ASSESSMENT OF A PLANT-BASED PROTEIN-POLYSACCHARIDE SCAFFOLD FOR CULTIVATING STEM CELLS IN CULTURED MEAT PRODUCTION

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I. INTRODUCTION

Cultured meat is a novel food technology utilizing stem cells obtained from animal biopsies. To replicate the texture of traditional meat, cells are grown in 3D structures such as scaffolds, microcarriers, and hydrogels. Scaffolds produced by freeze-drying hydrogels have sponge-like form, and cell-binding ligands [1]. Scaffolds using plant-based proteins or polysaccharides can offer desirable properties such as low cost, processability, and non-animal origin. Soy and pea protein are commonly used to make hydrogels due to their high gelation properties. However, additional crosslinking is necessary to form a stable 3D structure. Polysaccharides exhibit remarkable gelation properties and can maintain 3D structure, although their cell adhesion ability is low without chemical modifications [2]. We aimed to determine the optimal combinations for producing hydrogel scaffold using plant-based proteins (soy and pea) and polysaccharides (agarose and agar powder), and evaluating the physical characteristics, cytotoxicity, and cell adhesion properties using porcine adipose tissue-derived stem cells.

II. MATERIALS AND METHODS

Soy and pea protein solutions were prepared with water (5% (w/v)). Agarose and agar powder were mixed with water (2% (w/v)). The protein solution was preheated and mixed with the polysaccharide solution, and cooled to form the scaffold. Adipose-derived stem cells (ADSC) were obtained from porcine adipose tissue and cultured in Dulbecco′s Modified Eagle′s Medium/F-12 media containing 10% fetal bovine serum, and 100 ng/mL of basic fibroblast growth factor. The physical properties of the scaffold were evaluated using water absorption test, compressive test, Fourier transform infrared, and scanning electron micrographs (SEM). Cytotoxicity of the scaffolds and cell adhesion tests were conducted. Cell adhesion properties were evaluated by FE-SEM and media cell count.

III. RESULTS AND DISCUSSION

Solid hydrogels were formed by mixing protein and polysaccharide solutions, as shown in Figure 1A. Pea and soy proteins contain various amino acids, including glutamine. The amide groups of glutamine can form hydrogen bonds with the hydroxyl groups on agarose, contributing to the hydrogel formation. Hydrogels containing agar powder (SAP and PAP) showed a more yellowish hue than agarose (SA and PA) (Fig. 1B). The hydrogels containing agarose (SA and PA) displayed higher absorption bands at 2986 cm^{-1} and 1073 cm^{-1} than agar powder (SAP and PAP) (Fig. 1C). Moreover, pea protein hydrogels displayed higher absorption peaks at 2986 cm^{-1} and 1073 cm^{-1} than soy protein hydrogels, indicating an increase of chemical bonds. Pea protein has higher levels of lysine and arginine than soy protein, which can interact with the negatively charged carboxylate groups in agarose. Soy protein showed higher water absorption rate than pea protein (Fig. 1D). Agarose exhibited higher compressive strength than agar powder (Fig. 1E). All hydrogels showed a porous structure (Fig. 1F).

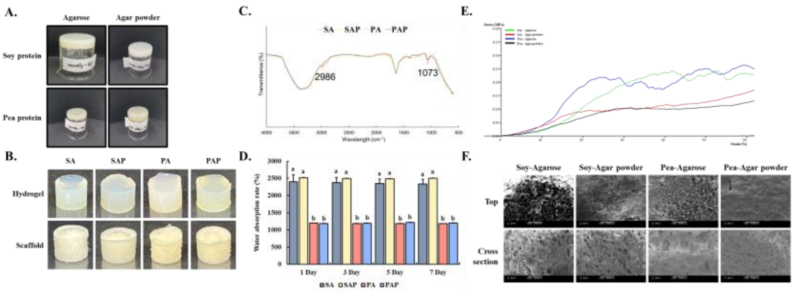

Figure 1. Gelation and physical properties of protein-polysaccharide scaffolds

All scaffold showed no significant cytotoxidity in 3-(4,5-Dimethylthiazol-2-Yl)-2,5-diphenyltetrazolium bromide and lactate dehydrogenase release, and live cell staining tests (Fig. 2A and 2B). Adhered ACSC cells were detected in all scaffolds in FE-SEM (Fig. 2C). Our data suggest that pea protein-agarose scaffolds using soy or pea protein have potential for providing a 3D structure for cultured meat production.

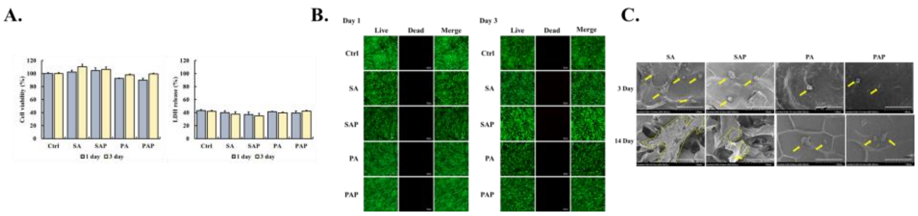

Figure 2. Cytotoxicity and cell adhesion properties of protein-polysaccharide scaffolds

IV. CONCLUSION

Soy and pea protein solutions exhibited higher gelation and porous properties when mixed with agarose and agar powder solutions. Scaffolds utilizing pea protein exhibited slightly higher water absorption properties and a smaller porous structure than soy protein scaffolds due to a higher number of hydrogen bonds. Scaffolds utilizing agarose displayed higher compressive strength than agar powder. Soy protein scaffolds exhibited slightly higher cell adhesion than pea protein. Overall, our data suggest that the soy protein-agarose scaffold exhibits the best potential for use in cultured meat.

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