Characterization of Cell-Seeded PEGDA Hydrogels Adapted for Tissue Engineered Heart Valves

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Statement of Purpose: Current clinical options for valve replacement are plagued by a variety of problems, which inspires development of enabling technologies to create a tissue engineered heart valve (TEHV). Poly(ethylene glycol) diacrylate (PEGDA) hydrogel scaffolds permit greater biological and biomechanical customization than do non-woven mesh scaffold technologies. However, the material characterization of PEGDA hydrogels has been predominantly limited to compression and tension, as opposed to bending. Since large flexural deformations result in points of maximum stress in native valves as well as TEHVs, it is crucial to evaluate any potential scaffold material in this mode.

Methods: The effect of formulation parameters on the bending mechanics of cell-seeded, heparinized PEGDA hydrogels were investigated using a custom designed bending tester. Three molecular weights (3.4, 6, or 8 kDa) and 3 weight fractions (5, 10, 15 %, w/v) were subjected to three-point bending tests and the flexural stiffness was calculated. Valvular interstitial cells (VICs) were seeded into heparinized gels at 22 million cells/mL. Methacrylated heparin was used to functionalize the gels to maintain VIC phenotype. Network properties of the gels were calculated using Flory-Rehner theory to determine the mesh size, molecular weight between crosslinks, and mass swelling ratio of the hydrogels. Additionally, basic immunostaining was used to verify the preservation of VIC phenotypic markers when seeded in these gels.

Results: Manipulating the composition of the hydrogels resulted in flexural stiffnesses comparable to native tissues (15-220 kPa, Figure 1) with varied mesh sizes and swelling ratios. Hydrogels containing encapsulated valve cells, methacrylated heparin (Hep-MA), or both were less stiff than acellular hydrogels.

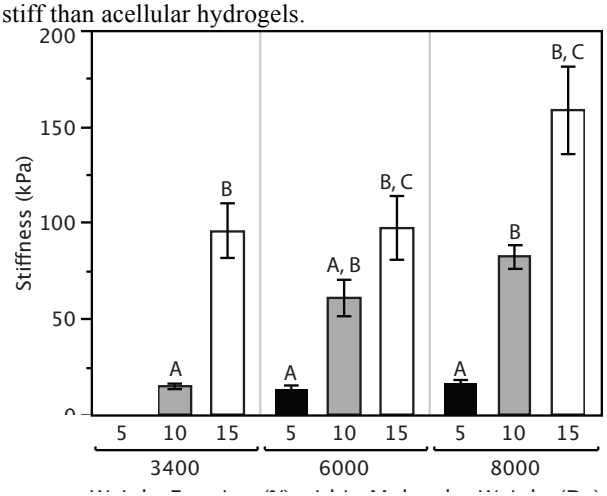

Figure 1. Flexural stiffness of Cell Seeded PEGDA Hydrogels with Crosslinked Hep-MA. Samples marked with different letters are significantly different at α=0.05. N=6 for all sample groups.

VICs seeded within gels with lower stiffnesses (5 and 10 %) showed more expression of alpha-smooth muscle actin than did those within gels of higher stiffness (15%, Figure 2).

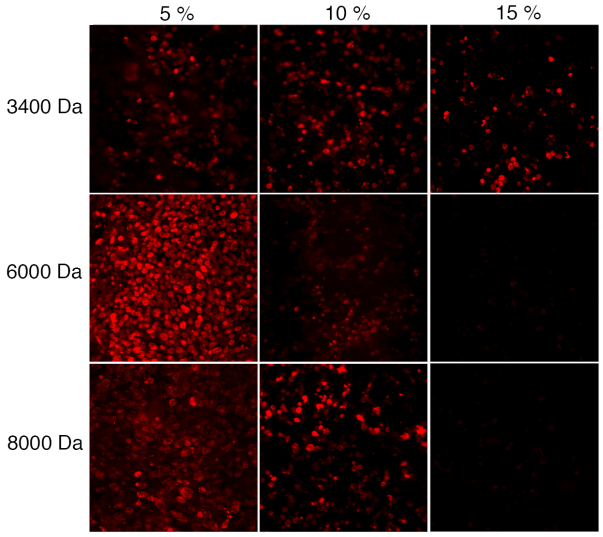

Figure 2. Representative immunostaining images for alpha-smooth muscle actin across all sample groups.

Conclusions: In conclusion, PEGDA hydrogels are an attractive potential scaffold system for TEHVs because they are not only cytocompatible and modifiable but can also withstand bending deformations. Network property data showed that inclusion of Hep-MA in the bulk of the gel resulted in disruption of hydrogel network, and these gels were less stiff in flexure than gels that contained solely PEGDA. Additionally, the incorporation of cells into the hydrogels profoundly decreased flexural stiffness. This study is the first to explore the encapsulation of valvular interstitial cells in pure PEGDA hydrogels as well as to investigate the bending properties of PEGDA gels.