

## DLC Surface Treatment Decreases Biofilm Burden by *S. aureus* on Titanium Implants *in vitro* – A Pilot Study

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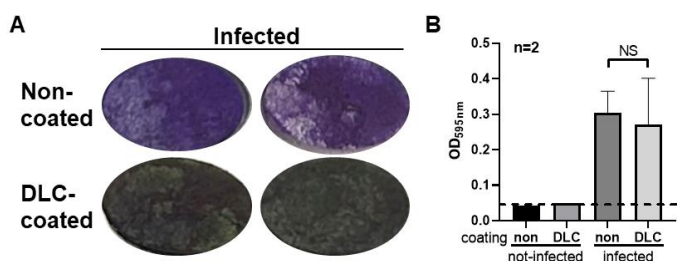
**INTRODUCTION:** Total joint arthroplasty is a common surgery for patients with end-stage osteoarthritis and rheumatoid arthritis with positive outcomes in quality of life and function. Although a majority of patients live a complication-free life, infections are one of the most common complications in total joint arthroplasty. With a 5-year mortality rate of over 26%, costing over \$1.6 billion dollars with over a 31%-63% treatment failure rate, generation of coatings that can prevent infection is paramount. Diamond-like carbon (DLC) is an ideal coating for implants as they are wear resistant, anatomically smooth, corrosion resistant, and are unable to be scratched by wear particles. These coatings can also be carriers for ions and can act as local antibacterial agents with implications in both primary and revision joint replacements. The purpose of this study was to test the efficacy of DLC surface treatment in prevention of biofilm on titanium discs infected with *Staphylococcus aureus* (*S. aureus*) *in vitro*.

**METHODS:** Titanium alloy discs were either left uncoated or DLC-coated and gamma sterilized. Discs (n=4 non-coated and n=4 DLC-coated) were placed in a 12 well plate and infected with 5x10<sup>5</sup> colony forming units (CFU) of *S. aureus* in 4mL media. Two mL of media was changed every other day taking care as to not disturb biofilm formation. After two weeks, discs were washed in phosphate buffered saline (PBS) to wash away non-adherent planktonic bacteria prior to analysis. For crystal violet, n=2 samples per group were submerged in crystal violet for 20 mins, aspirated, and imaged. Discs were subsequently soaked in 30% acetic acid and measured at optical density (OD) 595nm for biofilm quantification. For scanning electron microscopy, n=2 samples per group were fixed with 4% paraformaldehyde, washed, and subsequently dehydrated using an alcohol dilution series. After overnight drying, samples were gold sputtered, and 20 images were collected at 1500x magnification using predetermined imaging locations on the surface of the implant. Images were analyzed for biofilm coverage using Trainable Weka Segmentation in Fiji.

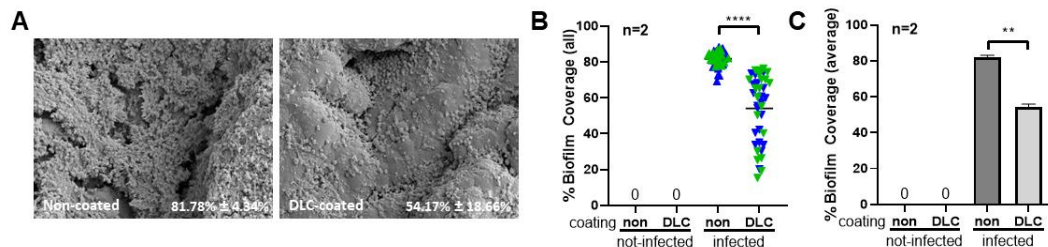
**RESULTS:** Crystal violet analysis yielded differences in the appearance of biofilm on the surface of the implant where DLC-coated samples had a clumpier appearance but no difference in biofilm quantification was observed between non-coated and DLC coated samples after two-week culture (Figure 1). Interestingly, this clumpy appearance did lead to differences in SEM biofilm coverage where significantly less biofilm coverage was found in the DLC-coated discs (81.78% vs. 54.17%, p<0.003; Figure 2).

**DISCUSSION:** DLC-coated titanium alloy implants may have preventative properties in *S. aureus* infection. The CFU dose used in these *in vitro* experiments are well above the upper limit of what is necessary to seed an implant associated infection clinically. Nevertheless, observing differences in biofilm coverage does warrant additional testing including CFU titration and biofilm kinetics with eventual use in an animal model of periprosthetic joint infection (PJI).

**SIGNIFICANCE/CLINICAL RELEVANCE:** DLC-coating on implants may be useful in preventing biofilm-associated PJI. This will be useful as current treatment strategies still have high failure rates and infection prevention methods are needed.



**Figure 1. Crystal Violet Biofilm Assay** A) Images after crystal violet staining of infected discs after 2 weeks of biofilm growth. Entire media was changed every other day. B) Optical density graph of dissolved crystal violet. n=2 per disc coating, n=1 non-infected (historical control). Dashed line is background OD limit of detection.



**Figure 2. Scanning Electron Microscopy (SEM) Assay** A) Representative SEM images (1500x magnification) taken of non-coated and DLC coated discs two weeks after *S. aureus* infection. B) Percent biofilm coverage plotted of all images taken from two replicates \*\*\*\*p<0.0001 C) Percent biofilm coverage from the average of 20 images taken from two replicates \*\*p<0.01