

## Minimum Bromelain Treatment Time for Effective Biofilm Dissolution

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**Introduction:** Orthopedic implant surgeries experience implant-associated infections (IAI) in up to 5% of cases due to biofilm formation. While current treatment approaches such as irrigation and debridement are common, enzymatic debridement using bromelain could present as an alternative by breaking biofilm's glycosidic bonds. In previous lab experiments, applying a 1 mg/mL bromelain solution for 20 minutes successfully dissolved biofilms on infected implants. This study aims to investigate whether shorter application times can be just as effective, potentially making the method more practical for use during surgery.

**Methods:** Six surgical grade cortical bone screws (10 mm x 3.5 mm) were incubated in methicillin-resistant *Staphylococcus aureus* (MRSA) broth, supplemented with 10% fetal bovine serum (FBS), for 120 hours. After incubation, each screw was rinsed with 1X phosphate-buffered saline (PBS) and divided into treatment and control groups. Screws in the treatment group were exposed to a bromelain solution (1 mg/mL) while those in the control group were exposed to 1X PBS for either 15 or 10 minutes in a shaking incubator. Following exposure, all screws were scrubbed for 30 seconds and rinsed with 1X PBS. The screws were stained using 0.25% crystal violet (CV) dye for 25 minutes to assess the remaining biofilm. After staining, the biofilm was removed from the screws using 33% acetic acid, and the resultant solution was analyzed by measuring optical density (OD) at 600 nm. The OD values were compared between the treatment and control groups using t-tests, while comparisons across different treatment durations (including previously obtained 20-minute data) were made using analysis of variance (ANOVA) with Bonferroni correction for multiple comparisons.

**Results:** The mean optical density (OD) after 15-minute bromelain liquid exposure was 0.13 +/- 0.007 and following 10-minute exposure was 0.192 +/- 0.021. Both exposure times led to significantly lower ODs compared to their respective controls, indicating reduced biofilm on the treated screws than control screws ( $p = 0.0023$  for 15 minutes,  $p = 0.0418$  for 10 minutes). Previously collected data for 20-minute exposure showed a mean OD of  $0.045 \pm 0.014$ , also indicating less biofilm than controls. Comparisons between 20-, 15-, and 10- minutes exposure groups indicated that 20-minute exposure resulted in lower optical density measurements compared to 15-minutes ( $p > 0.001$ ) and 10-minutes ( $p = 0.013$ ). There was no difference between 15- and 10-minute exposures.

**Conclusion:** Both the 15- and 10-minute bromelain exposure times showed biofilm dissolution when compared to control treatments with PBS. The previously collected data for 20-minute exposure indicated even lower raw optical densities, suggesting that a longer exposure time may be more effective. Notable in this data, the 10-minute exposure yielded a lower reading than the 15-minute exposure, which was unexpected. The next steps for this project will involve increasing the sample size and exploring alternative methods to reduce the required time for improved biofilm dissolution.