Biopolymer Hydrogel Microfibers with Internal Alignment via Electrospinning

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Statement of Purpose: Inducing cellular alignment on substrates with uniaxial alignment features is an important approach to control cell organization, tissue microarchitecture, and biological function.[1] Electrospun fiber meshes are a popular choice for generating aligned cellular substrates, but the 2D nature of these meshes limits their utility in regenerating 3D tissue constructs. Polymeric hydrogels have been widely used for 3D cell culture and tissue regeneration due to their tunable biochemical and physicochemical properties as well as their high water content, which resembles the aqueous microenvironment of the native extracellular matrix.[2,3] Here we report a new electrospinning strategy to generate hydrogel microfibers with internal alignment using aqueous biopolymer solutions, chosen for their biocompatibility, degradability, functionality, bioactivity, and ability to encapsulate cells during fiber generation, and examine the effect of internal alignment on the morphology and organization of encapsulated cells in 3D.

Methods: The hydrogel microfibers were electrospun using cell friendly aqueous solutions of biopolymer such as alginate, fibrin, gelatin, or hyaluronic acid in combination with polyethylene oxide as a viscosifier to maintain stability of the polymer jet. A low electrical potential of 3-5 kV was applied to the biopolymer solution, initially stretching of the polymer jet as it is extruded from the syringe tip towards the grounded rotating collection bath containing the crosslinking agent. Crosslinking agents used include: ionic crosslinkers such as Ca^{+2} for rapid fixation of alginate, thrombin for enzymatic crosslinking of fibrin, Michael addition using PEGDA to crosslink thiolated hyaluronic acid, and the combination of 365 nm wavelength UV light and photoinitiator (Irgacure 2959) to photocrosslink methacrylated gelatin microfibers. Control over fiber diameter can be achieved through alteration of biopolymer composition and changing the rotational velocity of the collector.

Results: The hydrogel microfibers prepared contain both high water content (>95%) and porosity characteristic of hydrogels in addition to uniaxial alignment along fiber axis (Fig. 1). Fibers from different biopolymers, including alginate, fibrin, gelatin, and hyaluronic acid can be prepared suing the same method. Due to the high degree of alignment, these hydrogel fibers exhibited higher tensile moduli than those non-aligned hydrogel fibers of the same geometry prepared in a mold with 500 µm diameter (Fig. 2). Isotropic hydrogels of the other biopolymers were too weak to mechanically test using the same geometry. Fibers show several orders of magnitude increase in modulus when dry compared to the initial wet state. After rehydration, fibers show an intermediate level of strength between the initial wet and dried conditions.

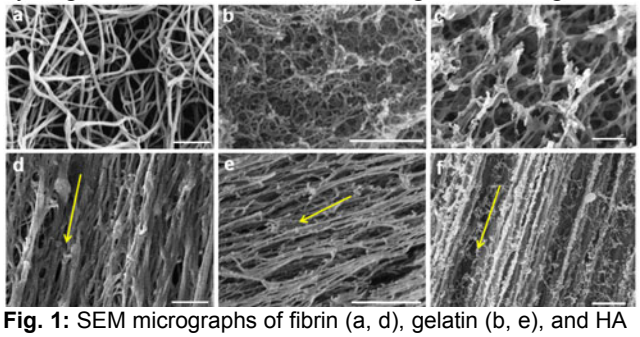

Fig. 1: SEM micrographs of fibrin (a, d), gelatin (b, e), and HA (c, f) showing microstructure of extruded isotropic hydrogel (upper row) and electrospun hydrogel fibers (lower row). Scale bars represent 1 µm in (a, b, d, e), 2 µm in (c, f).

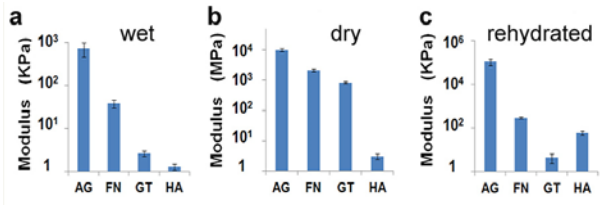

Fig. 2: Tensile moduli of alginate (AG), fibrin (FN), gelatin (GT), and HA hydrogel fibers in wet (a), dried (b), and rehydrated state (c). Error bars represent mean ± s.d. (n = 3).

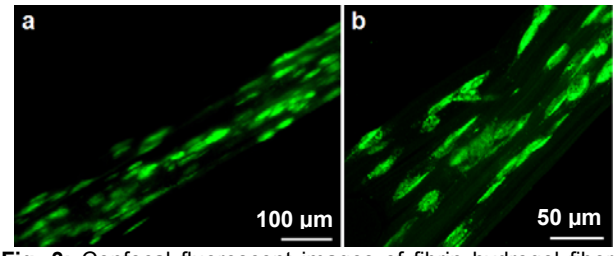

Fig. 3: Confocal fluorescent images of fibrin hydrogel fibers seeded with ASCs showing cellular alignment (labeled with CellTracker™ green CMFDA) induced by internal alignment cue after 2 d (a) and 5 d (b) of culture.

Adipose derived stem cells (ASCs) were encapsulated within dual component alginate-fibrin microfibers with high cell viability. Fibers were then treated with 1 U/ml alginase overnight following fiber formation to facilitate removal of alginate and increase hydrogel fiber porosity. Following 2–5 days of culture, cells showed robust proliferation and aligned along the fiber axis (Fig. 3).

Conclusions: A method for developing internally aligned hydrogel biopolymer microfibers was established. The variety of polymers compatible with the process allows the tuning of fiber mechanical properties. Cells encapsulated within the fibers have shown proliferation and alignment along the oriented microfibers, indicating its potential as an improved *in vitro* platform for investigating more complex cellular systems in 3D.

References:

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