Evaluating Bromelain's Effects on NIH-3T3 Fibroblasts

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Background: Bromelain, a proteolytic enzyme extract derived from pineapple stems, has gained interest for its role in enzymatic wound debridement. In addition to its debriding capabilities, bromelain possesses antimicrobial and anti-inflammatory properties that may further support wound healing. While in vivo studies have demonstrated its therapeutic potential, limited research exists on bromelain's cellular effects in vitro. This project aims to investigate bromelain's influence on cells within the wound milieu, focusing on its interaction with myofibroblasts, a key player in wound repair and fibrosis. Using NIH3T3's, a murine fibroblast cell-line, we will determine the potential cytotoxicity of bromelain and use that data to perform myofibroblast differentiation assays using sub-cytotoxic bromelain concentrations. Differentiation assays will be performed, with or without sub-cytotoxic bromelain concentrations, to determine if bromelain modulates myofibroblast differentiation and/or fibrosis development in vitro.

Methods: MTT assays were performed to test bromelain's cytotoxicity. NIH3T3's were plated in 96-well plates and allowed to adhere for 24hrs. The growth medium was then replaced with increasing concentrations of bromelain-treated medium (0-1mg/ml). MTT assays were performed post-treatment to estimate cytotoxicity by measuring relative absorbance at 570nm compared to untreated cells. One-way ANOVA followed by Dunnett's post-hoc test was used to determine significant effects and specific differences compared to control, respectively (α=0.05). Next, we measured the mechanism of cytotoxicity using lift assays. NIH3T3's were cultured in-vitro in 4-wells of a 24-well plate and allowed to adhere for 24hrs. Cells were treated with bromelain at a concentration of 1mg/ml. The plates were imaged using live imaging every 5-minutes for a period of 90-minutes. Using live cell imaging, we determined the time-course for bromelain's cell detaching effect. Finally, we investigated the effects of bromelain on cell differentiation. NIH-3T3's were cultured on Poly-L-Lysine (PLL) coated coverslips placed inside the well of 6-well plates and induced towards myofibroblasts at 90% confluency with 5nM TGF-ß at 0, 10, and 30µg/ml bromelain. Cells were fixed and processed for Immunocytochemistry (ICC) at 48hrs post-induction to visualize and quantify αSMA and Collagen1 as indicators of myofibroblast differentiation.

Results: In accordance with the MTT data, NIH/3T3 fibroblasts showed a dose-dependent decrease in relative absorbance, starting at 100µg/ml. By 72 hours, significant and meaningful reductions in relative absorbance (<50%) were found at concentrations above 300µg/ml. The results of the lift assays showed a steady decrease in cell count with respect to time progression when exposed to Bromelain at a concentration of 1mg/ml. The 12-minute time point was the first time point in which we noticed a significant decrease in the number of attached cells. At the 30-minute mark an average of 14.42% of the cells remained adhered to the wells, and by the 60-minute mark no cells remained attached. The results from the cellular differentiation assays are yet to be determined as the PLL coated coverslips were insufficient in maintaining consistent cellular adherence. Cellular differentiation assays will be re-performed using Poly-D-Lysine (PDL).

Conclusion: Bromelain treatment demonstrated cytotoxic effects on NIH3T3 cells at concentrations above 100µg/ml. However, bromelain appears to lift cells from the plate which may confound our in-vitro results. Future work will re-evaluate bromelain's effect on myofibrogenesis using more robust culture ware coatings.