Controlling Stromal Cell-Derived Factor-1α Delivery through Norbornene Hyaluronic Acid Microgels

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Statement of Purpose: Stromal cell-derived factor-1α (SDF-1α; also known as CXCL12) is a potent chemotactic molecule that works in conjunction with its receptors CXCR4 and CXCR7 to promote central nervous system repair following injury [1–3]. SDF-1α has been shown to influence neural stem cell recruitment, angiogenesis, and inhibitory tone in the central nervous system [2, 4]. Exogenous delivery and manipulation of SDF-1α signal in the nervous system may allow for increased stem cell recruitment following traumatic brain injury, stroke, and other neurological diseases. Current efforts to alter the presentation of SDF-1α in the nervous system are limited by lack of tunability of SDF-1α release, its inherent short half-life, and the use of materials that contribute to inflammation. Advances in hyaluronic acid (HA) functionalization and thiol-ene click chemistry allows for precise cross-linking and molecule functionalization of hydrogels [5]. Here, we present an HA-based injectable, biomaterial microgel system that accommodates precise tuning and delivery of SDF-1α and SDF-1α analogs.

Methods: HA injectable microgels were generated with norbornene HA (NorHA) and a microfluidic flow-focusing device (MFFD) equipped with a UV light source downstream to crosslink microgels and encapsulate thiol-containing biomolecules [5]. Our design also incorporates a matrix metalloproteinase (MMP) cleavable peptide sequence (VPMSMRGG) to tether a SDF-1α peptide to NorHA (Fig 1). Specifically, the oil phase consisted of light mineral oil (Sigma) supplemented with Span80 (2% v/v; Sigma) and aqueous phase containing: NorHA (0.1% w/v), Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (0.05% w/v LAP; Sigma), MMP cleavable SDF-1α peptide (33ng/µl; Watson Bio), and dithiol (0.4 stoichiometric ratio to Norbornene; Sigma). Flow rates ratios between the oil and aqueous phase were kept 2:1. Here, we assessed the microgel size, degradation characteristics, and enzymatic release of SDF-1α peptide. Size distribution was evaluated via incorporating sulfo-Cy3 tetrazine (100uM; Fisher) into the aqueous phase to visualize the microgels under fluorescent microscopy. MMP mediated release of SDF-1α was examined by using an SDF-1α peptide coupled to a biotin molecule. Microgels were incubated at 37C with or without 200U/ml type IV collagenase (MP Biomedicals). Supernatants were collected after 48 hours.

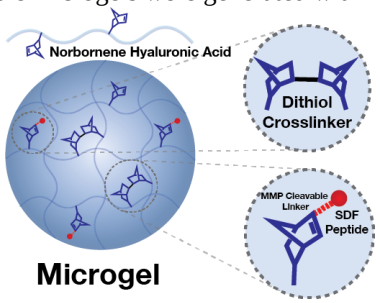

Fig 1: Norbornene HA microgels use free norbornene groups to tether MMP cleavable SDF-1a peptide.

and biotin levels quantified via a fluorescence biotin quantification kit (Thermo-Fisher). To assess microgel degradation, microgels were incubated at 37C with or without 0.01mg/ml hyaluronidase (Sigma). Supernatants were collected across 10 days and HA mass was quantified with a carbazole uronic acid assay.

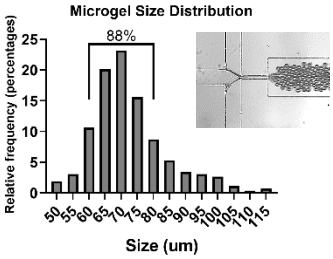

Fig 2: Microfluidic flow focusing device generates monodisperse microgels. Microgels are generated via flow focusing microfluidics (inset) where 88% of microgels fall within a 20µm size distribution.

Results: We readily generated monodisperse microgels less than 100µm (Fig 2). Furthermore, we established the capability to release biomolecules in an MMP enzyme (collagenase) dependent manner (Fig 3) within the first 48hrs of assessment. Studies underway will determine the 30-day release profile. We demonstrated the sensitivity of these microgels to hyaluronidase enzyme and their *in vitro* degradation characteristics (Fig 4). Detection of HA from microgels incubated without hyaluronidase may stem from microgel particulates persisting in the supernatant prior to sample collection.

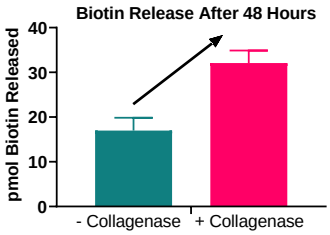

Fig 3: Addition of collagenase leads to release of biotin conjugated SDF-1a *in vitro*. n=3

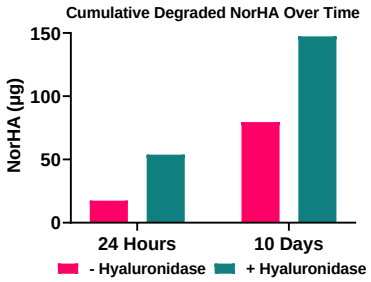

Fig 4: Microgels degrade in presence of hyaluronidase over time.

Conclusions: We successfully created <100µm, injectable, monodisperse, microgels capable of controlled delivery of SDF-1α. This size is critical as it allows for microgel injection via 26-gauge needle or larger, necessary for neural applications. Moreover, these microgels offer improved control of SDF-1α delivery as a tool for neural regeneration and repair.

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References:

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