Dual-Crosslinked Porous Alginate Scaffolds for use in Cultured Meat Production

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Statement of Purpose: With the rising harmful effects of climate change, a novel, emerging field of technology called cellular agriculture has arisen to explore 'cultured meat' - an artificial meat substitute developed *in vitro* using muscle cells and tissue scaffolds. Cultured meat is an advantageous alternative to traditional animal agriculture, which contributes to methane production, deforestation, and use of natural resources. In this study, our goal was to create an ionically and covalently crosslinked alginate scaffold that supports muscle tissue development with the overall goal of achieving a cultured bovine steak for food.

Methods: Herein, we synthesized: 1) methacrylated alginate (Alg-MA) using methacrylic anhydride to enable covalent crosslinking via visible light exposure;[1, 2] and 2) arginine-glycine-aspartic acid (RGD) conjugates (Alg-MA-RGD) using carbodiimide chemistry to provide cell-binding sites onto the material.[3] Inherently alginate is susceptible to ionic crosslinking via divalent cations.[4, 5] Chemical modification was verified using proton nuclear magnetic resonance (^{1}H-NMR) and Fourier transform infrared (FTIR) spectroscopy. Alginate hydrogels of different concentrations (1% and 3%, w/v) and cross-linking types (ionic, covalent, ionic + covalent) were created. Hydrogels were made by injecting hydrogel precursor solutions into custom molds, freezing at -20 °C, and crosslinked with 1M calcium chloride ($CaCl_2$). Covalently crosslinked alginate hydrogels were made by mixing precursor solutions with 1mM eosin Y (photo-sensitizer), 125 mM triethanolamine (photo-initiator), and 20 mM 1-vinyl-2-pyrrolidinone (catalyst) and exposing to green light (525 nm, SuperBrightLEDs) for five minutes. Dual cross-linking was achieved by combining the two types of crosslinking. Scaffolds were characterized by scanning electron microscopy, rheometry, and unconfined uniaxial compression tests. murine C2C12 cells were cultured in the presence of the scaffolds to access viability.

Results: We demonstrated that modifying the material structure chemically and physically via different crosslinking techniques resulted in different material properties between groups, such as gelation kinetics (data not shown) and scaffold stiffness. The scaffolds demonstrate interconnected pores in the range of The gelation time of the varied materials did not vary broadly, although the shear and compression moduli demonstrated significant differences between groups. 20-200 µm (**Fig.1**). The hydrogel groups with the significantly higher compressive moduli were the ionically crosslinked 3%Alg and dual crosslinked 3%Alg-MA groups. The data indicates sequential control of the mechanical properties (**Fig.2**).

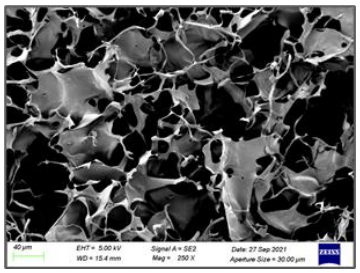

Fig.1. SEM image of 3% Alg-MA scaffold structure.

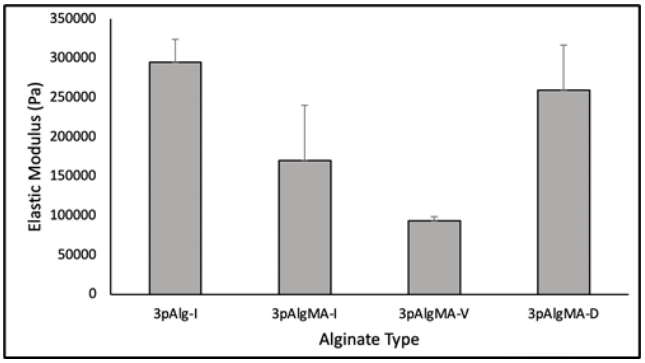

Fig.2. Compressive stiffness of crosslinked alginate scaffolds

Conclusion: Our unique method of synthesizing alginate hydrogels and the associated pilot data indicates the efficacy of dual-crosslinked alginate hydrogels for the production of cultured meat. Ongoing work includes primary bovine muscle cell response and myogenic differentiation on the alginate scaffolds, and investigating the effects of stiffness, pore size, and chemical composition.

References:

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