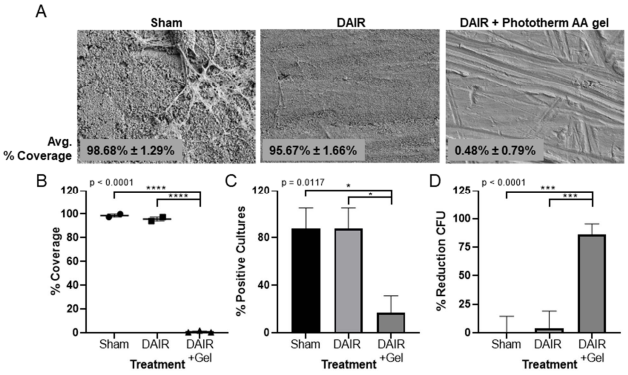

Figure 1. Efficacy of DAIR + PhotothermAA gel treatment two weeks after treatment *in vivo*
(A) Representative SEM images at 1,500x magnification with mean percent biofilm coverage ± standard error of the mean of combined 20 images per implant (n=2 Sham, n=2 DAIR, n=3 DAIR + PhotothermAA gel) (B) Average SEM quantification of biofilm coverage of 20 standardized locations on the top surface of the implant. 1,500x magnification. Biofilm coverage was acquired using Trainable Weka Segmentation plugin in Fiji. (C) Periprosthetic tissue (n=3) and synovial fluid were isolated from a single animal, sonicated, and incubated for one week prior to plating overnight. Data depicted are the percentage of samples from a single animal that grew bacteria. (D) Percent reduction of CFU count compared to the average of Sham. Each CFU was quantified in triplicate and the average was used for calculation.