Fabrication and Mechanical Evaluation of Anatomically Inspired Advanced Hydrogel Structures

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Statement of Purpose: Many traditional hydrogel scaffolds are being investigated for use in heart valve tissue engineering. Fibrin, collagen, hyaluronic acid and synthetic gels, such as poly(ethylene glycol) diacrylate (PEGDA), are being investigated to create a tissue engineered heart valve (TEHV). This class of materials is interesting because they are intrinsically biologically compatible and permit the rapid encapsulation of cells in a cytocompatible environment. Furthermore, synthetic hydrogels can be modified extensively to tune their biochemical and biomechanical characteristics. However, these hydrogels do not possess sufficient mechanical strength for use in animal models or clinical applications of heart valve replacement, Furthermore, a PEGDA hydrogel is a fundamentally elastic, isotropic material, while heart valve leaflets are viscoelastic and demonstrate anisotropic material behavior. These unique mechanical characteristics of the valve leaflet allow the valve to undergo many loading-unloading cycles and a complete reversal of curvature upon opening. Furthermore, the layered structure of the leaflet is believed to aid in shielding the cells from high stresses. We have developed methods to fabricate quasilaminate structures with each layer having distinct cellularity and material properties. The resulting gels have different bulk material properties than single-component, single-layer (slab) gels.

Methods: Multilayer quasilaminate composites were created by first polymerizing a slab layer of PEGDA in a glass mold, then increasing the space in the mold, and injecting a second, different prepolymer solution to the mold. This second prepolymer was allowed to diffuse into the slab for 0.5-16 hrs and was then photocrosslinked. This procedure was iterated to create trilayer quasilaminates. Interface strength was tested by creating two-component quasilaminates and cutting out a dogbone shaped sample in which the interface was located in the center of the neck. These samples were then mechanically tested in uniaxial tension. Flexure tests of acellular and cellular "stiff-soft-stiff" trilayer structures were performed using a custom three-point bending tester. Cellularized quasilaminate structures were prepared with aortic valvular interstitial cells (VICs) located in the interior "soft" layer. Live/Dead staining was used to verify that, after gel fabrication, the cells were still viable.

Results: Interfacial tensile testing demonstrated that hydrogel failure stresses were greater than 100 kPa, and that failure did not occur at the interface. This pattern of failure was observed for quasilaminate hydrogels generated with secondary prepolymer diffusion times as low as 30 minutes. Flexure tests demonstrated that trilayer quasilaminates had altered bending stiffnesses in comparison with single layer bulk testing. Lastly, Live/Dead staining showed that VICs remained viable throughout the fabrication process.

Conclusions: These studies demonstrate methods for fabricating quasilaminate hydrogel structures with distinct cellularity and material properties in each layer. These techniques are inspired by anatomic structures, and can be applied to generate engineered versions of diverse layered structures, including but not limited to heart valves.

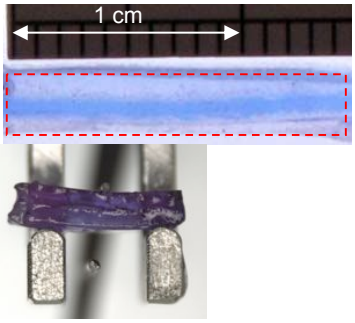

Figure 1. Photographs of trilayer quasilaminate PEGDA hydrogels. In top photo, inner layer (purple) is a stiffer composition than outer two layers (clear). In lower photo, a trilayer quasilaminate is loaded onto the bendinf tester

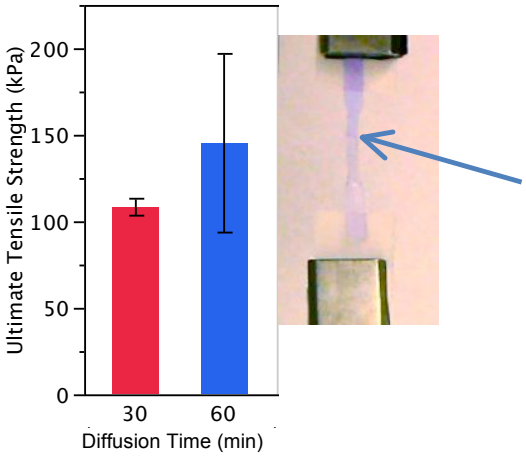

Figure 2. Left) Ultimate tensile stress of two layer quasilaminates tested to failure. Right) Photograph of two layer quasilaminate after dogbone shaped sample has been cut. Interface is visible in the middle of the thin sample neck.