Incorporation of Sulfated Hyaluronic Acid Macromers into Fibrous Hydrogels for Sustained Molecule Delivery

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Statement of Purpose: Hyaluronic acid (HA) is a naturally occurring glycosaminoglycan that plays a critical role in many cellular pathways. Synthetically sulfated HA has been shown to bind heparin-binding proteins (HBP) with high affinity [1]. We have recently covalently modified and crosslinked sulfated HA into hydrogels for tunable release of HBPs [2]. Electrospinning has recently gained much interest due to its ability to mimic the nanofibrous nature of the extracellular matrix [3]. The resulting fibrous scaffolds have a high porosity and surface area to volume ratio; these properties may allow for increased cellular infiltration, which is especially important for many acellular therapies, in comparison to non-fibrous, uniform hydrogels. Here, we focus on electrospinning sulfated HA into hydrolytically degradable, fibrous hydrogels for controlled release of stromal cell-derived factor-1 alpha (SDF-1), a potent chemoattractive cytokine involved in regulating progenitor cell trafficking.

Methods: Hydroxyethyl methacrylate-modified HA (HeMA-HA) and sulfated HeMA-HA (HeMA-SHA) macromers were synthesized as described in [2]. Briefly, HeMA succinate (HEMA-COOH) was coupled to a tetrabutylammonium (TBA) salt of HA in the presence of 4-dimethylaminopyridine and di-tert-butyl dicarbonate to form HeMA-HA. For HeMA-SHA synthesis, a TBA salt of HeMA-HA was dissolved in DMF (0.25 w/v%) and reacted with a SO3/DMF complex (20:1 molar ratio). HeMA-HA and HeMA-SHA macromers were characterized through ^{1}H NMR, gel permeation chromatography, a modified dimethylmethylene blue (DMMB) assay (for sulfate content) [4], and zeta potential measurements. Blends of HeMA-HA and HeMA-SHA (100/0 HeMA-HA/HeMA-SHA and 90/10 HeMA-HA/HeMA-SHA) were dissolved at a final combined concentration of 4 w/v% with 2 w/v% polyethyleneoxide in 0.05% w/v I2959 in deI H_2O and electrospun with a voltage of 22 kV, a distance of 15 cm, and a flow rate of 1.4 mL/hr. The fibrous samples were then crosslinked under UV light and imaged while dry (SEM) and swollen (confocal microscopy). For confocal imaging, a methacrylated rhodamine dye (MeRho) was included prior to electrospinning. For SDF-1 release studies, SDF-1 was added to each polymer blend at a concentration of 1.67 µg/mL prior to electrospinning. Samples were cut from the mats (1.5x1.5cm) and incubated in 1 w/v% BSA at 37°C, and released SDF-1 was quantified using an ELISA (R&D Systems).

Results: Both HeMA-HA and HeMA-SHA macromers had a % HeMA modification of 15% and a number average molecular weight (M_n) of 80 kDa. Using the DMMB assay, a significant increase in sulfate content was observed with HeMA-SHA compared to HeMA-HA. Further, the sulfate content of HeMA-SHA was comparable to that of heparin (Figure 1A). The addition of sulfate groups enhanced the negative charge of HeMA-SHA macromers compared to HeMA-HA macromers, as observed with zeta potential measurements (data not shown). Electrospinning both HeMA-HA/HeMA-SHA blends resulted in fiber diameters of ~200 nm dry and ~700 nm swollen, with no significant differences between the two blends. When incubated with DMMB dye, fibrous samples with HeMA-SHA stained purple whereas samples without HeMA-SHA remained blue, indicating a significant difference in sulfate content (Figure 1D). To demonstrate controlled release, SDF-1 was encapsulated as a model HBP in HeMA-HA/HeMA-SHA blend fibrous scaffolds. SDF-1 release was significantly slowed in the 90/10 HeMA-HA/HeMA-SHA group (Figure 1C).

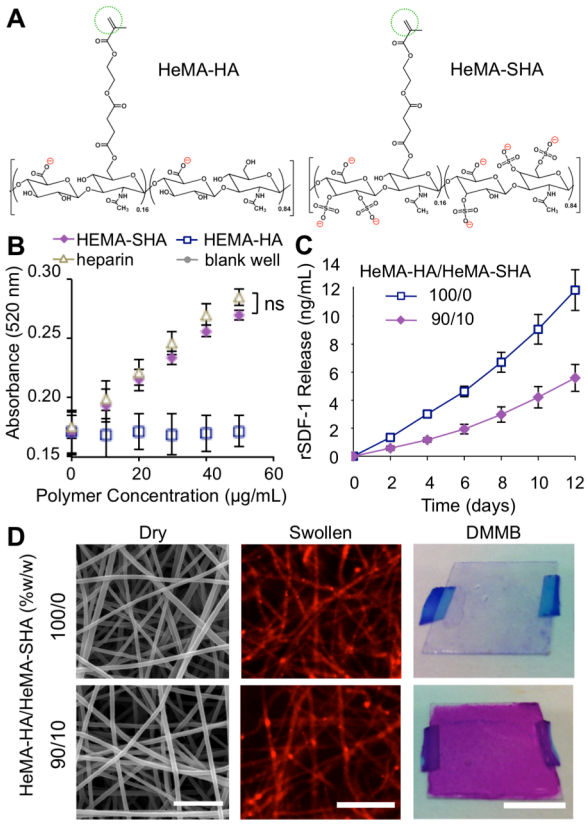

Figure 1. (A) Chemical structures of HeMA-HA and HeMA-SHA, (B) Sulfate content of HeMA-SHA and HeMA-HA macromers and heparin using a modified DMMB assay, (C) Release of encapsulated SDF-1 from electrospun HeMA-HA/HeMA-SHA blends, (D) Left column: SEM images of dry fibers (scale bar = 2 µm), Middle column: confocal images of swollen fibers with MeRho (scale bar = 10 µm), Right column: fibrous scaffolds stained with DMMB dye (scale bar = 1 cm).

Conclusions: This work demonstrates the ability to specifically control release of HBPs within a chemically versatile, HA-based fibrous scaffold. Ongoing work includes tuning the release profiles of SDF-1 along with other factors using multi-polymer systems, and investigating the synergistic effects of these growth factors on MSC migration and phenotype *in vitro*.

References: [1] Hintze V, et al. Biomacromolecules. 2009; 10:3290-97. [2] Purcell BP, et al. Biomaterials Science. 2013. In press. [3] Mauck RL, et al. Tissue Engineering. 2009; 15:171-193. [4] Farndale RW, et al. Biochimica et Biophysica Acta. 1986; 883 :173-77.