SUITABILITY OF ECM MATERIALS FOR COLLAGEN-BASED SCAFFOLD REPLACEMENT IN CULTURED MEAT

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I. INTRODUCTION

Population growth and rapid growth in meat consumption are raising questions about the sustainability of food supplies [1]. Meat alternatives produced in the laboratory based on animal muscle stem cells are called cultured meat [2]. Many scientists see cultured meat as potential alternative protein sources for the future of mankind [3]. For the commercialization, development and economical production of cultured meat, various extracellular matrix (ECM) as a scaffold for tissue reconstruction are being studied [4]. Manufacture using ECM is essential in the cultured meat production [5]. These experiments were investigated the suitability of ECM like alginate, carboxymethyl cellulos (CMC) that replaces the most commonly used collagen scaffold for cultured meat production.

II. MATERIALS AND METHODS

Collagen (C), matrigel (M), alginate (Alg), and laminin (LM) are pre-coated on a 96-well plate. Fibronectin (FN) treatment groups were divided into non-added groups and 10 μg/ml added groups in each ECM coating treatment groups. Hanwoo myosatllite cells were cultured in 96-well plates coated with each ECM material in a humidified incubator at 37ºC and 5% CO_2. After 48 h of incubation, cell proliferation assay (MTS) is measured at 490 nm. MTS optical density (O.D.) is used to measure cell viability. Based on the experimental results, ECM mixture is prepared for each treatment groups. Dispense the ECM mixture and Hanwoo myosatellite cells into a mould that self-assembles into a ring structure. Those are observed the culture until the 4th day in the mould, and check the self assemble everyday. The self-assembled tissues on 4th day of mould culture were observed. All statistical analyses including Duncan's multiple range test were carried out using SAS Statistical Package 9.4 (SAS, 2003). *p*-values of <0.05 indicated significant differences.

III. RESULTS AND DISCUSSION

Cell proliferation of 0.1-1% Alg coating groups are not significantly different from C (Fig. 1A). Cell proliferation of LM 1ug/ml, 10ug/ml groups are significantly higher than C (p<0.05) (Fig. 1B). Cell proliferation of FN 10ug/ml added group showed significantly higher values in the C, M, Alg, and LM groups than the non-added group (p<0.05) (Fig. 1C).

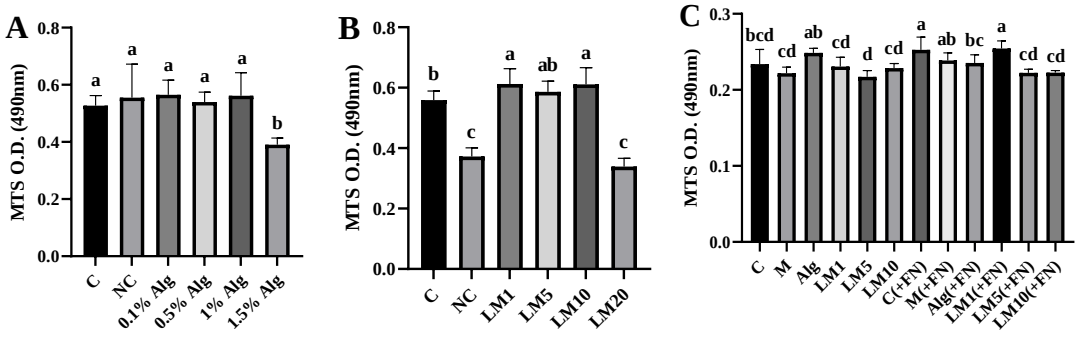

Figure 1. Hanwoo myosatellite cell proliferation according to ECM (alginate, laminin and fibronectin) material coating concentration. C: collagen coating, NC: Non-coating, Alg: alginate coating, LM(N): (N μg/ml) laminin coating, M: matrigel coating, FN: adding fibronectin. Data are presented as mean ± standard deviation. Different letters differ significantly (p < 0.05).

On the 3rd day of culture, cell proliferation of the treament groups was significantly higher than that of the control group (p<0.05) (Fig. 2A). On the 5th and 7th day of culture, cell proliferation of the CM(LM+FN) group was significantly higher than that of the control group (p<0.05) (Fig. 2B, 2C).

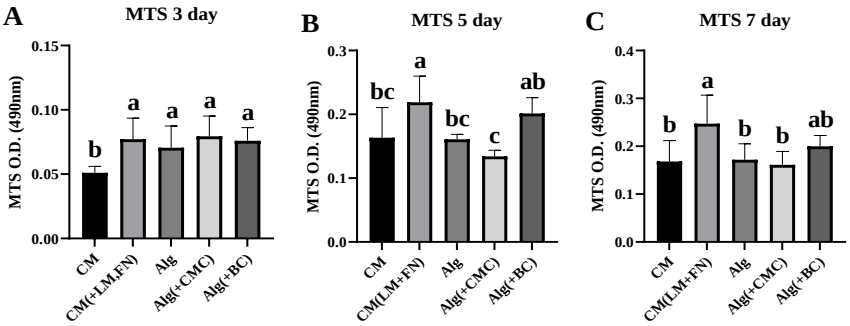

Figure 2. Hanwoo muscle cell proliferation according to ECM mixture. CM: collagen and matrigel coating, LM: laminin, FN: fibronectin, Alg: alginate, CMC: carboxymethyl cellulose, BC: bovine collagen. Data are presented as mean ± standard deviation. Different letters differ significantly (p < 0.05).

Control and treatment groups were seeding into mould (Fig. 3A). The seeded mixture was cultured in a mold for 4 days, and a ring structured tissue was observed through self-assemble (Fig. 3B). Hanwoo muscle cells were observed in the self-assembled ring structure tissues (Fig. 3C).

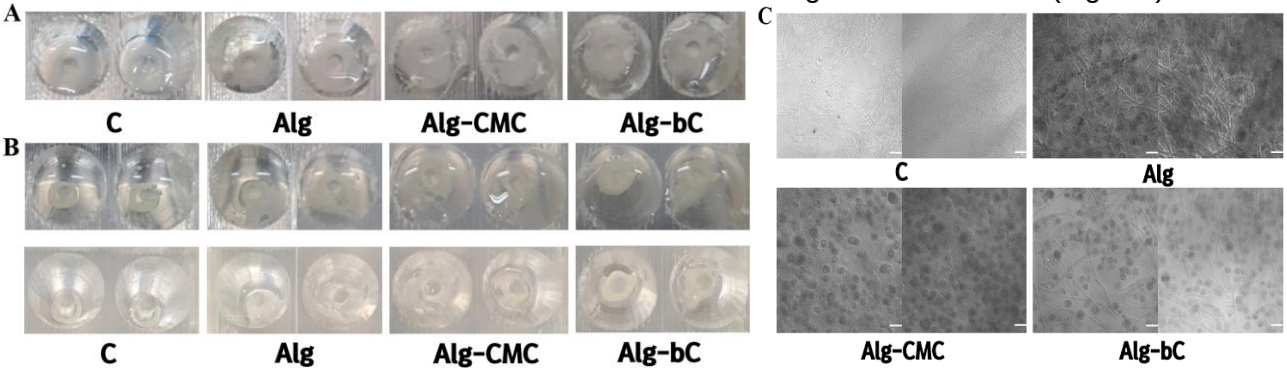

Figure 3. Self-assembled tissue of Hanwoo muscle cells and ECM in ring structure mould A: Seeding with ECM mixture B: Incubation for 96 h with ECM mixture C: Hanwoo muscle cells and ECM mixture in self-assembled tissue. Scale bar: 100μm

IV. CONCLUSION

In this experiment, the possibility of replacing collagen scaffold through self-assembling of various ECM was investigated for the development and economical production of cultured meat. Collagen and matrigel-based control achieved the best ring structure, and in the alginate replacement group, the group added with bovine collagen powder produced the best ring structure.

ACKNOWLEGEMENTS

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