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DEVELOPMENT AND CHARACTERIZATION OF GELATIN-BASED FOOD-GRADE MICROCARRIERS FOR CULTURED MEAT APPLICATIONS

Chan-Jin Kim1*, SI-Hoon An1, So-Hee Kim1, AMM Nurul Alam1, Young-Hwa Hwang2, Seon-Tea Joo1,2

[1]Division of Applied Life Science (BK21 Four), Gyeongsang National University, Jinju, 52828, South Korea

[2]Institute of Agriculture & Life Science, Gyeongsang National University, Jinju, 52828, South Korea

*Corresponding author email: stjoo@gnu.ac.kr

I. INTRODUCTION

Cultured meat offers a sustainable alternative to conventional livestock production, addressing global concerns related to food security, environmental impact, and animal welfare [1]. To enable large-scale production of cultured meat, the development of efficient and scalable cell culture systems is essential. Recently, edible microcarriers produced from food-grade biomaterials have gained attention as an integrated solution, serving both as scaffolds during cell expansion and as structural components of the final meat product [2,3]. Among these, gelatin—a food-safe materials with excellent biocompatibility and biodegradability—presents significant potential. Additionally, enzymatic crosslinking using microbial transglutaminase offers a non-toxic method to enhance the mechanical stability of gelatin scaffolds. This study aims to develop gelatin-based edible microcarriers through water-in-oil emulsification and enzymatic crosslinking, and to evaluate their physicochemical properties and functionality in muscle cell culture. We hypothesize that gelatin-based edible microcarriers are capable of facilitating cell adhesion, proliferation, and differentiation similarly to conventional non-edible microcarriers, with the added benefit of direct integration into the final cultured meat product.

II. MATERIALS AND METHODS

Gelatin-based edible microcarriers were prepared via water-in-oil emulsification using 10% (w/v) gelatin (Type A, 250 Bloom) dissolved in distilled water as the aqueous phase. Middle chain triglycerides (MCT) oil was used as the continuous oil phase, and lecithin was employed as the emulsifier. The gelatin solution was emulsified into the oil phase under stirring at 250–350 rpm using an overhead stirrer. Enzymatic crosslinking was performed by adding microbial transglutaminase and incubating the emulsion at 25 °C for 2 hours. The fabricated microcarriers were washed 3–5 times with 0.1% (v/v) Tween 80 aqueous solution to remove residual oil and unreacted components. Particle morphology and size distribution were analyzed by optical microscopy, and particle diameters were quantified using ImageJ software. Residual surface lipids were evaluated by LipidTOX fluorescence staining, and zeta potential was measured before and after the washing process. To assess cell compatibility, C2C12 myoblasts were cultured on the gelatin microcarriers, with Cytodex 1 microcarriers and 2D culture plates serving as controls. Cell proliferation was evaluated by calculating the doubling time during a 72-hour culture period based on cell counts at defined time points. Statistical analyses were performed using GraphPad Prism 10. One-way ANOVA with Tukey's post-hoc test and paired t-tests were used, with $P < 0.05$ considered statistically significant.

III. RESULTS AND DISCUSSION

Gelatin-based edible microcarriers were fabricated via water-in-oil emulsification, and their particle morphology was confirmed by optical microscopy, revealing spherical microcarriers with uniform size distribution (Fig. 1a). Optimization of the emulsification process using varying emulsifier concentrations and agitation speeds demonstrated that these parameters directly influenced microcarrier yield, as visualized through the response surface plot (Fig. 1b). These findings indicate that appropriate control of formulation conditions is essential for achieving high production efficiency of gelatin microcarriers. Residual surface lipids on the fabricated microcarriers were evaluated by LipidTOX fluorescence staining. After the washing process, a marked reduction in lipid-associated fluorescence intensity was observed (Fig. 1c), suggesting effective removal of residual oil from the microcarrier surfaces. In addition, zeta potential measurements showed an increase in surface charge stability following washing (Fig. 1d). These changes in surface properties are expected to enhance microcarrier dispersibility and improve cell attachment capability. The cell adhesion and proliferation performance of the gelatin microcarriers were assessed using C2C12 myoblast cultures. Phase-contrast microscopy demonstrated stable cell attachment and spreading on

the gelatin microcarriers, comparable to conventional Cytodex 1 microcarriers and 2D culture surfaces (Fig. 2a). Growth rate analysis further confirmed similar proliferation dynamics among the three conditions over a 72-hour culture period (Fig. 2b). Quantitative analysis of cell spreading area showed enhanced spreading on gelatin microcarriers compared to Cytodex 1 (Fig. 2c), while cell viability remained consistently high across all culture conditions (Fig. 2d). These results suggest that gelatin-based edible microcarriers can effectively support myoblast adhesion and growth, offering performance comparable to non-edible commercial microcarriers while providing the added benefit of food-grade compatibility.

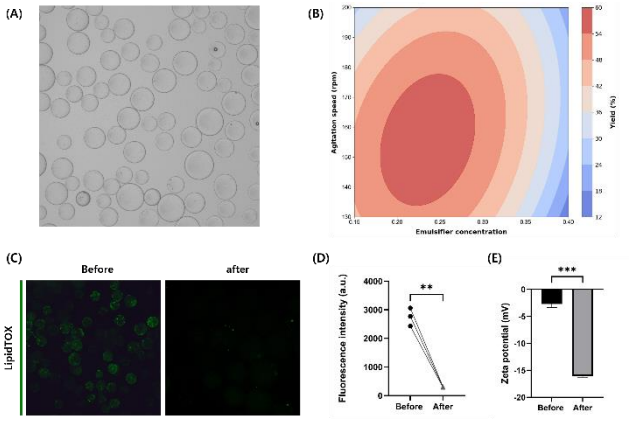


Fig 1. Fabrication and characterization of gelatin-based edible microcarriers. (a) Representative image of gelatin microcarriers produced via water-in-oil emulsification. (b) Response surface plot showing the effect of emulsifier concentration and agitation speed on microcarrier yield, optimized using response surface methodology (RSM). (c) Fluorescence images of LipidTOX-stained microcarriers before and after the washing process, indicating reduction of residual surface lipids. (d) Quantitative analysis of residual lipid content (left) and zeta potential measurements (right) before and after washing.

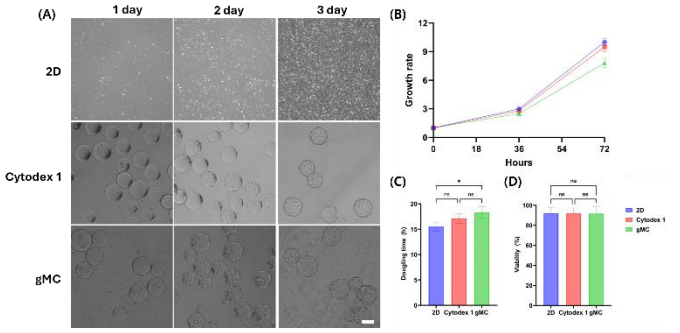


Fig 2. Cell adhesion and proliferation on gelatin-based edible microcarriers. (a) Phase-contrast microscopy images showing the morphology and attachment of C2C12 myoblasts cultured on 2D surfaces, Cytodex 1 microcarriers, and gelatin-based edible microcarriers (gMCs) at various time points. Scale bar, 100 µm. (b) Growth rate of C2C12 cells over a 72-hour culture period under each condition. (c) Cell spreading area analysis comparing 2D culture, Cytodex 1, and gMCs, indicating differences in cell morphology depending on the scaffold type. (d) Cell viability assessment after 72 hours of culture across the three culture conditions.

IV. CONCLUSION

Gelatin-based edible microcarriers were successfully fabricated via emulsification using MCT oil and lecithin, achieving uniform particle formation with reduced residual lipids. The optimized microcarriers demonstrated stable surface properties and supported effective myoblast adhesion and proliferation. These results suggest the potential of gelatin microcarriers as functional and food-compatible scaffolds for cultured meat applications.

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