Robust Approaches to Interface External Tubing to Freestanding Microfluidic Hydrogels

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Statement of Purpose: The fabrication of 3D cell-laden tissue constructs is among the forefront of emerging bioengineering technologies, with considerable potential to facilitate scientific discovery and revolutionize regenerative medicine. The development of three-dimensional tissue scaffolds approaching physiological scale, however, requires incorporating means to accommodate increasing cellular metabolic demands no longer adequately supported by diffusion. Perfusable 3D hydrogel scaffolds promote continuous nutrient/metabolite exchange, and, although facile and cell-compatible fabrication is still a substantial engineering challenge, significant progress is being made in this field.

While substantial effort has been devoted to optimizing microfluidic interconnect technologies for devices formed in rigid and elastomeric materials, to date there has been no effort devoted to establishing robust connections between freestanding microfluidic hydrogels and external pumping systems. Due to the fluidic resistance of the microfluidic network within such vascularized engineered tissue constructs, a mechanically robust, cell-friendly interface to external tubing is critical for reliable implementation. We have developed a range of techniques to connect tubing to fabricated channels in hydrogels. The optimization of such interconnect systems will further enable exciting developments in microfluidic cell-laden hydrogels.

Methods & Materials: We conducted a thorough battery of tests to determine an optimal fluidic interface assembly capable of withstanding the pressures produced during perfusion of high fluidic resistance microchannels. We sought water-tight, mechanically robust interface modalities that did not reduce cell viability. In addition to microchannel network devices, single-channel test devices were produced as a simplified model of pressures generated by stark reduction in channel diameter between the large inlet channel and microchannel network. We used intersecting needles of 2.8 mm and 300 μm diameter, resulting in a single channel with a 10 fold diameter reduction. Interface modalities subjected to testing are referred as: fish glue, dopa-glue, dopa-fish glue, fibrin glue, barb, and tubing friction fit. Superglue (Loctite 4641), barb fittings (Upchurch) and 0.125"OD silicone tubing (VWR) required no preparation. Fish glue was composed of 1:2 30% high molecular weight fish gelatin (Norland) and 60% transglutaminase (mTG, Moo Gloo TI, Modernist Pantry). Dopa-glue[1] was prepared using 1μg/ml tyrosinase (Sigma) and 100 mM $FeCl_3$ (Sigma) in 10% gelatin. Bovine fibrinogen and thrombin were combined (1:1) to yield fibrin glue. Adhesives were applied to the hydrogel surface to secure NanoPort (Upchurch) fittings. Up to two additional layers of adhesive were applied to the outer surface of the port interface.

Test device compositions included gelatin/mTG (7%, 10%, or 12% w/v), 3% (w/v) alginate (FMC BioPolymer), or PDMS (Sylgard 184, Dow-Corning). Microchannel devices were fabricated using sacrificial melt-spun fibers cast in 10% gelatin/mTG.[2] Burst pressure measurements were obtained using a pressure sensor connected in series with a syringe-pump (NE-300, New Era Pumps). Tensile measurements were obtained using an Instron 5944 at a vertical extension rate of 2mm/min. Cytocompatibility assays involved GFP-expressing HUVECS (Angio-Proteomie) cultured in channels and observed for 72 hours using confocal microscopy (LSM710, Zeiss).

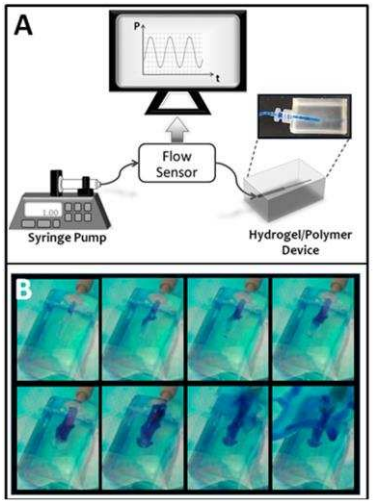

Figure 1. Testing fluidic interconnects. (A)Burst pressure setup (B) Example experiment

Results & Discussion: Fibrin and dopa-glue lacked the structural robustness for complete mechanical assessment and were thus excluded. Superglue, a strong, rapidly curing adhesive, proved unsuitable due to its cellular toxicity. While both barb and friction fit connections were promising options, hydrogel fragility, swelling propensity, deformation, and degradation over time threatened long-term integrity. Fish glue was both structurally robust and compliant to environmental changes, however, the assembly required up to 30 minutes, inducing cell stress, thus threatened cell viability. Incorporation of dopa-glue to initially stabilize port attachment reduced assembly times by up to 30%, greatly enhancing the overall success of the microfluidic system.

Conclusions: We identified a novel combination of L-DOPA modified porcine gelatin and fish gelatin adhesives as an optimal medium for attaching external tubing to freestanding hydrogels. Not surprisingly, material composition was most influential in the connective strength. However, connective efficacy was also largely dependent upon flexibility, long term integrity, and cytocompatibility of the interfacing technique. This report highlights the importance of selecting the appropriately tailored components and adhesives for fluidic hydrogel systems.

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References:

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