Profiling the Mechanical and Biological Properties of Sustainable Chitosan Films via NaOH Neutralisation for Skeletal Muscle Tissue Engineering

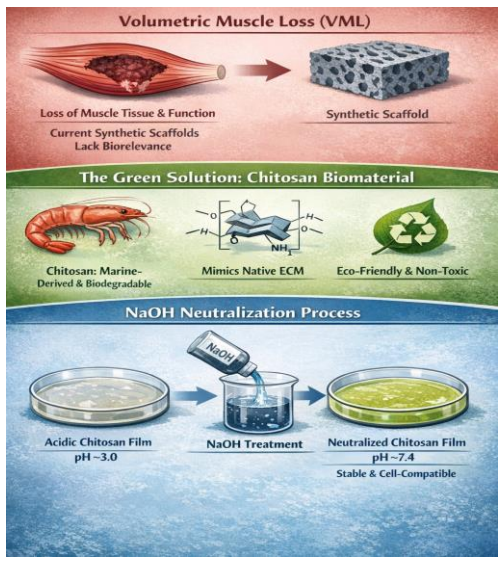
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INTRODUCTION



Volumetric Muscle Loss (VML) is a severe condition characterised by irreversible skeletal muscle loss and long-term functional impairment. Current synthetic scaffolds fail to fully replicate the biological and mechanical complexity of native tissue. **Chitosan**, a marine-derived polysaccharide, offers a **sustainable** and **scalable** alternative due to its biocompatibility and structural similarity to the extracellular matrix (ECM). However, **further characterisation** in relation to material fabrication and processing, material properties and **myogenic potential** require further investigation.

AIMS AND OBJECTIVES

The aim of this investigation was to characterise the fabrication, processing, and myogenic differentiation potential of chitosan films. To achieve this, the effects of NaOH neutralisation on material properties was explored. A comparison of chitosan from mollusc vs. crustacean sources under identical processing conditions, was also explored, alongside evaluating cytocompatibility using C2C12 myoblasts.

MATERIALS AND METHODS

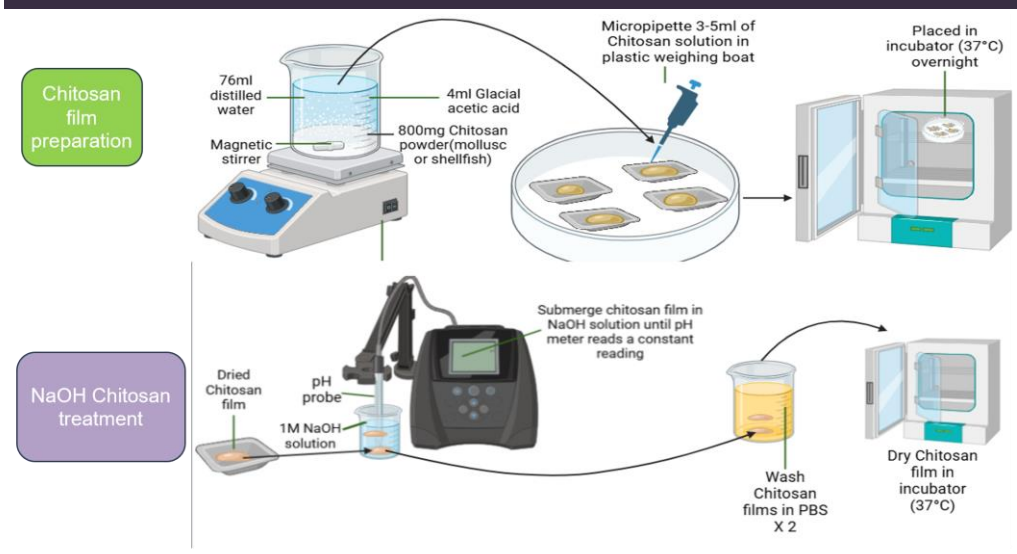


Figure 1: Schematic of chitosan film preparation and treatment

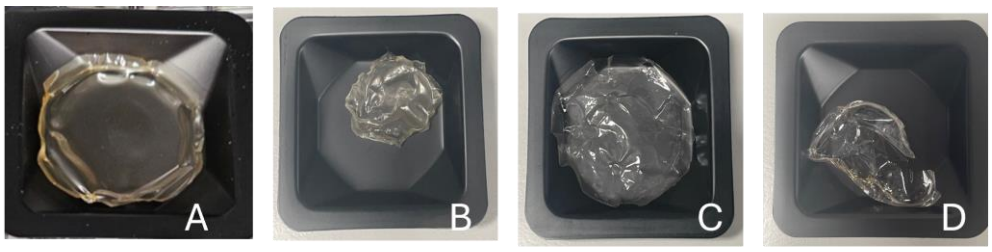


Figure 2: Mollusc Chitosan Untreated (A), Mollusc Chitosan w/ NaOH(B), Crustacean Chitosan Untreated(C), Crustacean Chitosan w/NaOH (D).

- **Mechanical Testing** at 37°C to assess stiffness and failure
- Surface: **AFM** and **microscopy**
- Chemical: **FTIR**
- **Biological**: C2C12 assays for viability and differentiation

RESULTS I: MECHANICAL & SURFACE TRANSFORMATION

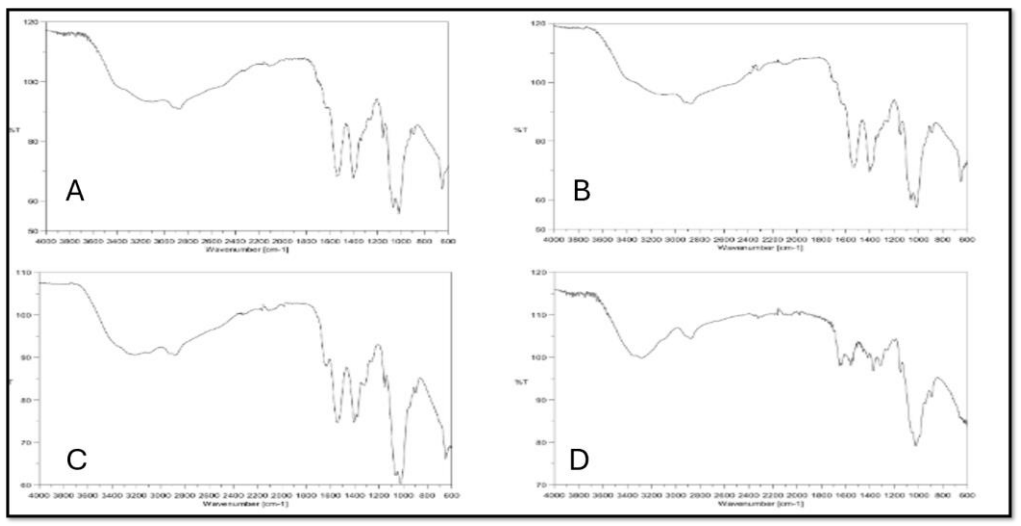


Figure 3: FTIR of Mollusc Chitosan untreated (A) and treated with NaOH(B), as well as Crustacean Chitosan untreated(C) and treated with NaOH(D)

Chitosan Condition	Young Modulus(GPA)	Stiffness (N/mm)	Max force at break (N)	Max displacement at break (mm)
Mollusc(untreated)	2.286	56.52	3.82	0.07
Mollusc (treated with NaOH + PBS)	1.31	33.15	3.93	0.24
Crustacean (untreated)	0.78	19.40	10.34	0.79
Crustacean (NaOH+PBS)	3.8	57.79	5.28	0.10

Table 1: Mechanical testing results of Chitosan films after static loading test

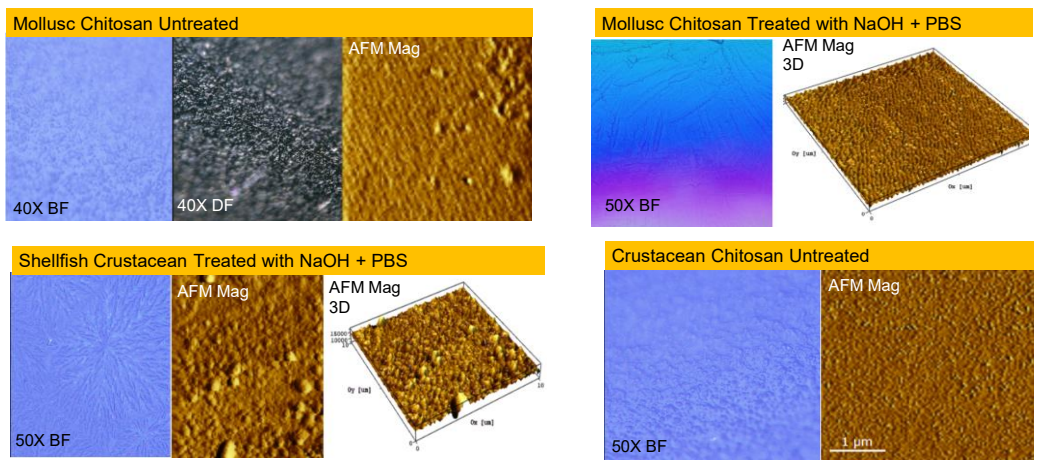


Figure 4: Light microscopic and AFM surface topography analysis

RESULTS II: EVIDENCE OF MYOGENESIS

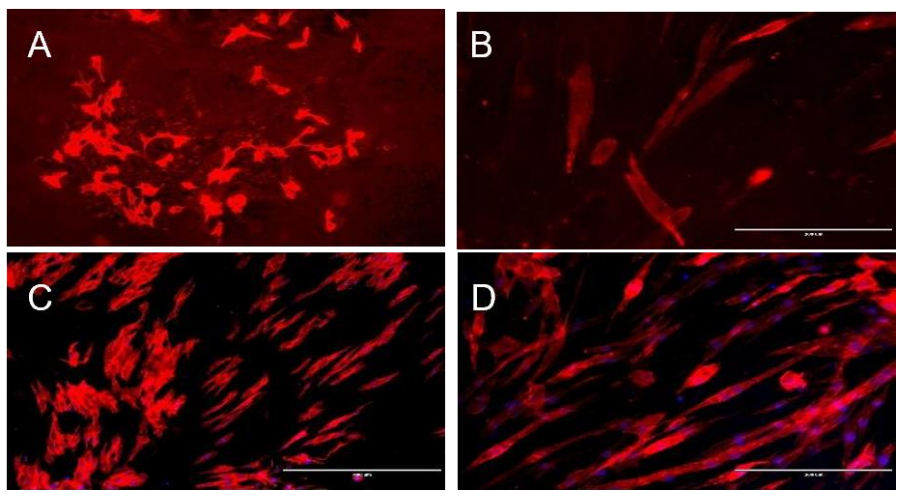


Figure 5: Day 7 Phalloidin (Red) and DAPI (Blue) stain (A) Crustacean Treated (B) Crustacean Untreated (C) Mollusc Treated (D) Mollusc Untreated.

DISCUSSION AND CONCLUSION

The findings presented here demonstrate reliable, sustainable chitosan film production that supports high C2C12 viability and myogenic differentiation, establishing chitosan films as a robust green biomaterial platform for translation towards VML repair. Future work will seek to apply this work in an *in vitro* human skeletal muscle injury model, prior to pre-clinical testing.