The Angiogenic Role of Hyaluronic Acid Molecular Weight in Biomaterials for Spinal Cord Repair

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Statement of Purpose: There are currently no treatments that can fully restore function and regenerate tissue after spinal cord injury (SCI). This is due to the complex pathophysiology of SCI. The primary injury is the initial mechanical insult. This is followed by the secondary injury which presents several barriers to functional repair that need to be addressed such as chronic inflammation and glial scar formation. The injury also induces enzymatic degradation of the spinal cord extracellular matrix (ECM), which is rich in high molecular weight hyaluronic acid (>300 kDa, HMW HA). Despite its relatively simple structure, the MW of HA has profound effects on its bioactivity[1,2]. HMW HA in the intact spinal cord acts to maintain quiescence of neural stem cells, astrocytes and immune cells and established neuronal circuits through its presence in perineuronal nets[1–3]. After injury, native, HMW HA is degraded into low MW (LMW) fragments (<200 kDa) that initiate the wound healing response. As healing progresses, the HMW HA ECM re-establishes resolving the inflammatory response. In cases of chronic inflammation, like after SCI, the wound healing process essentially stalls here[2]. HA hydrogels, a promising method for SCI treatment, have been shown to significantly reduce secondary injury. Although HA hydrogels have shown promise in laboratory studies, the MW-dependent functions of HA have yet to be fully explored. One major limitation of any biomaterial strategy is vascularization of the regenerated tissue. Vascularization of biomaterials has been shown to improve SCI outcomes. Endothelial cells (EC), the main building blocks of blood vessels, experience MW-dependent bioactivity in response to HA, where LMW HA promotes EC proliferation and migration, activities inhibited by HMW HA. This study aims to thoroughly characterize the HA MW-dependent bioactivity of human brain microvascular endothelial cells (HBMVECs), and how this bioactivity is affected when incorporating HA into annealed macroporous, microparticle scaffolds (AMMS) made up of annealed HA microparticles. AMMS have been shown to promote increased tissue integration after implantation[4].

Methods: HBMVECs were purchased from Applied Biological Materials (ABM, T0259), and used in experiments between passages 6-9. HA was purchased from Lifecore Biomedical in the four following MW ranges: 10-20 kDa (10K), 41-65 kDa (40K), 100-150 kDa (100K), and 750-1000 kDa (1M). For migration studies, cells were seeded into 2-well wound healing inserts (Ibidi) with a 500 µm gap. Once attached, inserts were removed, and cells were cultured in complete Prigrow I (Prigrow Comp, ABM) or 1 mg/ml HA in Prigrow I. Cell migration was tracked for 36 hours using an IncuCyte S3. For Tube Formation studies, HBMVECs were seeded onto 10 µL of Geltrex™ in complete EC Media (ECMC, ScienCell) or 1 mg/ml HA in ECMC without the EC growth supplement and observed for 18 hours using time lapse microscopy.

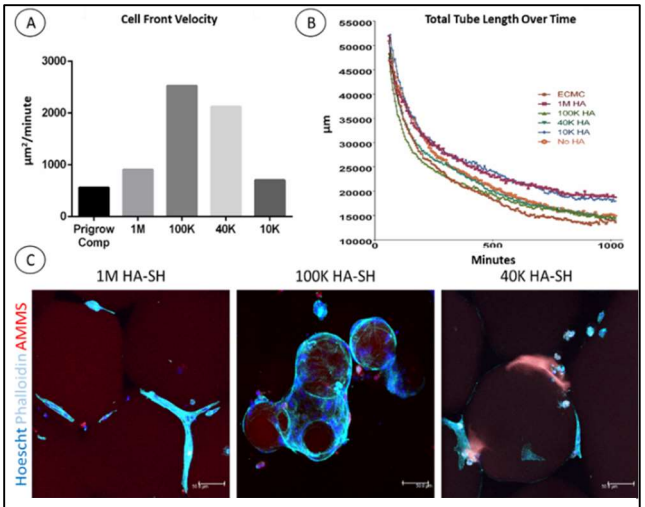

Figure 1: HBMVECs were cultured in 2-well wound healing inserts with a 500 µm gap and allowed to migrated for 36 hours (A). HBMVECs were cultured on Geltrex™ and allowed to form tubes for 18 hours (B). HBMVECs were cultured for 2 days in AMMS made from 1M, 100K, and 40K HA-SH. Vessel morphology was characterized using Hoescht (nuclei) and phalloidin (actin cytoskeleton) (C). Scale bar = 50 µm.

Image sets for migration and tube formation were analyzed using the FastTrack AI software (MetaVi Labs). To form AMMS, thiolated HA (HA-SH) was made by replacing carboxyl groups with free thiols using EDC/NHS chemistry and cysteamine[4]. HA-SH was then crosslinked with 4-arm vinyl sulfone-terminated polyethylene glycol modified with 0.3 mM RGD-SH and 10 uM Flamma 552-SH, for visualization. AMMS are formed by flowing these two solutions through a step-emulsification microfluidic device and then incubating particles at 37°C to anneal[4,5]. HBMVEC morphology in 3D AMMS cultures was captured using a Leica SP5 confocal microscope.

Results: In our migration studies, 1M and 10K HA exhibited 2-3x slower cell front velocities (µm²/minute, Fig 1A) than 100K and 40K, but maintained greater total tube lengths (µm, Fig 1B) over an 18-hour period than 100K and 40K. HBMVECs in AMMS made with 1M HA-SH exhibited longer, more nucleated vessels with more branch points, while HBMVECs in AMMS made with 100K and 40K HA-SH formed less and shorter vessels.

Conclusions: Since LMW HA promotes EC proliferation and migration, activities inhibited by HMW HA, and HMW HA is required for full wound repair, we hypothesized HMW HA treatment would decrease migration speeds, but increase tube formation in both solubilized and biomaterial formats, while LMW HA would induce the opposite responses. Our results partially supported this hypothesis.

References: [1] Sherman LS. Int. J. Cell Biol. 2015;2015:368584. [2] Gaudet AD. Exp Neurol. 2014;258:24-34. [3] Su W. Matrix Biol. 2019;78-79:272-283. [4] Ehsanipour A. APL Bioeng. 2021;5:16104.