

Bromelain as a Source of Debridement for Infected Orthopaedic Implants

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Disclosures: Matthew B. Bratton (N), Jaclyn P. Murphy (N), Jessica C. Rivera (AAOS: Board or committee member, AAOS Now: Editorial or governing board, Limb Lengthening and Reconstruction Society: Board or committee member, Orthopaedic Research Society: Board or committee member)

INTRODUCTION: The growth of biofilms on orthopedic implants is of major surgical concern, with infection rates estimated to be up to 2 percent for all orthopedic procedures. All bacteria can produce biofilms, but the rate and size vary between species. Biofilms are created by a cluster of bacteria that produce extracellular polymeric substance (EPS) which surrounds the colony, allows adherence to surfaces, and protects the colony from host immune cells. Due to the protective nature of EPS, biofilms are resistant to most antibiotic therapies that are targeted towards planktonic bacteria. Currently, manual scrubbing accompanied with a saline wash is the most common method of eradication. However, enzymatic debridement has emerged as an alternative option. Bromelain is an enzyme derived from pineapple stem and has been previously used in several studies as a method of biofilm dissolution. In addition to its intrinsic antimicrobial properties, bromelain is capable of hydrolyzing the complex carbohydrate shell of EPS and destabilizing the biofilm. As a result, we hypothesized that bromelain may be used for the debridement of infected orthopaedic implants.

METHODS: In our study, 10 mm x 3.5 mm surgical grade cortical bone screws were incubated in methicillin resistant *Staphylococcus aureus* (MRSA) inoculated broth for 120 hours with 10% fetal bovine serum (FBS). Treatment groups were exposed to low dose bromelain solution (200 µg/mL), high dose bromelain solution (1mg/mL), or bromelain powder (3 U/mg) for 20 minutes. The screws were then either rinsed with 1X phosphate buffer saline (PBS) or briefly scrubbed for thirty seconds prior to rinsing. The screws were then stained with 0.25% crystal violet (CV) dye for 25 minutes to determine the amount of biofilm remaining. The stained biofilm was removed from the screws using 33% acetic acid (Figure 1). Resultant effluents were analyzed by optical density (OD) read at 600nm. Optical density means were compared between each treatment group and respective controls with Students t-test. The percent of biofilm dissolution was determined using absolute OD values in the following formula: % BD = $[(\text{OD Control} - \text{OD Treated}) / \text{OD Control}] \times 100$.¹

RESULTS SECTION: Three screws were used for each group. The average optical densities of the low dose bromelain solution (0.348 ± 0.068) and low dose + scrub (0.061 ± 0.021) groups were no different compared to respective controls ($p=0.5610$; $p=0.1738$). The average optical densities of high dose bromelain solution (0.056 ± 0.009) and high dose + scrub (0.055 ± 0.012) were not different from their respective controls ($p=0.0791$; $p=0.2234$). The average optical densities for screws in the powder treatment group (0.041 ± 0.011) trended towards being lower than their respective controls ($p=0.0529$); and screws treated with powder + scrub did have lower optical densities compared to controls (0.032 ± 0.005 ; $p=0.0002$). The powder + scrub treatment resulted in 91% biofilm dissolution (Figure 2).

DISCUSSION: Based on our work, bromelain is a promising alternative option for the debridement of infected orthopedic implants. However, an increased sample size is needed to more accurately assess the reliability of our results. In addition, further investigation is required to assess how different concentrations and exposure times may affect the percent biofilm dissolution. In the future, this experiment should be replicated in vivo to determine if treating infected implants with high dose bromelain yields any toxic side effects to the surrounding tissue.

SIGNIFICANCE/CLINICAL RELEVANCE: Bromelain enzymatic debridement has the potential to be used as an alternative option to effectively treat infected orthopaedic implants and reduce the risk of further revision surgeries.

REFERENCES: Carter CJ, *et al.* Dissolution of biofilm secreted by three different strains of *Pseudomonas aeruginosa* with bromelain, N-acetylcysteine, and their combination. Appl Sci, 2021; 11: 11388.

IMAGES AND TABLES:



Figure 1: Bromelain powder + scrub treatment group (top row) Resulted in less crystal violet stained residual biofilm versus Control screws (bottom row)

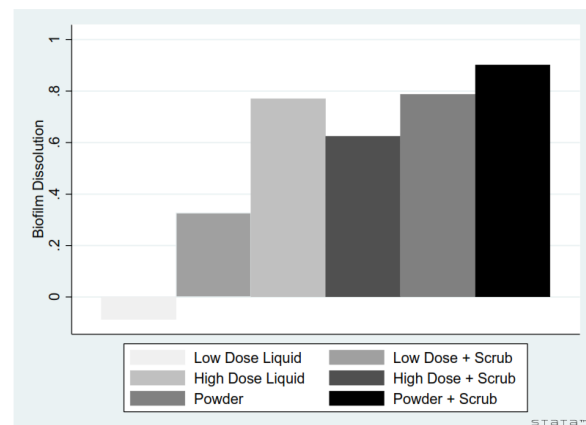


Figure 2: Calculated biofilm dissolution per treatment group