Pore Formation Mediated by Polysaccharide Incorporation for 3D Skeletal Myotube Formation

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Introduction: Porous matrices have been extensively used as a biomaterial for three-dimensional tissue engineering. It is well agreed that interconnectivity of pores in the porous matrix is essential for cell seeding, migration into pores, transport of biomolecules, and, ultimately, tissue formation. Many methods to introduce interconnected pores rely on the use of porogens, such as salt, and/or organic solvents, which must be rinsed away before use with cells, or complex fabrication methods. These methods also result in poor interconnectivity. Another common method of creating interconnectivity is freeze-drying. Freeze-drying allows one to avoid the use of porogens, organic solvents, and complex fabrication processes; however, the quality and diameter of pores from freeze-drying are highly dependent on the choice of polymer and freezing temperature. We have developed a new method to control the porosity of the material by incorporating a polysaccharide into the polymer network. The introduction of the polysaccharide increases the amount of water bound to matrix, thereby increasing the size of the ice crystals formed during the freezing process. Consequently, the size of the pores formed during the drying process are larger and have greater interconnectivity. This greater interconnectivity facilitates better cell engraftment and three dimensional myotube formation in the matrix.

Materials and Methods: The authors prepared poly(ethylene glycol) diacrylate (PEGDA) hydrogels via a radical polymerization with photoinitiator and swollen for 24 hours in deionized water. To facilitate polymerization with PEGDA and cell culture, we modified polysaccharides with methacrylate groups and RGD peptide respectively.

This involved varying the concentration of polysaccharide to examine its affect on porosity while keeping RGD concentration constant across all conditions. Matrices were analyzed for porosity using scanning electron microscopy (SEM) and microtomography (μCT). Bound water content and ice crystals were examined using magnetic resonance imaging and microscopy respectively. Each matrix was then seeded with primary myoblasts and cultured at 37° C and 5 % CO_2 for 2 days in proliferation media followed by 8 days in differentiation media prior to analysis of myosin expression (confocal microscopy) and muscle creatine kinase (MCK) activity (MCK assay). Cell viability was also analyzed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay at days 1, 4, 7, and 10.

Results and Discussion: Varying the amount of polysaccharide in the pre-polymer solution, allowed us to control the porosity of the freeze-dried matrix. Changing the polysaccharide pre-gel concentration from 0% to 1% resulted in a two-fold increase of bound water to the matrix. The increase in bound water resulted in larger ice crystals during the freezing process. This was validated through SEM images in which no pores were visible in the absence of polysaccharide. Further analysis with μCT demonstrated that by increasing the prepolymer concentration of polysaccharide from 0% to 1%, while maintaining a constant total polymer concentration, resulted in an increase in porosity from 17% to 79%. Due to the increased porosity, it was possible for more cells to be incorporated into the matrix as demonstrated with 3D confocal microscopy and MTT assay. Further image and biochemical analysis examining the expression of myosin (in red in **Figure 1**) and MCK, two late stage myogenic markers, demonstrated an increase in myogenic expression and MCK activity as the amount of polysaccharide in the matrix was increased.

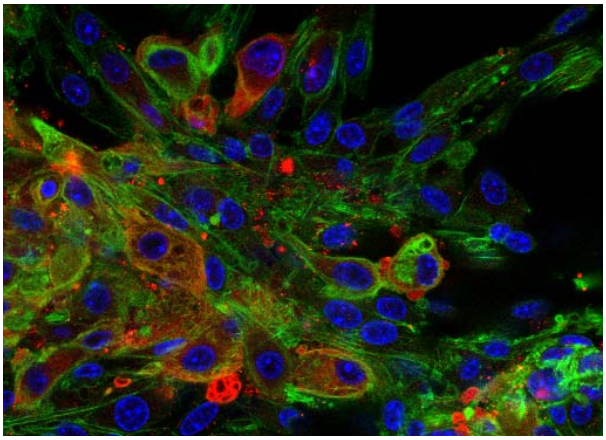

Figure 1: Fluorescent image of primary myoblasts cultured in matrix with 0.5% polysaccharide stained for actin (green), dapi (blue), and myosin heavy chain (red).

Conclusions: The incorporation of polysaccharides into a PEGDA hydrogel increases the fraction of bound water, thereby increasing the size of ice crystals created during freezing and porosity of the resulting porous matrix. The greater interconnectivity of the matrix facilitates a larger number of cells being incorporated into the matrix and a higher level of myogenic differentiation. This study implicates an important role of pore architecture and extracellular matrix on regulating emergent behavior related to myogenesis.

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