

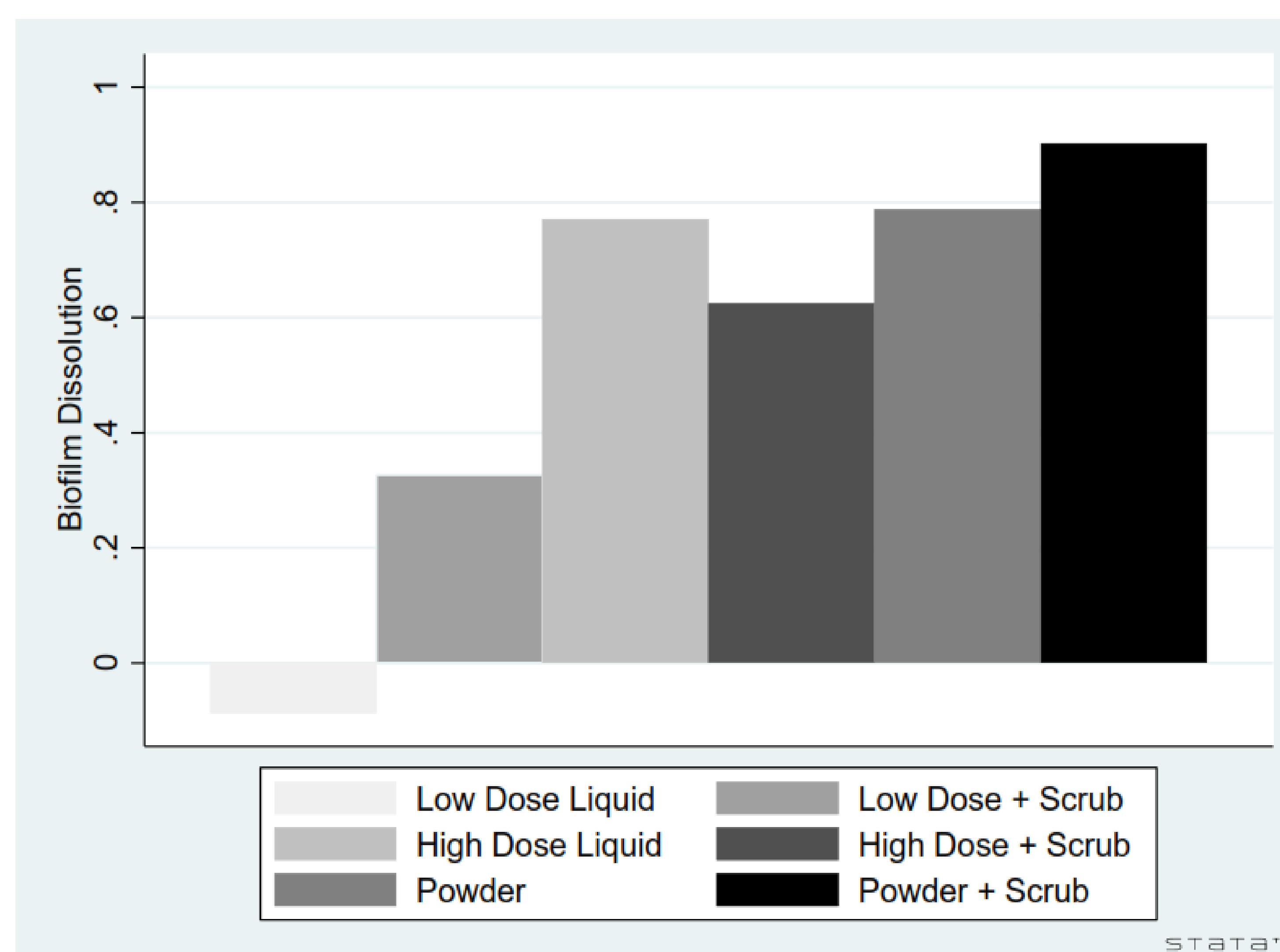
Introduction

- Infection rates are estimated to be up to 2 percent for all orthopedic procedures.
- Bacteria produce extracellular polymeric substance (EPS) which surrounds the colony, allows adherence to surfaces, and protects the colony from immune cells.
- Biofilms are resistant to most antibiotic therapies
- Manual scrubbing accompanied with a saline wash is the most common method of eradication.
- Bromelain is an enzyme derived from pineapple stem and has been previously used in several studies as a method of biofilm dissolution.
- Bromelain is capable of hydrolyzing the complex carbohydrate shell of EPS and destabilizing the biofilm.
- We hypothesized that bromelain may be used for the debridement of infected orthopaedic implants.

Methods

- 10 mm x 3.5 mm surgical grade cortical bone screws were incubated in MRSA inoculated broth
- Treatment groups were exposed to low dose bromelain solution (200 µg/mL), high dose bromelain solution (1 mg/mL), or bromelain powder (3 U/mg) for 20 minutes.
- The screws were 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 (Figure 3).
- Resultant effluents were analyzed by optical density (OD) at 600 nm.
- OD means were compared between each treatment group and respective controls with Student's t-test. The percent of biofilm dissolution was determined using absolute OD values (Figure 2).

Figure 1



Calculated Biofilm Dissolution by Treatment Group

Figure 2

$$\% \text{ BD} = \frac{[\text{OD Control} - \text{OD Treated}]}{[\text{OD Control}]} \times 100$$

Figure 3



Bromelain powder + scrub treatment group (top row) resulted in less crystal violet-stained residual biofilm versus Control Screws (bottom row)

Results

- 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 optimal 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 1).

Conclusion

- **Bromelain is a promising alternative option for the debridement of infected orthopedic implants.**
- 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

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.