ENHANCING BOVINE SATELLITE CELL GROWTH WITH CHITOSAN-MODIFIED MICROCRYSTALLINE CELLULOSE SCAFFOLDS

Ingrida Pauliukaityte[1*], Asta Krings[2], Dainius Martuzevicius[1], Darius Ciuzas[1], Edvinas Krugly[1], Alvija Salaseviciene[1], Stig Skrivergaard[2], Margrethe Therkildsen[2]

[1] Kaunas University of Technology, Kaunas, Lithuania.

[2] Aarhus University, Agro Food Park 48, 8200 Aarhus N, Denmark

*Corresponding author email: ingrida.pauliukaityte@ktu.lt

I. INTRODUCTION

In recent years, there has been a burgeoning interest in developing cellulose structures with enhanced characteristics for biomedical applications [1, 2]. These structures are particularly sought after for their biodegradability, biocompatibility, and extensive surface area. Cellulose's ability to support high cell density and modulate pore size, along with its capability to structure in both random and aligned fiber arrangements, has positioned it as a favorable scaffold material for cultivated meat production [3]. While previous studies have largely focused on cellulose derivatives, our research aims to harness the potential of pure cellulose by utilizing electrospun nanofiber matrices for muscle cell cultivation, leveraging their structural benefits for improved cell proliferation.

II. MATERIALS AND METHODS

2.1. Development of electrospun microcrystalline cellulose scaffold

2.1.1. Electrospun microcrystalline cellulose scaffold

Microcrystalline cellulose was dissolved in a 1:1 ratio of 1-butyl-3-methylimidazolium acetate (an ionic liquid) and dimethyl sulfoxide (co-solvent) and then electrospun using a wet-type apparatus to form a smooth nanofibrous scaffold (eMCS). The eMCS underwent a three-stage elution with deionized water and ethanol for purity [4], followed by lyophilization to enhance its porosity and surface-area-to-volume ratio.

2.1.2. Chitosan-modified electrospun microcrystalline cellulose scaffold

Surface modifications were applied to the eMSC (2.1.1.) to create the chitosan-modified scaffold. The eMCS was immersed for 1-3 hours in a 0.02 g/ml chitosan solution prepared using 2% wt. acetic acid solution to produce a chitosan-modified electrospun microcrystalline cellulose scaffold (eMCSch).

2.2. Testing of the electrospun microcrystalline cellulose scaffolds for primary bovine satellite cell proliferation

2.2.1. Bovine satellite cell proliferation

Bovine Satellite Cells (BSCs) were isolated from a one-month-old Holstein bull calf weighing 59 kg, sourced from Aarhus University Cattle facility (DKC, 8830 Tjele). The M. semimembranosus muscle was then excised from the carcass and transported on ice to the Cell Laboratory at the Department of Food Science, Aarhus University. Cell isolation commenced approximately 2 hours postmortem, following the protocol established by Skrivergaard et al. (2021) [5]. Isolated BSCs were resuspended in 37°C growth media (GM) consisting of Dulbecco's Modified Eagle Medium (1X) + GlutaMAXTM (DMEM) (61965-026, Gibco) supplemented with 1% Pen Strep (1:1) (15140122, Gibco), 0.2% Gentamicin sulfate (L0012100, Biowest), 1% Amphotericin B (15290026, Gibco), 1% sodium pyruvate (11360-070, Gibco), 10% FBS (10082147, Gibco), and 10% HS (26050-088, Gibco). The cell solutions were seeded on the cellulose matrix with cell densities of 5,000-50,000 cells per well in cell repellent

96-well plates (174925, Thermo Scientific). The seeded BSCs were cultured for three days at 37°C in a 5% CO_2 humidified atmosphere.

2.2.2. Bovine satellite cell viability

The viability of BSCs cultured on the eMCS and eMCSch was assessed using a metabolic assay with Cell proliferation Reagent WST-1 (11644807001, Roche). The WST-1 reagent was added in a 1:10 ratio and incubated at 37°C in a 5% CO_2 humidified atmosphere for 1h. The assay measured the absorbance at a wavelength of 440 nm for formazan and 650 nm for reference using Cytation 5 Cell Imaging Reader (BioTek), which indicates relative cell viability.

III. RESULTS AND DISCUSSION

3.1. Development of the electrospun microcrystalline cellulose scaffolds

Research determined that the optimal cellulose concentration for electrospinning microcrystalline cellulose scaffolds (eMCS) is 10% wt, with a solvent/co-solvent ratio of 1:1. Under these conditions, the resulting eMCS exhibited a smooth structure and consisted of cylindrical fibers. The optimal conditions for wet-type electrospinning were found to be a flow rate of 6.28 ml/h, a voltage of 10-13 kV, and a distance of 2 cm from the 22-gauge steel needle to the collector.

The research on the development of a chitosan-modified scaffold (eMCSch) determined that optimal parameters for the chitosan solution used in the modification process were determined to be a concentration of 0.02 g/mL in 2% acetic acid, with the eMCS scaffold immersion time of 3 hours. This concentration effectively ensured complete coverage of the eMCS surface and allowed penetration into the deeper layers of the eMCS without compromising its inherent fibrous structure.

3.2. Testing of the electrospun microcrystalline cellulose scaffolds for primary bovine satellite cell proliferation

The efficacy of both the eMCS and eMCSch scaffolds in supporting primary bovine satellite cell (BSC) proliferation was assessed. The chitosan-modified scaffold (eMCSch) significantly enhanced cell viability, showing a corrected absorbance increase to 0.090 ± 0.022, compared to the unmodified scaffold (eMCS), which recorded an absorbance of 0.048 ± 0.009. This data indicates an improved cellular environment due to the chitosan modification.

While initial cell adhesion and proliferation were observable on the eMCSch, the presence of clearly defined cells was limited. Neither scaffold exhibited significant cytotoxic effects, as evidenced by cell counts in the medium with scaffold residues, which were comparable to control values. These findings underscore the potential of chitosan-modified scaffolds in tissue engineering, although further optimization may be necessary to enhance cellular integration and proliferation.

IV. CONCLUSION

This study has demonstrated the potential of chitosan-modified microcrystalline cellulose scaffolds (eMCSch) to enhance the initial viability and growth of primary bovine satellite cells (BSCs). The findings revealed that the eMCSch significantly improved cell viability, as evidenced by the increase in corrected absorbance measurements compared to the unmodified electrospun microcrystalline cellulose scaffold (eMCS). These results underscore the effectiveness of chitosan modifications in creating a more conducive cellular environment for tissue engineering applications.

REFERENCES

1. Ciuzas, Darius, et al. "Electrospun cellulose fibers from ionic liquid: Practical implications toward robust morphology." *Journal of Applied Polymer Science* 139.3 (2022): 51525.
2. Verma, Chandrabhan, et al. "Dissolution of cellulose in ionic liquids and their mixed cosolvents: A review." *Sustainable Chemistry and Pharmacy* 13 (2019): 100162.

3. Santos, Ana Elisa Antunes dos, et al. "Random cellulose acetate nanofibers: a breakthrough for cultivated meat production." *Frontiers in Nutrition* 10 (2024): 1297926.
4. Krugly, Edvinas, et al. "Cellulose electrospinning from ionic liquids: The effects of ionic liquid removal on the fiber morphology." Carbohydrate Polymers 285 (2022): 119260.
5. Skrivergaard S, Rasmussen MK, Therkildsen M, Young JF. 2021. Bovine Satellite Cells Isolated after 2 and 5 Days of Tissue Storage Maintain the Proliferative and Myogenic Capacity Needed for Cultured Meat Production. Int J Mol Sci 22.