Evaluating Bromelain's Effects on C2C12 Myoblast In Vitro

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Disclosures: No Disclosures

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Introduction

Bromelain, a proteolytic enzyme derived from pineapple stems, can be used for enzymatic wound debridement because of its ability to remove nonviable tissue (Schulz et al., 2017). However, its effect on viable tissue is less well studied. Muscle tissue is likely to be damaged by both superficial and deep wounds, and healing is improved by proper myoblast differentiation into myocytes. Given the lack of in vitro evidence regarding the effect of bromelain on cells within the wound environment and the importance of muscle repair after injury, this research aims to assess how bromelain influences myoblast differentiation and its potential cytotoxic effects.

Methods

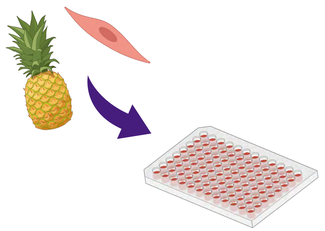
Cell Lines Used:
- C2C12 Myoblast

Cell Culture & Cytotoxicity Assay :
- Cells suspended in standard growth medium
- Plated in 96-well plates at 5–15 × 10^3 cells/well
- Growth medium replaced with bromelain-treated medium (0–1 mg/mL) after 24 hours
- MTT assays performed at 3-, 24-, and 48-hours post-treatment (3 hr MTT incubation)
- Relative absorbance @ 570 nm to estimate metabolic activity
- Results normalized to untreated controls
- Reductions in relative absorbance >30% considered cytotoxic

MTT Assay Statistical Analysis:
- One-way ANOVA used to assess overall treatment effects
- Dunnett's post-hoc test was used to compare individual bromelain concentrations vs. the control
- α = 0.05

Lift Assay:
- C2C12 cells are plated in 24-well plates
- Treated with 1 mg/mL bromelain after 24 hours
- Plates were imaged every 6 minutes for 120 minutes using live cell imaging
- Time-course analysis performed to assess bromelain's cell-detaching effect.
- Cells were quantified as % of cells attached over time relative to time zero.

Lift Assay Statistical Analysis:
- Attached cell counts over time were analyzed using repeated-measures ANOVA with Dunnett's test versus baseline.
- α = 0.05



Objective

To evaluate the in vitro cytotoxic effects of increasing bromelain concentrations on C2C12 myoblasts commonly found in the wound milieu, and to assess Bromelain's impact on healthy progenitor muscle cells. To investigate bromelain's selectivity and potential safety for enzymatic debridement applications.

Results

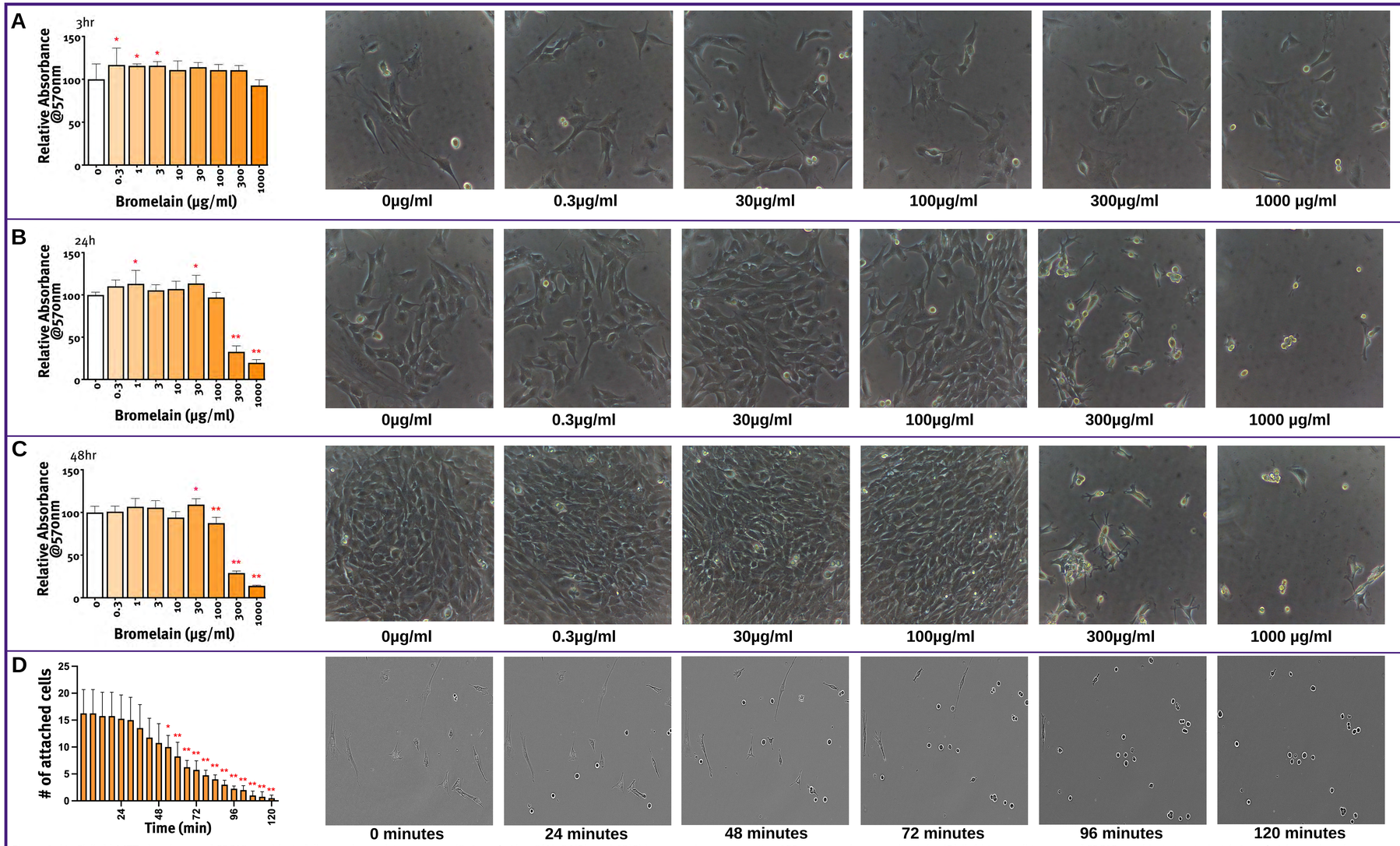


Figure 1: (A–C) (left) MTT absorbance of C2C12 myoblasts following bromelain exposure (0–1 mg/mL) at 3(A), 24(B), and 48(C) hours, with representative 100× images shown to the right. (D) (left) Live-cell imaging of C2C12 myoblasts treated with 1 mg/mL bromelain demonstrates progressive detachment over 120 minutes, with corresponding 100× images at indicated time points shown on the right. Statistical analysis was performed using one-way (A-C) or repeated-measures ANOVA (D) with Dunnett's post hoc test. (*) $p < 0.05$; (**) $p < 0.01$.

Discussion

Summary of Results

- MTT assays showed a concentration-dependent decrease in metabolic activity, with significant reductions in relative absorbance at bromelain concentrations ≥300 µg/mL after 24 hours.
- Corresponding 100× images showed a clear decrease in cell number, likely indicating cell death due to loss of adhesion.
- Live-cell imaging showed that 1 mg/mL bromelain caused progressive detachment of C2C12 myoblasts starting around 54 minutes, with over 90% detachment by 120 minutes.
- These findings suggest that bromelain quickly disrupts cell adhesion rather than directly causing cell death.

Limitations

- In vitro monolayer cultures lack the structural and biochemical complexity of wound tissue, including vascular and immune components.
- MTT assays give indirect measures of viability, and bromelain-induced detachment can artificially decrease absorbance values.

Conclusion & Future Directions

- Bromelain concentrations ≥300 µg/mL significantly decrease cell attachment and viability in fibroblast-like cells, likely by enzymatically breaking down adhesion molecules rather than causing apoptosis or necrosis directly.
- Future research will investigate how lower, non-cytotoxic concentrations affect myoblast differentiation and matrix remodeling on collagen-coated substrates, aiming to determine safe therapeutic levels for effective debridement and tissue healing.

Acknowledgements

This research was supported by the mentorship of Dr. Jessica Rivera and Dr. Matthew Scott, with additional thanks to the Department of Orthopedic Surgery at LSU Health New Orleans for providing resources and a collaborative research environment.

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.