Effect of Hyaluronic Acid Molecular Weight on Viscoelastic Properties and Glioblastoma Invasion

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Statement of Purpose

Hyaluronic acid (HA), the primary component of brain extracellular matrix, is increasingly used in culture models of brain disease, such as the invasion of the deadly brain tumor glioblastoma (GBM).[1] Current HA platforms typically differ in two key respects from HA networks in the brain. First, current platforms are typically composed of low-molecular weight (LMW) HA, whereas brain matrix is rich in higher-MW HA species. This MW difference is in turn expected to strongly influence bioactivity.[2] Second, in vitro HA networks are often formed by covalent crosslinking, giving rise to highly elastic rather than viscoelastic networks, where viscoelasticity has shown to influence 2D cell migration.[2-3] Here we introduce new hydrogel matrices formed from either LMW or HMW HA to investigate the effects of HA MW on hydrogel viscoelastic properties and the 3D invasion of GBM cells.

Methods

We created hydrogels from either 60 kDa (LMW) or 1.5 MDa (HMW) methacrylated HA polymers crosslinked via Michael Addition with di-thiol species, such as DTT or an MMP-degradable peptide sequence capped with a cysteine on each end. To characterize the mechanical properties of the different gels, we performed dynamic rheology measurements, including frequency sweeps (50 – 0.5 rad/s at 0.1% strain) and stress relaxation measurements (15% strain). We also encapsulated GBM U87 tumor spheres in 3D hydrogels and used phase contrast imaging to observe differences in invasive phenotypes.

Results

We first measured bulk rheological properties of our hydrogels. First, we matched the storage modulus of the HMW and LMW hydrogels to be similar to one another and native brain tissue (100-1000 Pa) by modulating crosslinker density.[1] The HMW hydrogels exhibited greater viscoelastic character than LMW hydrogels, including greater stress relaxation capabilities (Fig 1). The moduli were also strongly frequency-dependent (not shown), as expected for a viscoelastic material and consistent with past measurements of whole brain tissue. We next investigated 3D cell migration within these hydrogels using a spheroid invasion assay. U87 GBM tumor spheres infiltrated HMW HA hydrogels more rapidly and diffusely than LMW HA hydrogels, with spheroids in LMW HA remaining circumscribed even after 5 days (Fig 2).

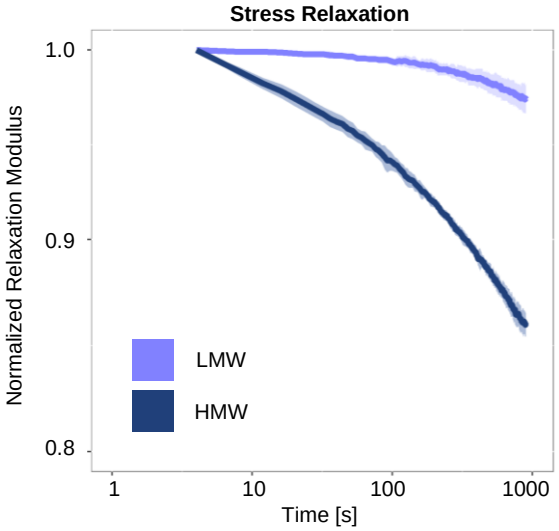

Figure 1: HMW HA gels exhibit faster stress relaxation than LMW HA gels (n=4)

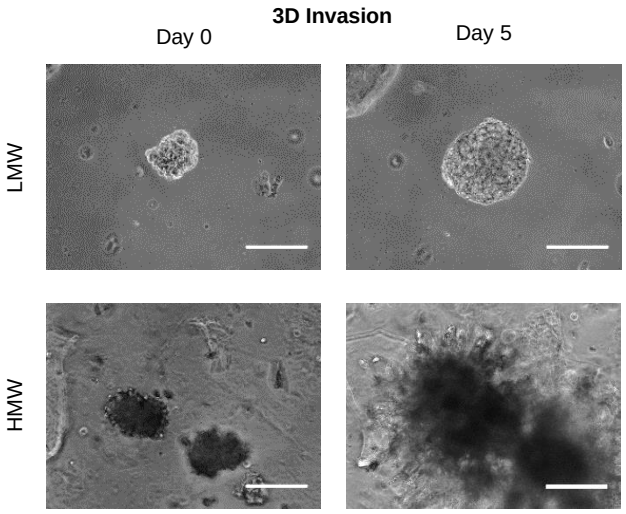

Figure 2: U87 GBM cells invade HMW HA hydrogels more rapidly and diffusely than LMW HA hydrogels (scale bar = 200 μm)

Conclusion

Traditional HA hydrogel models of brain tissue based on crosslinked LMW HA often fail to capture the HMW HA-rich and viscoelastic nature of native brain tissue. Here we show that hydrogels composed of HMW HA exhibit much greater viscoelastic character than LMW HA hydrogels as measured by stress relaxation. Moreover, HMW HA hydrogels support much more rapid and diffuse 3D invasion of GBM tumor cells than do LMW HA hydrogels. Our results support deeper exploration of the mechanisms linking HA MW to 3D invasion and argue for greater consideration of HA MW in culture models of brain matrix.

References

[1]Wolf et al., *Nat. Rev. Mat.*, 2019
[2]Wolf and Kumar, *ACS BioMat. Sci. & Eng.*, 2019
[3]Chaudhuri et al., *Nat Mat.*, 2016