PLANT CELLULOSE AS AN ECONOMICAL 3D SCAFFOLD FOR CHICKEN MUSCLE TISSUE CULTURE

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I. INTRODUCTION

Many researches discusses tissue engineering strategies for developmental biology using chemical, biomechanical, and biophysical processes to simulate tissue-like structures [1-3]. Scaffold materials are required for creating 3D cell-laden structures in vitro, and plant-derived scaffolds are cost-effective and suitable for 3D cell culture platforms [4-6]. Here we established culture conditions for chicken myogenic cells derived from chick embryo and developed a plant-based scaffold for generating 3D tissue-like structures. This bioengineered 3D muscle culture system could provide insights into how chicken myogenic cells interact with porous scaffolds and how tissue-like structures are efficiently produced.

II. MATERIALS AND METHODS

Myoblast cell line were established from chick myoblasts isolated by pronase digestion and characterized by marker expression. Celery tissue was sliced using mandolin slicer and decellularized by 1% SDS solution. Decellularized scaffolds were sterilization with EtOH and then lyophilization. Isolated myoblasts were co-cultured with scaffolds until adhered structure developed and further cultured in serum free culture medium.

III. RESULTS AND DISCUSSION

To generate 3D muscle tissue-like structure, myoblasts grown on scaffold require a medium that can induce proliferation and differentiation of myoblast. Myoblasts were cultured in different medium conditions supplemented with a combination of FBS, HS, CEE, and bFGFs. Myoblast proliferation rate was analyzed using CCK-8 after initial culture on 96-well plate in the corresponding culture medium for 3 days (Fig. 1A). The optimum condition for myoblasts proliferation was found to be a medium supplemented with 10% HS and 5% CEE. Medium supplemented with chick embryo extract (CEE) and horse serum (HS) significantly increased proliferation and myotube (MYH1E+) differentiation of myoblasts (Fig. 1B). Scratch assay with myoblasts also showed the promoted proliferation ability of CEE + HS medium (Fig. 1C).

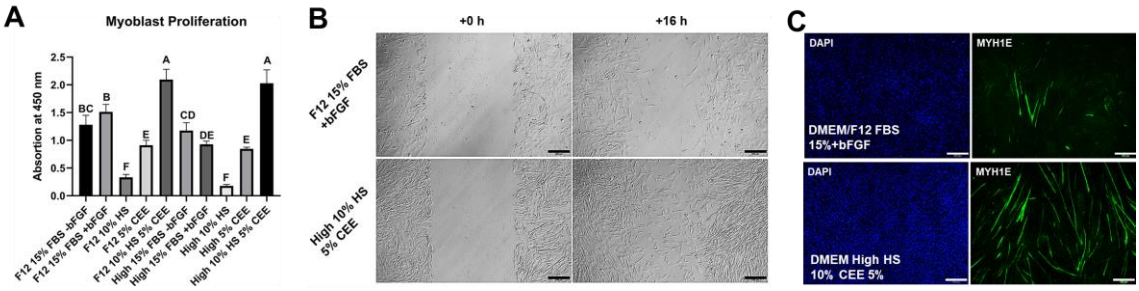

Figure 1. Establishment of culture medium condition for proliferation and differentiation of myoblasts

When decellularized scaffold was cut into 15 mm diameter round disc and cultured with myoblasts on scaffolds for 2 weeks, cells began to form dense cell masses, covering the surface of the scaffold. Each myotube mass was interconnected to form networks and showed spontaneous contractions (Fig. 2). This study presents compelling evidence for the successful production and cultivation of 3D chicken skeletal muscle-like structures capable of exhibiting contractile properties similar to myofibers. These findings highlight the feasibility of generating muscle tissue-like structures through a straightforward and cost-effective protocol, thus paving the way for the advancement of practical methodologies for large-scale 3D tissue production.

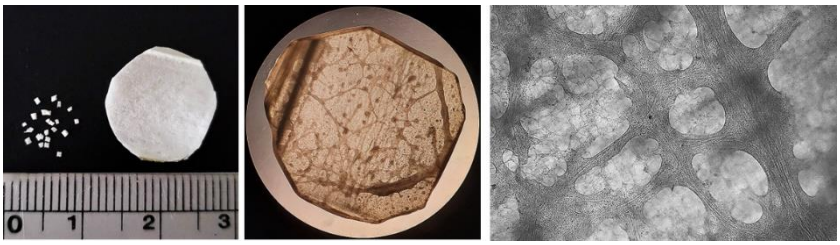

Figure 2. Formation of muscle tissue like structure on decellularizedscaffolds

IV. CONCLUSION

Chicken myoblasts highly proliferate and differentiate in CEE/HS supplemented medium. Celery derived decell-scaffolds are compatible for myoblast 3D culture. Muscle tissue like structure for cultured meat production can be developed by 3D culture on decell-scaffolds.

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