Tunable Fibrous Hyaluronic Acid Scaffolds for Cartilage Tissue Engineering

Iris L. Kim, Matthew B. Fisher, Brendon M. Baker, Robert L. Mauck, and Jason A. Burdick
Department of Bioengineering, University of Pennsylvania, Philadelphia, PA, USA

Statement of Purpose: Electrospinning has recently gained much interest due to its ability to mimic the nanofibrous nature of the extracellular matrix [1]. While an abundance of materials have been electrospun, many of these systems lack versatility in important factors for tissue engineering applications, including mechanics, adhesivity, and growth factor delivery. Here, we electrospin modifications of hyaluronic acid (HA), a naturally occurring glycosaminoglycan, into swollen gel fibers. Unlike many other electrospun materials, HA is electrospun from a water-based solution, which is important for retaining activity of sensitive growth factors, such as TGFβ3. In this work, we demonstrate the tunability of fibrous HA hydrogels, with precise control over mechanics and adhesivity and, through both *in vitro* and *in vivo* studies, sustained release and bioactivity of encapsulated TGFβ3.

Methods: Methacrylated HA (MeHA) (both 35% modified and 100% modified) were synthesized as described in [2] and then coupled to RGD through a Michael addition reaction. HA-based macromers were dissolved at 4 w/v% in 0.05% w/v I2959 and electrospun with a voltage of 22 kV, flow rate of 1.4 mL/hr, and a distance of 16 cm. Samples were then crosslinked with UV light. Fiber mechanics were assessed through atomic force microscopy, and three-point bending moduli of single fibers were calculated as described in [3]. To assess cell morphology, mesenchymal stem cells (MSC) were seeded onto fibrous scaffolds, and fixed, stained, and imaged after overnight culture in standard growth media. For TGFβ3 release studies, dual polymer fibrous scaffolds were created by separately electrospinning MeHA and hydroxyethyl methacrylate-modified HA (HeMA-HA); HeMA-HA was synthesized as described previously [4]. Three scaffolds were electrospun: MeHA only, dual polymer MeHA/HeMA-HA, and dual polymer MeHA/HeMA-HA with TGFβ3. For the latter group, 100 ug of TGFβ3 was loaded into the HeMA-HA solution prior to electrospinning. For *in vitro* release studies, samples were incubated at 37°C in 0.1 w/v% BSA, and releasate was collected at each timepoint. MSC pellets were cultured either with exogenous TGFβ3 (10 ng/mL) or dual polymer MeHA/HeMA-HA scaffolds with or without encapsulated TGFβ3, and GAG production was quantified after 2 and 4 weeks of *in vitro* culture to assess TGFβ3 bioactivity. For *in vivo* studies, 4-mm defects were created in the hind limb trochlear grooves of mini-pigs, and fibrous scaffolds were placed within the defects

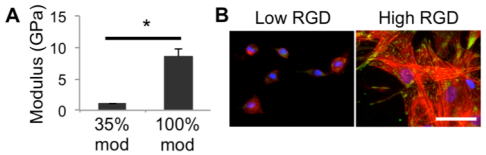

Figure 1. (A) Moduli of single 35% and 100% modified MeHA fibers, (B) MSCs seeded on fibrous scaffolds with varying RGD densities and stained for nuclei (blue), actin (red), and vinculin (green).

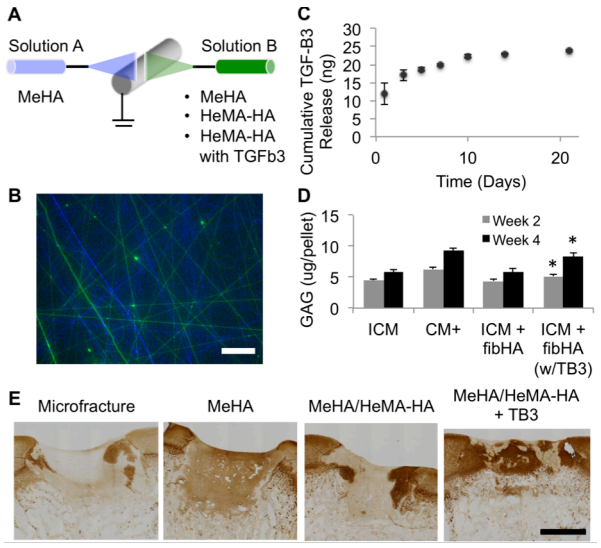

Figure 2. (A) Schematic of dual-jet electrospinning setup, (B) Fluorescent image of dual polymer MeHA (blue)/HeMA-HA (green) scaffold (scale bar = 50 μm), (C) Release profile of encapsulated TGFβ3, (D) Total GAG (μg/pellet) with exogenous or released TGFβ3 (* indicates statistical significance compared to ICM+fibHA group at same timepoint), (E) IHC for type 2 collagen in porcine osteochondral defects (scale bar = 2 mm).

directly after microfracture (n=5). Animals were sacrificed after 6 weeks, after which samples were harvested and processed for histology.

Results: Both the single fiber moduli and adhesivity of HA fibers were successfully controlled (Fig 1), and these variables had a significant impact on MSC chondrogenesis *in vitro* (data not shown). For TGFβ3 release studies, MeHA and HeMA-HA fibers (both with diameters of ~200 nm dry and ~800 nm swollen) were combined to produce scaffolds with long-term stability (MeHA) and sustained, full release of encapsulated factors (HeMA-HA). Dual jet electrospinning produced composite scaffolds with relatively equal amounts of both polymer fibers (Fig 2B). MeHA/HeMA-HA samples with encapsulated TGFβ3 exhibited a sustained release over 10 days *in vitro* (Fig 2C). Electrospun TGFβ3 retained bioactivity, as MSC pellets cultured with MeHA/HeMA-HA samples releasing TGFβ3 exhibited similar amounts of GAG production compared to MSC pellets cultured with exogenous TGFβ3 (Fig 2D). Importantly, this retained bioactivity was also translated to *in vivo* studies, with more intense staining for type 2 collagen within the MeHA/HeMA-HA with TGFβ3 group (Fig 2E).

Conclusions: This work demonstrates the versatility of fibrous HA-based hydrogels and then applies the system toward a specific application. Activity of released TGFβ3 was validated through both *in vitro* and *in vivo* studies. Ongoing work includes further tailoring and investigating these scaffolds through long-term *in vivo* studies.

References: [1] Mauck RL, et al. Tissue Engineering. 2009;15:171-193. [2] Burdick JA, et al. Biomacromolecules. 2004;6:386-91. [3] Kim IL, et al. Biomaterials. 2013;22:5571-80. [4] Tous E, et al. Biomacromolecules. 2011; 12:4127-35.