Fabrication of Freestanding Alginate Microfibers and Microstructures for Tissue Engineering Applications

John M. Szymanski, and Adam W. Feinberg
Carnegie Mellon University, Pittsburgh, PA

Statement of Purpose: Interest in polysaccharide hydrogels such as alginate has spurred the development of new approaches to engineer these materials at the micro- and nano-scales to better control cell interactions. Current methods such as electrospinning and micromolding are able to fabricate alginate fibers and structures at these dimensions, but are limited in terms of fiber diameters and scaffolds architectures that can be obtained. In this study, we developed a method to fabricate freestanding alginate-based microfibers and microstructures with improved geometric control. This was accomplished by using micromolding in combination with a sacrificial release surface. With this approach we were able to precisely control the morphology of the engineered alginate microfibers and microstructures down to ~1 μm in XYZ. Since alginate is inherently non-adhesive to cells and serum proteins, we also engineered alginate-fibrin microfibers to create freestanding, bioactive scaffolds that supported cell adhesion and directed uniaxial cell alignment.

Methods: Alginate microfibers and microstructures were fabricated using a surface-based assembly process. First, a droplet of alginate solution in DI water was deposited on a poly(N-isopropyl-acrylamide) (PIPAAm) coated coverslip heated to 40°C. Next, the alginate was micromolded by pressing a topographically patterned polydimethylsiloxane stamp onto the alginate solution and kept in conformal contact with the PIPAAm surface until the alginate dried. The stamp was then removed and the alginate was crosslinked by hydration in 40°C calcium chloride solution. Cooling the solution to room temperature caused the dissolution of the thermally sensitive PIPAAm surface and the nondestructive release of the micromolded alginate. Alginate-fibrin microfibers and microstructures were fabricated by first blending alginate and fibrinogen prior to micromolding and adding thrombin in addition to calcium chloride to the crosslinking solution. Cell adhesion was evaluated using the C2C12 cell line cultured under standard conditions and fluorescently stained for the nuclei (DAPI) and the actin cytoskeleton (phalloidin).

Results: To demonstrate the tunability of this system we engineered alginate and alginate-fibrin microfibers with uniform dimensions as well as more complex microstructures. As proof-of-concept, 30 μm wide, 3 μm thick alginate-fibrin microfibers showed high fidelity in the pre-release state after micromolding and floating in solution after thermally-triggered release (Fig 1). Use of the PIPAAm layer was deemed critical to the nondestructive release of these thin hydrogel materials. Similar micromolding and release of a star-like microstructure demonstrated the ability fabricate unique scaffold geometries. While the pure alginate microfibers did not support cell adhesion, the alginate-fibrin blends supported cell attachment and alignment of C2C12 myoblasts along the microfiber.

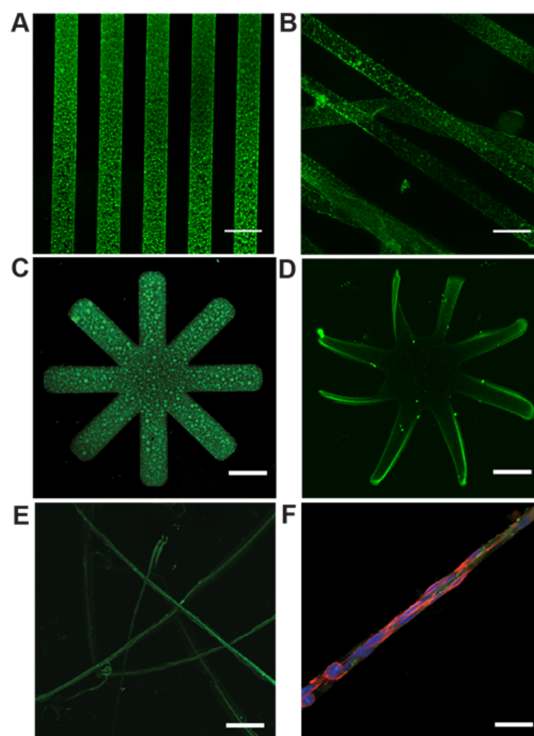

Figure 1: Micromolded alginate-fibrin microfibers (A) prior to release and (B) after thermally triggered release. An example of a multi-arm alginate star (C) pre-release and (D) post-release. (E) Pure alginate microfibers were unable to support cell adhesion. (F) alginate-fibrin microfibers supported cell adhesion and uniaxial alignment. Labeled for green (fluorescent alginate), blue (cell nuclei) and red (F-actin). Scale bars are 50 μm.

Conclusion: We have developed a method to fabricate alginate-based microfibers and microstructures with tunable geometries using surface-based assembly. Future work will use these as microstructured scaffolds to direct the C2C12 myoblasts to form skeletal muscle threads that can be engineered into functional muscle tissue constructs.

©2014 Society for Biomaterials