Driving Oligodendrocyte Progenitor Cell Differentiation in 3D Hydrogels via Biomaterial Stiffness and Topographical Cues

Rachel A. Mazur[1], Hannah E. Hockensmith[1] and Kyle J. Lampe[1]
[1]Department of Chemical Engineering, University of Virginia, Charlottesville, VA 22903

Statement of Purpose: Therapeutically regenerating myelin may ultimately address and treat demyelinating disorders and injuries. However, a knowledge gap persists in determining factors that influence oligodendrocyte (OL) differentiation and myelin formation. Environmental cues such as matrix stiffness influence the morphology and proliferation of oligodendrocyte progenitor cells (OPCs) encapsulated in 3D hydrogels[1]. We encapsulated OPCs within norbornene-functionalized hyaluronic acid (NorHA) hydrogels at storage moduli representative of native brain tissue (G' = 200-2000 Pa). Since the topographical cues of native axons serve as important features in driving OPC differentiation into oligodendrocytes,[2] we investigated the effects of co-encapsulated axon-mimicking HA fibers in the 3D hydrogel on OPC fate.

Materials and Methods: Hyaluronic acid (HA) was functionalized with norbornene groups to allow radical-mediated crosslinking in the presence of dithiothreitol (DTT) crosslinker. Shear rheology was used to quantify material properties of 1.5 wt% NorHA gels through on-stage gelation during UV exposure in the presence of LAP photoinitiator. Methacrylated hyaluronic acid (MeHA) fibers were electrospun from a solution of 2 wt% MeHA, 3 wt% polyethylene oxide (PEO), and I2959 photoinitiator, crosslinked under UV light, and rehydrated in phosphate buffered saline (PBS). Fiber mats were broken up into individual fibers by passing repeatedly through first an 18 gauge, then a 23 gauge needle. GFP+ MADM OPCs were encapsulated in 1.5% (w/v) NorHA gels containing 1 wt% fiber (experimental group) or no fibers (control group) and cultured at 37C for up to 7 days. Gels at each timepoint were incubated in EdU solution for one hour to stain for newly synthesized DNA, followed by fixing and staining with DAPI to detect nuclei. Z-stack confocal images were analyzed to determine the effects of fiber co-encapsulation on OPC proliferation. ATP/DNA assays assessed changes in encapsulated cell viability and metabolic activity, associated with cell death and proliferation, respectively.

Results: NorHA gels demonstrated storage moduli of 1395 Pa (1.5 wt%), which accurately reflects intermediate conditions in the range of native brain tissue stiffness. Stiffness was not significantly affected by the inclusion of electrospun fibers at 1 wt%. Confocal imaging of gels stained with DAPI and EdU revealed that cells in fiber-free, amorphous control gels exhibited high rates of proliferation over the course of 7 days, with a peak in EdU signal at culture day 4. Cells also began to clonally expand and grow as large multicellular spheroids, with proliferation occurring at a particularly high rate within these cell clusters. These cells within fiber free gels tended to retain an immature, rounded morphology. This indicates that OPCs encapsulated in NorHA tend to proliferate, but not differentiate, in the absence of topographical cues. By contrast, cells encapsulated in gels containing 1 wt% electrospun fibers exhibited consistently lower proliferation rates across all timepoints, with a roughly two-fold lower rate of proliferation at culture day 7. Cells encapsulated in fiber-containing gels extended multiple processes, dramatically maturing in their morphological appearance. Process extension in OPCs is associated with progression towards differentiation, also reflected in the downregulation of proliferation and EdU incorporation in the fiber-free samples. Therefore, the incorporation of topographical cues from electrospun fibers appears to drive differentiation of OPCs at the expense of cell proliferation. At day 7, ATP/DNA ratio was roughly 4-fold higher in fiber-free gels as compared to fiber-containing gels. This indicates a higher level of average metabolic activity per cell for the fiber-free gels, consistent with higher rates of proliferation.

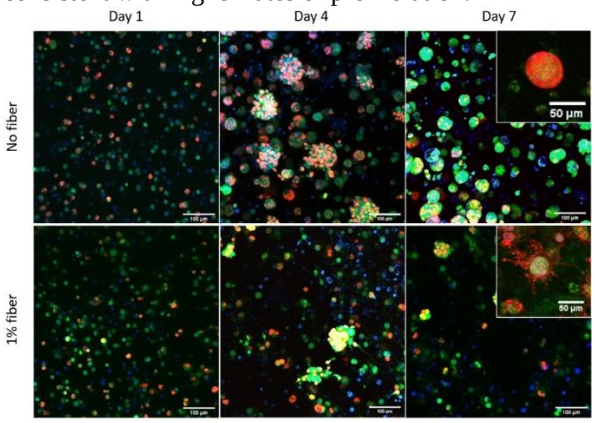

Figure 1. Maximum intensity projections of OPCs encapsulated in 1.5 wt% NorHA gels with or without fibers. Cells constitutively express GFP (green) and were stained with DAPI (blue) and EdU (red) as markers of nuclear DNA and newly synthesized DNA, respectively. Cells in fiber free gels exhibit more cell clustering and greater rates of proliferation, while cells in fiber-containing gels show lower proliferation rates and more morphological changes associated with differentiation (i.e. process extension). Inset: maximum intensity projections of OPCs in 1.5 wt% NorHA gels with and without fibers, Cells constitutively express GFP. Nuclei were stained with DAPI (blue) and f-actin was stained with phalloidin (red) to visualize process extension at culture day 7.

Conclusions: The incorporation of electrospun fibers in 3D hyaluronic acid hydrogels provides encapsulated OPCs with topographical cues which drive differentiation and reduce cell proliferation. Inclusion of electrospun fibers does not affect the bulk stiffness of the hydrogels, although mechanical effects at the cellular scale are currently being investigated via nanoindentation.

Acknowledgements: UVA Dean's Fellowship to RAM; NSF CMMI-1904198 to KJL.

References: 1) Russell LN. *ACS Biomater Sci Eng*, 2017; 3:3459–3468. 2) Lee S, *Nat Methods*, 2012; 9: 917-922.