Effects of Bromelain on Fibroblast Viability and Myofibroblastogenesis

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INTRODUCTION: Bromelain, an enzymatic wound debridement tool, features beneficial antimicrobial and anti-inflammatory properties. While in vivo studies have highlighted its therapeutic potential, much less is known about how bromelain affects cells required for wound healing. This project explores bromelain's impact on fibroblasts found within the wound milieu, with particular attention to bromelain's potential cellular cytotoxicity. In addition, we aim to evaluate myofibroblast differentiation, a key process in tissue repair and fibrosis.

METHODS: To determine cytotoxicity, NIH3T3 cells were seeded in 96-well plates, treated with escalating concentrations of bromelain (0–1 mg/ml), and assessed with MTT assays. Following bromelain treatment, absorbance at 570 nm was compared to untreated controls, and statistical analysis was performed using one-way ANOVA with Dunnett's post-hoc test (α=0.05). To evaluate bromelain's potential to cause cell detachment, lift assays were performed at a concentration of 1mg/ml bromelain. Cells were cultured in 24-well plates and images were acquired every 5 minutes over a 90-minute period to track cell detachment. Cellular detachment statistics were analyzed using one-way ANOVA with Dunnett's post-hoc test (α=0.05). Finally, we investigated bromelain's effect on myofibroblast differentiation. NIH3T3 cells were cultured in 4-well, collagen coated, chamber slides. Cells were grown to 90% confluency and serum deprived (75% reduced serum growth media) for 24 hours. After 24 hours of serum deprivation, myofibroblastogenesis was induced with 5 nM TGF-β in the presence of 0, 3, or 10 µg/ml bromelain. After 48 hours, cells were fixed and processed for immunocytochemistry (ICC). ICC was performed using an initial incubation with anti-αSMA mouse monoclonal antibody followed by a secondary co-incubation with anti-mouse Alexa 594 and DAPI. Cell monolayers were imaged using a confocal microscope with excitation at 358 nm to visualize DAPI-stained nuclei and 590 nm to visualize αSMA. Image analysis was performed using Fiji (Image J) to quantify nuclei and smooth muscle actin expression as a measure of myofibroblastogenesis. Mean fluorescence intensity for αSMA was normalized to nuclei count, and one-way ANOVA followed by Tukey's post-hoc test was used for statistical analysis (α=0.05).

RESULTS: MTT results indicate bromelain reduced NIH3T3 viability in a dose-dependent manner, with decreased absorbance observed beginning at concentrations of 100 µg/ml. At 72 hours, concentrations above 300 µg/ml demonstrated a significant reduction in relative absorbance (<50% of control). Lift assay results showed bromelain treatment led to progressive cell detachment. Cellular attachment was significantly reduced by 55.2% after 12-minutes, with an average of only 14.4% of cells remaining attached at the 30-minute time point, and complete detachment was observed by 60 minutes. Analysis of αSMA signal intensity, adjusted by nuclei number, showed no significant differences between control, TGF-ß treated cells, and bromelain treated cells ($p>0.05$).

DISCUSSION: Bromelain demonstrates cytotoxic effects on NIH3T3 fibroblasts at concentrations exceeding 100 µg/ml and induces relatively rapid cell detachment at 1mg/ml. Outcomes for NIH3T3 differentiation, adjusted αSMA signal intensity, did not reveal a significant difference between control and TGF-ß treated cells, excluding the potential to determine an effect of bromelain on myofibroblastogenesis in this study. In the context of enzymatic wound debridement, these results suggest that bromelain, at appropriate concentrations, could selectively facilitate removal of devitalized tissue while minimizing harm to viable fibroblasts. However, exposure to high concentrations (1 mg/ml) for long duration may affect normal wound healing by damaging and even debriding healthy cells found within the wound milieu. The cytotoxic response observed demonstrates the importance of optimizing both bromelain concentration and exposure duration to balance debridement efficacy with tissue preservation. Future work will focus on optimizing our myofibroblastogenesis experiments for the purpose of addressing bromelain's potential to modulate fibroblast differentiation.

SIGNIFICANCE/CLINICAL RELEVANCE: The results of this experiment hold clinical importance for enzymatic wound debridement, as several FDA approved formulations already include bromelain due to its potent debridement potential. Further characterizing bromelain's effect on myofibroblast differentiation and activity will better inform is use in a clinical setting.

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