Advanced Biotechnologies for Sustainable Food: *Insect Cell Lines* as an Innovative Nutritional Solution

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Background

The growing global demand for meat is placing significant pressure on natural resources, contributing to increased greenhouse gas emissions, freshwater consumption, and deforestation[1]. In this context, *cultured meat (CM)* represents a promising sustainable alternative; however, current approaches based on mammalian cells are limited by high costs and poor scalability[2]. Insect cells are emerging as a viable solution due to their high proliferative capacity, reduced nutritional requirements, and ability to grow under simplified culture conditions[3-4]. Among insect species, *Hermetia illucens* (*Black Soldier Fly*) stands out for its ecological value and its capacity to produce protein-rich biomass from organic waste[5]. This study aims to establish a muscle cell line derived from *H. illucens* embryos, enabling the development of stable in vitro cultures suitable for insect-based cultivated meat. To support the formation of three-dimensional tissues, chitosan-based scaffolds derived from *H. illucens* will also be evaluated for their ability to promote cell adhesion, structural organization, and functional maturation[6-7].

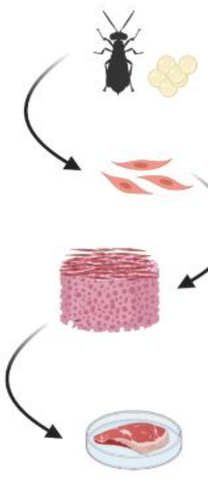

Figure 1: Insect-derived cultured meat: muscle cells isolated from *H. illucens* embryos are expanded and differentiated in *vitro*, and subsequently seeded onto a *chitosan-based scaffol*d derived from *H. illucen*s, aiming to produce a sustainable alternative to conventional meat..

Methods

Primary cell cultures were obtained from embryonated eggs incubated for 19 hours at 28 °C, an optimal condition for the isolation of myogenic precursors.

After homogenization and subsequent culture in a specific medium, muscle differentiation was induced using 20-hydroxyecdysone (20-HE) and assessed through immunofluorescence and observation of spontaneous contractile activity.

In parallel, 3D chitosan-based scaffolds were developed from larvae, pupal exuviae, and adults of *H. illucens*. The materials were produced by dissolution in acetic acid followed by freezing and lyophilization. The scaffolds were characterized in terms of porosity, structural stability, swelling capacity, and digestibility, while morphology was analyzed using scanning electron microscopy (SEM).

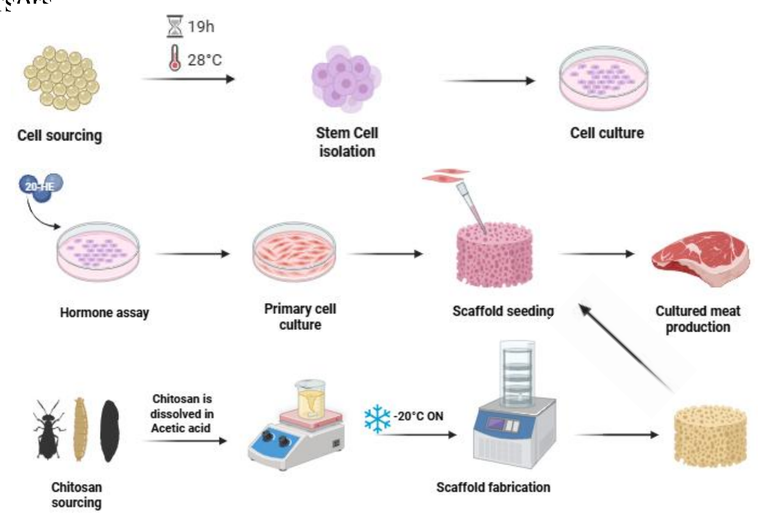

Figure 2: Schematic representation of the experimental workflow, from embryo processing and myoblast selection to myotube induction and scaffold-based 3D tissue formation.

Results

Cells isolated from *H. illucens* embryos demonstrated the capacity to proliferate and differentiate *in vitro*, with spontaneous contractile activity observed within 6–10 days of culture.

20-HE treatment did not significantly affect cell length but reduced cell viability. Although a slight increase was observed at 20 ng/mL, the high variability (SD) and the return to control-like cell length at higher concentrations (40–500 ng/mL) indicate the need for dose optimization.

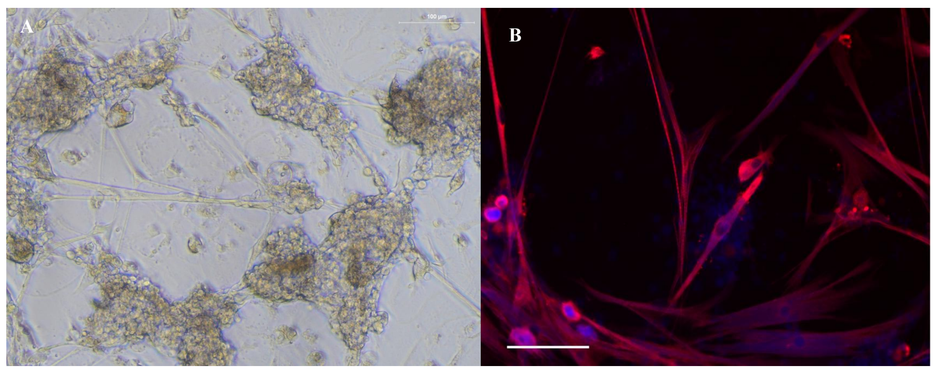

Figure 3: A. Phase-contrast image of *H. illucens* primary cell cultures isolated 19 h after egg collection, showing a heterogeneous population with myoblast-like cells displaying elongated morphology and intercellular projections. Scale bar: 100 µm.
B. Immunofluorescence staining for myosin heavy chain (red), confirming the presence of differentiated myogenic cells. Nuclei are counterstained with DAPI (blue). Scale bars: 100 µm.

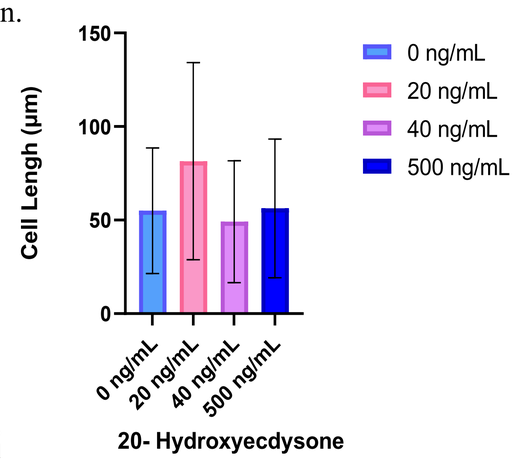

Figure 4: Effect of 20-HE (0, 20, 40, 500 ng/mL) on *H. illucens* cell length measured 5 days after seeding. Data are presented as mean ± SD. Statistical analysis: one-way ANOVA with Tukey's post hoc test (n = 3 wells/condition, 40 measurements per well); no statistically significant differences were observed.

The fabricated scaffolds exhibited porous and interconnected architectures, with properties dependent on the developmental stage of the source material. Swelling tests showed that scaffolds derived from larval and pupal exuviae exhibited good stability after 24h in PBS, with a swelling index (23–26%) comparable to or lower than that of commercial chitosan controls. Exuviae-derived scaffolds showed significantly reduced swelling compared to low-molecular-weight chitosan, indicating a more compact matrix. In contrast, adult-derived scaffolds were unstable and completely dissolved within 2h. Overall, larval and exuvial sources appear to be the most promising for stable biomaterial applications..

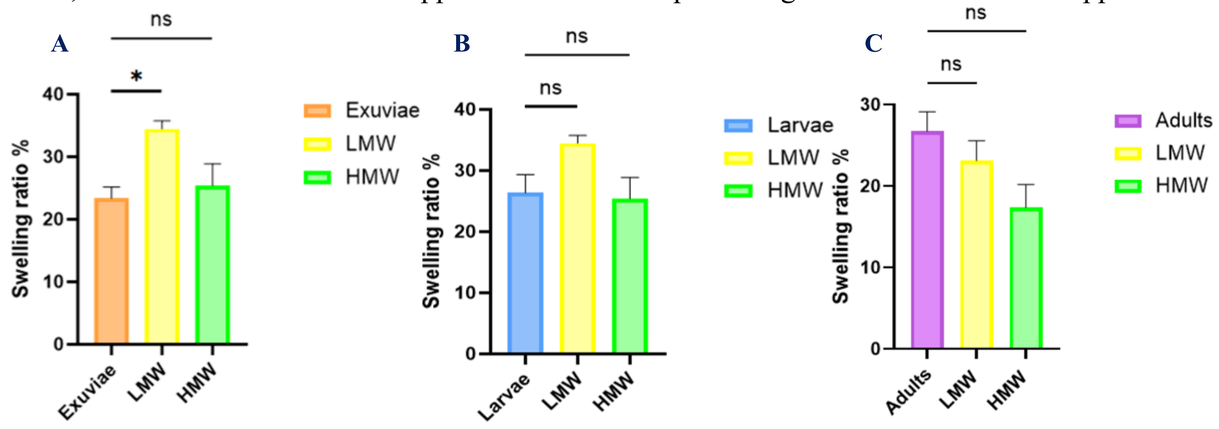

Figure 5: The graphs compare scaffolds derived from pupal exuviae (A), larvae (B), and adults (C) with commercial chitosan controls (LMW and HMW). A and B were measured after 24 h in insect cell medium, while C reflects the initial phase before complete dissolution within 2 h. Data are presented as mean ± SD. Statistical analysis: one-way ANOVA with Tukey's post hoc test (n = 3 samples/condition); pupal exuviae-derived scaffolds showed significantly lower swelling than LMW chitosan.

SEM analysis showed that all scaffolds had a highly porous, interconnected structure suitable for cell adhesion and nutrient transport. Exuviae-derived scaffolds displayed the most organised morphology with well-defined secondary microporosity, whereas larval and adult samples showed a more irregular lamellar structure. Commercial chitosan controls exhibited larger, smoother pores. Overall, exuviae-derived chitin produced the most structurally complex scaffolds, with potential advantages for tissue engineering.

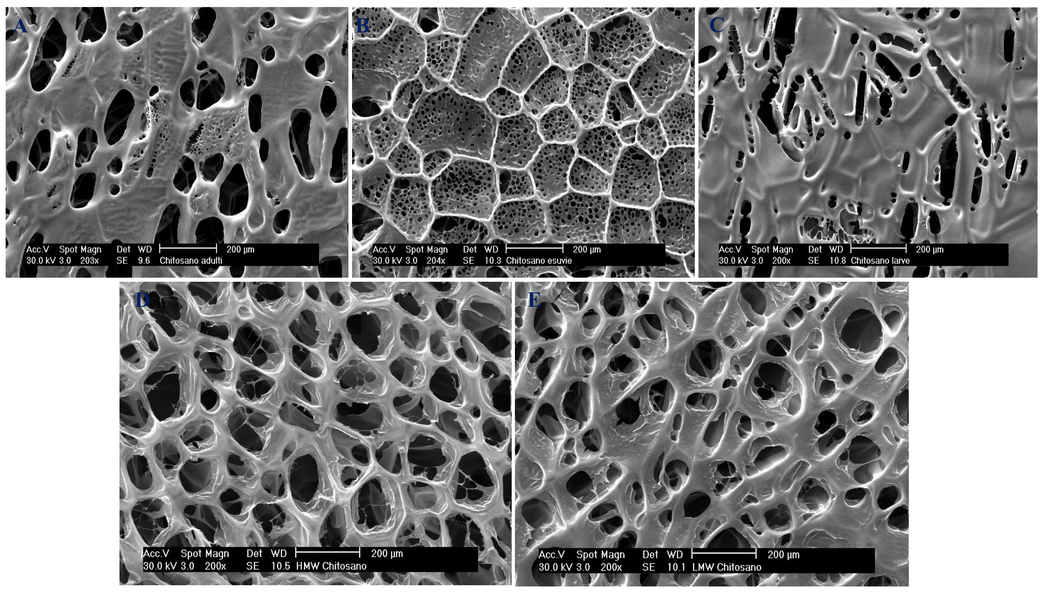

Figure 6: SEM images of chitosan-based scaffolds highlighting their interconnected porous architecture. Differences in pore morphology, including size and distribution, are observed among scaffolds derived from different sources: adults (A), exuviae (B), larvae (C), and high- and low-molecular-weight chitosan (D–E). Scale bar: 200 µm.

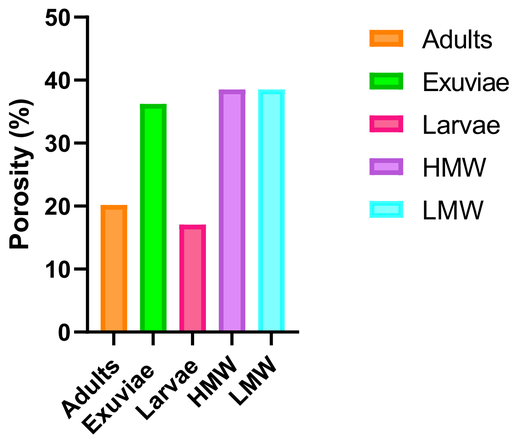

Figure 7: Porosity (%) of chitosan scaffolds, evaluated from SEM images (Scale bar 200 µm) using ImageJ analysis. Scaffolds derived from pupal exuviae exhibit porosity values comparable to those of scaffolds obtained from commercial chitosan (HMW and LMW).

Conclusions

These results highlight the potential of *H. illucens* muscle cells as a sustainable platform for cultivated meat production. Their contractile activity indicates successful functional differentiation, while their integration with chitosan-based scaffolds represents a promising approach for scalable insect-derived tissue engineering. Future work will focus on improving differentiation efficiency, supporting long-term cell proliferation, and optimizing scaffold properties to enable the formation of structured 3D muscle tissue. Particular attention will be given to the role of methoprene, a juvenile hormone analog, in enhancing early-stage cell proliferation. In addition, further studies will assess cell adhesion and viability on chitosan scaffolds.

Acknowledgements

References

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