"Evaluating Bromelain's Effects on C2C12 Myoblasts In Vitro"

Ty Gregory[1], Richard Harkrider[1], Julianne Guercio[1], John Carleton[2], Mathew Scott[2], Jessica Rivera[2]
[1]LSUHSC School of Medicine, [2]Department of Orthopaedic Surgery,
tgreg1@lsuhsc.edu

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INTRODUCTION: Bromelain, a proteolytic enzyme derived from pineapple stems, can be utilized for enzymatic wound debridement due to its potential for nonviable tissue removal (Schulz et al., 2017). However, its effect on viable tissue is less studied. Muscle tissue is likely to be damaged by both superficial and deep wounds, and healing is benefited by adequate myoblast differentiation into myocytes. Considering the lack of in vitro evidence related to the effect of bromelain on cells within the wound milieu and the importance of muscle repair in response to injury, the purpose of this research is to evaluate the impact of bromelain on myoblast differentiation as well as its potential cytotoxic effects.

OBJECTIVE: To investigate the effects of increasing bromelain concentrations on C2C12 myoblast viability, detachment, and differentiation capacity in vitro.

METHODS: C2C12 myoblasts were exposed to increasing concentrations of bromelain ranging from 0-1000µg/mL. Viability was assessed using MTT assays at 3, 24, and 48 hours to determine dose-dependent cytotoxicity. Additionally, live cell imaging was performed, with images acquired every 6 minutes, to monitor morphology and detachment while cells were exposed to 1 mg/mL bromelain. For differentiation studies, C2C12 cells were cultured in 2% horse serum differentiation medium supplemented with 1µM insulin and plated on poly-L-lysine (PLL) coated coverslips. Differentiation will be quantified as the number of multinucleated cells and the average number of nuclei per multinucleated cell, using a DAPI-phalloidin staining.

RESULTS/EXPECTED RESULTS: MTT assays demonstrated a concentration-dependent decrease in cell viability, with significant reductions observed at ≥300 µg/mL after 24 hours. The image assay at 1000 µg/mL revealed a time-dependent loss of adherence, with attached cell number beginning to decline significantly by 54 minutes ($p = 0.0495$) and continuing through 120 minutes ($p < 0.0001$). By the end of the 2-hour exposure, more than 90% of cells had detached compared to baseline. Differentiation assays were performed; however, PLL coating was insufficient to maintain proper adherence of myotubes. We anticipate reduced myotube formation at near-cytotoxic concentrations, while lower doses are expected to have a limited impact.

CONCLUSION: Differentiation assays will be re-performed on collagen-coated coverslips to provide a sufficient adherence substrate for myotube formation. These studies aim to determine the concentration thresholds of bromelain that compromise muscle cell viability, attachment, and differentiation. The observed concentration-dependent and time-dependent cytotoxicity further emphasize the importance of defining therapeutic windows for enzymatic debridement that minimize collateral damage to regenerating muscle tissue.

REFERENCES:

1. Schulz, A., Shoham, Y., Rosenberg, L., Rothermund, I., Perbix, W., Christian Fuchs, P., Lipensky, A., & Schiefer, J. L. (2017). Enzymatic Versus Traditional Surgical Debridement of Severely Burned Hands: A Comparison of Selectivity, Efficacy, Healing Time, and Three-Month Scar Quality. *Journal of Burn Care & Research*, *38*(4), e745–e755. https://doi.org/10.1097/BCR.0000000000000478