3D Printing Heterogeneous Anatomically Accurate Hydrogel Heart Valves Using Collagen and PEGDA Interpenetrating Networks

Laura A Hockaday [1], Kevin H Kang [1], Nicholas Colangelo[1], Phillip Y-C. Cheung [1], Jun Wu [3], Hod Lipson[2], Chih-Chang Chu [3], Jonathan T Butcher [1]. Biomedical Engineering, Cornell University (1); Mechanical Engineering and Aerospace, Cornell University(2); Human Ecology, Cornell University (3)

Introduction:

Heart valves have complex shape with non-self supporting features and heterogeneous internal regions[1]. Engineered hydrogel scaffolds that duplicate these features have not been demonstrated[1,2]. Functionalized or composite polyethylene glycol diacrylate (PEGDA) and PEG hydrogels can be mechanically tuned and biologically tailored to induce patterned differentiation and cell phenotype[3,4]. Several studies highlight PEGDA hydrogels as potential biomaterials for valve tissue engineering[3], but a suitable fabrication approach to utilize these biomaterials for implants has not been demonstrated. The objective of this study was to evaluate our fabrication approach for printing PEGDA composite gels into complex and heterogeneous tissues. We have developed a systematic approach that combines image guided 3D tissue printing with localized photocrosslinking. With this we fabricated scalable anatomically complex cell-seeded living valved conduits. Collagen was combined with 8000 Da PEGDA and 700 Da PEGDA to make printable interpenetrating network hydrogels of different mechanical properties. The morphology and viability of porcine aortic valve interstitial cells (PAVICs) on the different hydrogels were assessed at day 1,2, 7, and 21.

Materials and Methods:

The Fab@Home™ Model 1 (ver. 0.23b) 3D printer was modified to incorporate three deposition syringe tools traversing along the X-Y axis and a custom 365nm UV-LED crosslinking system. PEGDA, Irgacure ® 2959, alginate, and collagen were combined in different ratios to make composite gels. Uniaxial tensile and confined compression tests were carried out on different hydrogel blends to quantify scaffold biomechanical tunability. MicroCT scans of porcine aortic valves were converted to a printable sterolithography model (STL). The model was imported into the Fab@Home™ program, which generated print paths. The printer then extruded hydrogel along the paths while the UV system cured the hydrogel. The hydrogel valves were scanned with MicroCT and the shape fidelity was evaluated. PAVICs were cultured on hydrogel scaffolds statically and in a shaker to demonstrate viability and morphology using LIVE/DEAD staining. Scaffolds were fixed and sectioned for histological staining to determine cell infiltration and matrix deposition.

Results:

Gels were tuned to exhibit a broad range of mechanical properties in strain-to-failure tests (stress_UTS=[5,250]kPa, max_strain=[0.24,2.1]) Figure 1A. By varying the ratio of high and low molecular weight PEGDA in printed scaffolds, the aggregate modulus could be tuned between 20kPa to 300kPa. Hydrogel valves were printed using the integrated

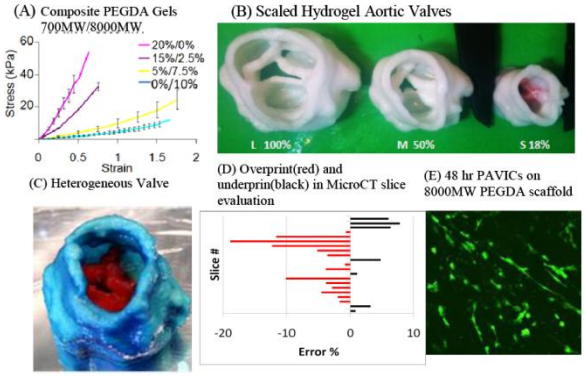

Figure 1: Mechanical Properties, Complex Printed Shapes, Shape Fidelity, and Scaffold Viability

photo-cross-linking system with 32mm, 22 mm, and 18 mm diameter bases Figure 1B. Spatially heterogeneous valves were printed with leaflets in a different material from the root, ostia, and sinus structures Figure 1C. Each valve was generated in less than 30 minutes. Slice by slice comparison of a MicroCT scan of a printed scaffold to the original model shows regions of over print (red) and underprint (black) Figure 1D. 100% and 50% scale valves had 85% to 90% average spatial accuracy when evaluated using MicroCT slice comparisons. 18% scale valve prints had significantly lower accuracy. After 2 days, PAVICs seeded onto scaffolds containing 8000MW PEGDA and collagen were almost 100% viable and showed spread morphology Figure 1E. When PAVICs were seeded onto printed hydrogels, viability increased if alginate (used as a viscosity modifier) was first leeched out of the scaffold. PAVICs were viable on valve scaffolds 7 days after surface seeding.

Discussion and Conclusions:

This study demonstrated the capability of a Fab@Home photo-crosslinking integrated system to fabricate hydrogel valves with complex anatomical features with high degree of shape fidelity. Live/Dead staining of composite gels show that collagen improves cell spreading. Collagen/PEGDA hydrogel is a potentially useful material for tissue engineering valve constructs. Two part mechanical heterogeneity was incorporated into valve scaffolds, but consequences of the heterogeneity (which we hypothesize is very important) has yet to be evaluated.

References:

1. Sacks M. On the biomechanics of heart valve function. J Biomech. 42,1804,2009.
2. Schmidt D, Hoerstrup SP. Minimally-invasive implantation of living tissue engineered heart valves. *J Am Coll Cardiol.* 56, 510, 2010.
3. Stephens E, Grande-Allen K. Mitral valvular interstitial cell responses to substrate stiffness depend on age and anatomic region. *Acta Biomater*. 2010.
4. Benton J, Anseth K. Characterization of valvular interstitial cell function in three dimensional matrix metalloproteinase degradable PEG hydrogels. *Biomaterials* 30, 6593, 2009.

Acknowledgments: National Science Foundation, Hartwell Foundation, Morgan Family Foundation, GAANN Fellowship