Sustained Growth Factor Delivery from Hyaluronic Acid Hydrogels for Spinal Cord Regeneration

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Introduction: Spinal cord injury (SCI) is a debilitating medical condition that affects approximately 291,000 people in the US [1]. Current experimental therapies include cell transplantation of either differentiated neurons or, more recently, neural progenitor cells, along with the use of biomaterial platforms. The use of therapeutic agents to treat SCI is limited by the difficulty to reach the target tissue area at an appropriate concentration while keeping the drug biologically active. Neurotrophin-3 (NT-3) is a growth factor that has gained increased attention due to its ability to promote functional recovery *in vivo* by increasing axonal growth in rodent SCI models [2]. Biomaterials, and more specifically polymeric scaffolds can be chemically modified to mimic the host tissue and provide spatio-temporal control of drug release. These scaffolds can also be functionalized with biochemical cues to study cell behavior *in vitro*, including drug response and biological activity. The Sakiyama-Elbert lab has previously used a hyaluronic acid (HA) hydrogel to transplant astrocyte-derived extracellular matrix and mouse embryonic stem cell derived interneurons *in vivo* [3]. More recently, we have tuned this HA hydrogel system to allow for sustained release of bioactive NT-3 *in vitro*, providing a promising platform for drug delivery in the central nervous system.

Materials and Methods: Hyaluronic acid hydrogels were synthesized in two steps: the HA backbone was first reacted with methylfuran to functionalize it, followed by a Diels-Alder reaction using a bifunctional polyethylene-glycol maleimide crosslinker [4]. The molar ratio of methylfuran to maleimide was 1.5:1, 1:3 or 1:5. The gels were characterized chemically via NMR and mechanically using rheology. Spinal cord tissue samples were collected from rats and analyzed under the same conditions. Release studies were conducted loading the hydrogel with NT-3 and recovering released NT-3 in PBS buffer at several time points, followed by protein quantification using ELISA. The bioactivity of NT-3 was measured first using a dorsal root ganglion (DRG) assay, in which the neurite outgrowth of primary neurons harvested from chick embryos could be measured and correlated to the NT-3 concentration. Mouse embryonic stem cell derived V2a interneurons and Hb9 motoneuron aggregates were seeded on top and inside of hydrogels containing either laminin, gelatin or fibronectin and the axonal growth was also measured 48h after seeding.

Results and Discussion: Hydrogels were successfully synthesized, confirming the correct functionalization via NMR and ideal gelation kinetics using rheology. The gels formed in 15 minutes, and the final stiffness could be controlled by tuning the methylfuran to maleimide ratio to match that of native rat spinal cord tissue. The release of NT-3 into PBS was found to be strongly controlled by electrostatic interactions between the positively charged NT-3 and negatively charged HA backbone, leading to slow release of NT-3 along with trapping of most of the protein even after 28 days (Fig. 1). When a high salt content elution buffer was used to disrupt these ionic interactions, the amount of drug released was almost doubled. The biological activity of NT-3 released at 24 hours and 7 days following encapsulation was shown to be mostly retained, when comparing the neurite outgrowth of DRGs supplemented with either release samples or fresh NT-3. V2a interneuron and Hb9 motoneuron aggregates presented robust neurite outgrowth when seeded within the scaffolds loaded with NT-3 (Fig. 2). The addition of laminin to the HA gel enhanced the axonal growth while the effect of gelatin and fibronectin was not beneficial when compared to the non-functionalized gels.

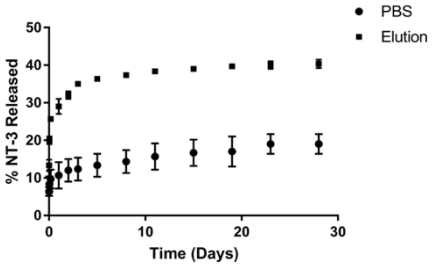

Figure 1. Buffer dependent release of NT-3 from HA hydrogels.

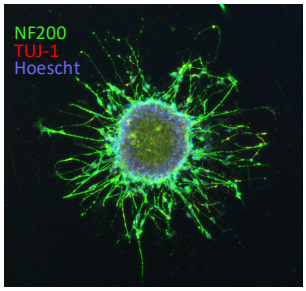

Figure 2: V2a interneuron aggregate seeded inside of the hydrogel.

Conclusions: We synthesized and tuned a HA-based hydrogel system for sustained delivery of NT-3 *in vitro* via affinity binding. The HA scaffold is injectable and gels in 15 minutes, presenting a promising platform to be used to treat SCI *in vivo*. The HA gel was optimized for both 2D and 3D culture of interneurons and motoneurons within the scaffold, suggesting it is a potential platform to study axonal growth *in vitro*. Future studies will focus on studying the effect of NT-3 loaded HA hydrogels in an *in vivo* model of SCI in rats.

References: [1] National SCI Statistical Center, UAB. 2019. [2] Baumann, M.D. *J. Control. Release.* 2009; 138, 205–213. [3] Thompson, R.E. *Biomaterials*. 2018; 162, 208-223. [4] Smith, L. J., *Biomacromolecules*, 2018; 19(3), 926-935.