Evaluating In Vivo Efficacy of Enzymatic Biofilm Dispersal from Orthopaedic Implants

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Introduction

Biofilm-related implant complications affect 0.5-2% of all orthopaedic procedures, highlighting the urgent need for effective cleaning methods. Recent in vitro studies have shown that debridement with bromelain, an enzyme derived from pineapple stem, can effectively break down biofilms on infected implants, but its efficacy in vivo has yet to be investigated. Through utilization of the nucleic acid stain Sytox Orange, we can assess the bioburden on both infected and uninfected explanted in vivo orthopaedic implants. By fluorescently labeling sessile bacteria in biofilm, this stain allows for a comparison of bacterial burden in vitro as well as in vivo.

Objectives

- To gain insights into bromelain's capability to remove biofilms outside of culture through an in vivo model.
- Visualize the bioburden of infected orthopedic implants using the nucleic acid stain Sytox Orange.

Methods

- Stainless steel bone pins were incubated at 37℃ in tryptic soy broth with 10% fetal bovine serum and inoculated with methicillin-resistant Staphylococcus aureus (MRSA).
- Biofilm washed pins were first infected in the same fashion then soaked in 1000 µg/mL bromelain for 20 minutes and washed in PBS.
- A second set of pins prepared as above were placed into the intramedullary space of adult rats for 7 days and explanted.
- All pins were fixed, washed with PBS, stained with Sytox Orange, and washed again with PBS.
- Pins were mounted on slide spacers with antifade media and mounted on a slide.
- Pins imaged at 100x magnification using confocal microscopy.
- The bacterial burden was quantified using SlideBook 5.0 software (3i).
- Statistical comparison was done using one-way ANOVA and $\alpha = 0.05$.

Results

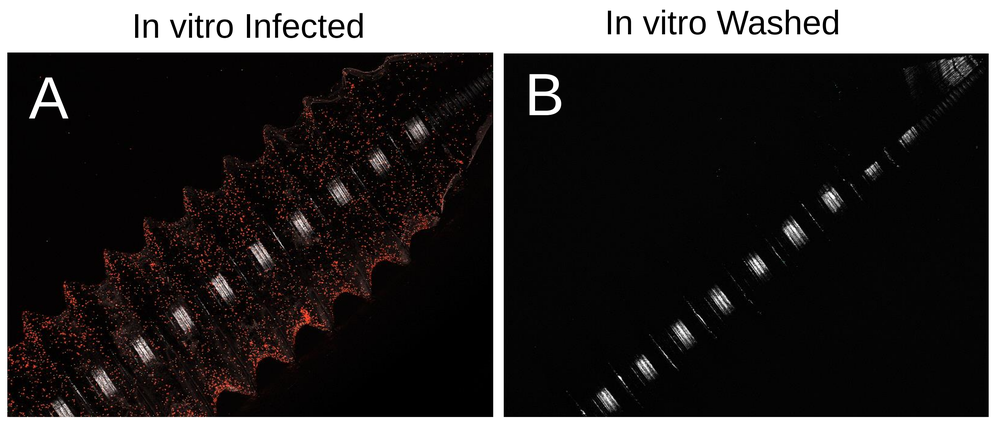

Figure 1: Representative 100x confocal 3D images of (A) in vitro infected stainless steel bone pin and (B) in vitro bromelain washed stainless steel bone pin.

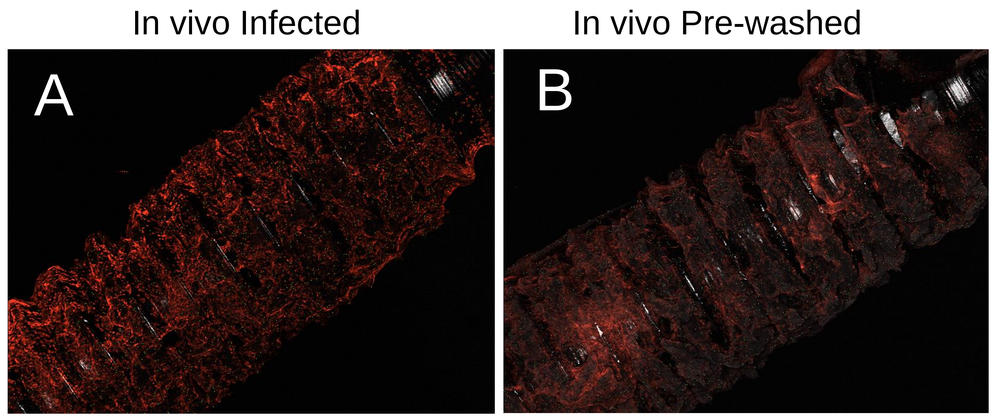

Figure 2: Representative 3D images of (A) in vivo infected stainless steel bone pin and (B) in vivo bromelain pre-washed stainless steel bone pin.

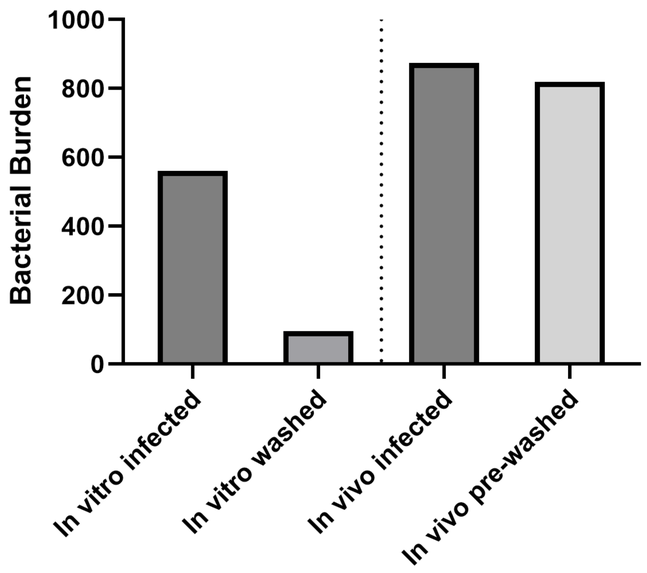

Figure 3: The mean bacterial burden CFU was compared between groups. The in vitro infected pins (n=6) exhibited a mean bacterial burden of 560.83 CFU, while the in vivo infected pins (n=3) displayed a higher mean burden of 873.7 CFU (p=0.3944). The pre-washed pins (n=3) had a mean bacterial burden of 819.3 CFU compared to the in vitro bromelain-washed pins (n=4), demonstrating a vastly lower bacterial burden of 96.5 CFU (p=0.0195). $\alpha = 0.05$.

Discussion and Limitations

- Bromelain washes effectively reduced bacterial burden in vitro but did not maintain low bacterial levels in vivo.
- A larger sample size is necessary to validate findings.
- Stricter sterile technique must be implemented in future research to prevent contamination.

Conclusion

- Bromelain shows promise as a viable option for cleaning infected orthopedic implants, but further exploration beyond in vitro culture is necessary.
- Testing with different concentrations of bromelain in vivo may be beneficial to achieve low bacterial burden levels.

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References

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