Collagenase Delivery from Multi-Polymer Fibrous Scaffolds To Promote Cellular Mobility and Meniscus Repair

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Statement of Purpose: Over one million meniscal surgeries are performed in the United States every year, where the most common treatment option following a meniscal tear is a partial meniscectomy [1]. Meniscal tissue removal, however, results in a proportional increase in contact stresses on the tibial plateau and predisposes the patient to osteoarthritis [2]. As with other dense fibrous connective tissues, the intrinsic healing of the meniscus is severely limited due to poor vascularity and hypocellularity. Previous research indicates that the high extracellular matrix (ECM) density in the mature meniscus serves as a physical hurdle to cell migration and proliferation, ultimately limiting endogenous repair [3]. Here, we use electrospun scaffolds to deliver collagenase, a matrix-degrading enzyme, in combination with a chemokine for enhancing cell mobility and recruitment, respectively. Compared to traditional hydrogels, electrospun scaffolds better model the fibrous nature of the native ECM and multiple jets can be used to finely control the release profiles of multiple biomolecules.

Methods: Fibrous scaffolds were synthesized through tri-component electrospinning with the following components: (1) 14.3 wt% poly(ε-caprolactone) (PCL) in a 1:1 mixture of DMF and THF; (2) 8 wt% poly(ethylene oxide) (PEO) in 50% ethanol; and (3) 4 wt% hydroxyethyl methacrylate functionalized hyaluronic acid (HA) with 2 wt% PEO and 0.05% w/v Irgacure 2959 in water. Electrospinning was performed using the following approximate conditions for each component (Fig 1a): (PCL) +13 kV, 2.0 mL/hr, 15 cm; (PEO) +13 kV, 2.5 mL/hr, 15 cm; and (HA) +27 kV, 1.4 mL/hr, 16 cm, corresponding to potential difference between spinneret and mandrel, polymer flow rate, and spinneret to mandrel distance. Distinct fiber fractions were imaged fluorescently by doping the PCL, PEO, and HA solutions with rhodamine, fluorescein, and 4',6-diamidino-2-phenylindole (DAPI), respectively. After electrospinning, fibrous scaffolds were immersed in PBS to study fiber stability and collagenase release over time, where the PEO fiber component was electrospun with and without 1500 U/mL collagenase type II. Collagenase release was quantified by measuring the fluorescence intensity of the release samples at 280/350 nm excitation/emission. Matrix metalloprotease (MMP) activity was evaluated using a fluorogenic peptide substrate assay. A second biomolecule to promote cellular recruitment, stromal cell-derived factor-1 (SDF-1), was encapsulated within the HA fiber fraction, where release was evaluated using ELISA. Ongoing studies are investigating biomolecule activity using previously described ex vivo models [3].

Results: Tri-component fibrous scaffolds were successfully electrospun and visualized fluorescently showing three distinct fiber fractions (Fig 1b). To probe collagenase release from the quickly dissolving PEO fiber fraction, PCL/PEO fibrous scaffolds were electrospun with and without collagenase. As expected the PEO fiber fraction completely dissolved in PBS within 24 hours, with a majority of the loaded collagenase releasing within one hour. In order to delay the release of collagenase, an extra PCL only layer was electrospun before and after electrospinning the PEO fiber fraction. Adding an extra PCL only fibrous layer resulted in a significant reduction in the initial burst collagenase release, where less than 20% was released in the first hour (Fig 2a). The in vitro MMP activity of the release samples was confirmed using a MMP-sensitive fluorogenic peptide substrate and corresponded well to release data. Furthermore, ex vivo studies indicated the delivery of collagenase from fibrous scaffolds promoted tissue integration using a meniscus-sandwich assay (Fig 2b). Preliminary results investigating SDF-1 release from the HA fiber fraction show controlled delivery over several weeks, with ongoing work evaluating bioactivity.

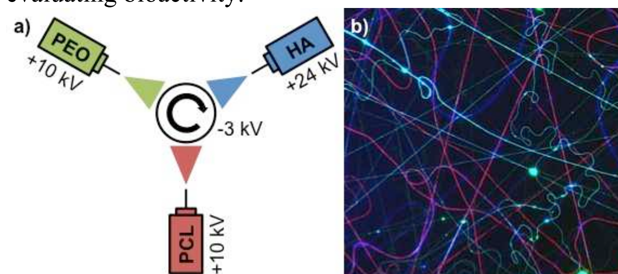

Fig 1. (a) Tri-jet electrospinning schematic. (b) Image showing three distinct fiber fractions: PCL (red), PEO (green), and HA (blue).

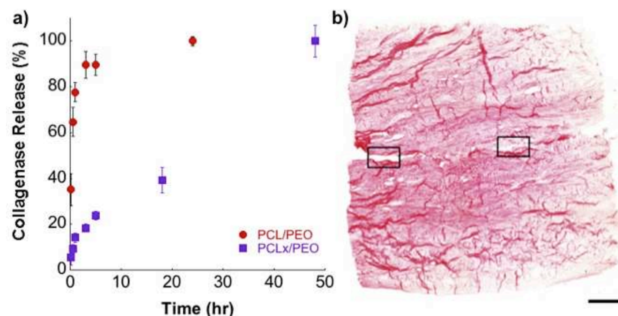

Fig 2. (a) Collagenase release from PCL/PEO fibrous scaffolds with and without an extra layer of PCL (denoted with x). (b) H&E staining showing improved integration after collagenase delivery using an ex vivo meniscus-sandwich repair model.

Conclusions: The combined delivery of collagenase and SDF-1 from electrospun fibrous scaffolds is a promising approach to promote tissue integration at the site of a meniscal tear, where the release profile of both biomolecules can be finely controlled individually. Ongoing work is evaluating the bioactivity of dual biomolecule delivery on cellular mobility and migration to the repair site.

References: [1]E.A. Khetia, B.P. McKeon. Sports Med Arthosc. 2007: 15, 114-20. [2]J.L. Cook. Clin Orthop Rel Res. 2005: 435, 88-95. [3]F. Qu, J.G. Lin, et al. Acta Biomaterialia. 2013: 9, 6393-402.