Controlled Guidance of Spinal Motor Axons through Synthetic Click Hydrogels

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Statement of Purpose: Since the initial studies on the CNS pioneered by Santiago Ramon y Cajal at the turn of the twentieth century, there has been an ever-expanding interest in the culture and manipulation of nerve cells *ex vivo*. Specifically, developing culture platforms that mimic cellular microenvironments through which axons navigate to their final target destination *in vivo* remains a daunting task. Previous work has demonstrated the feasibility of culturing embryonic stem cell-derived spinal motor neurons (ESMNs), one of the best-understood types of nerve cell, in 3D in synthetic PEG hydrogels, but axon outgrowth was shown to be isotropic. While this study proved the feasibility of culturing these cells in 3D and enabling them to extend axons, it did not attempt to recapitulate the directed and organized axon outgrowth observed *in vivo*. *In vivo* axons must extend from the cell body to specific synaptic targets along precisely defined paths for normal functioning and development. These paths are defined through a combination of extracellular matrix (ECM) presentation, physical channels, and axon guidance ligands. While extensive research has been done to characterize the fundamental aspects of both ECM- and ligand-driven axon guidance, physical guidance of axons is less well understood. It is known through electron microscopy and histological staining that axons extend through channels of diverse sizes in the extracellular space, but these channels remain physical and biochemically poorly characterized[1]. Presented here is a photodegradable hydrogel platform capable of supporting the viability of ESMNs that can be used for fundamental studies on motor axon outgrowth through channels of geometries that have been functionalized with different biomolecules.

Methods: ESMNs are differentiated from mouse embryonic stem (ES) cells in embryoid body (EB) culture using retinoic acid and smoothened agonist[2]. The resulting EBs contain approximately 50% motor neurons, 40% Oligo2$^+$ neural progenitors that likely go on to differentiate into oligodendrocytes, and 10% interneurons. The EBs were collected and encapsulated in a 10 wt% PEG hydrogel crosslinked through a strained octyne/azide copper-free click chemistry that incorporated ECM mimic peptides and a photodegradable nitrobenzyl ether moieties in each crosslink[3]. The cell-laden hydrogel were imaged on a confocal microscope (Zeiss LSM 710). Physical channels were degraded from the edge of the encapsulated EB to the target of interest using a two-photon laser tuned to 740nm. To quantify axon outgrowth, confocal image stacks are taken and imported into Fiji Simple Neurite Tracer.

Results: After encapsulation, motor neurons contained in EBs remain predominantly viable for more than 2 weeks in culture. Without degradation, the ESMNs still extend axons; however, they wrap around the EB rather than

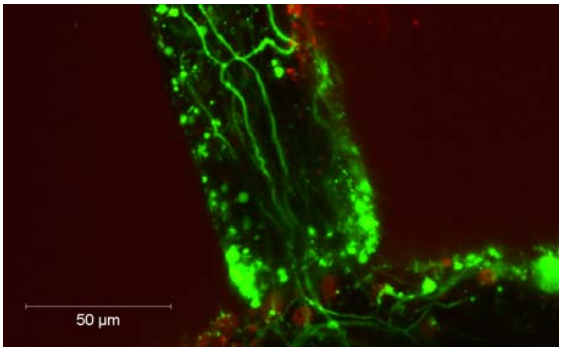

Figure 1: Spinal motor neurons (green) maintain high viability when encapsulated in an engineered PEG hydrogel (red) and extend axons through a physical channel (black) photo-eroded through the material.

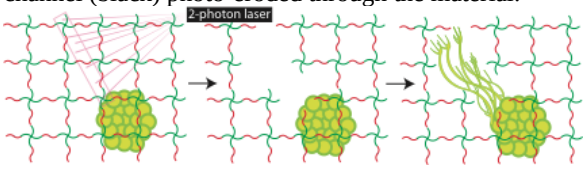

Figure 2: Schematic of degradation and axon outgrowth.

extend into the hydrogel. Conversely, many EBs exposed to a degraded channel of essentially any width, from 1 to 100 µm, extend axons through the channel for up to several millimeters. Often, this axon extension is accompanied by the migration of Oligo2$^+$ neural progenitor cells that compose the most of the non-motor neuron cells in the EB.

Conclusions: PEG hydrogels crosslinked through a copper-free click chemistry and functionalized with ECM-mimic peptides support the viability of encapsulated ESMNs through cell-material interactions and bioorthogonal gelation chemistry. Photodegradable aspect of the hydrogel platform and the versatility of two-photon confocal microscopy enable channels of sub-micron to macroscopic length scales to be eroded in 3D. *In vivo* axons extend through channels whose geometries span orders of magnitude in length scales from the microscopic intercellular spaces to the mesoscopic neuroepithelial endfeet channels to the macroscopic ventral root. This material platform enables the *in vitro* study and simulation of axon pathways and future work is focused on the assembly of a neural circuit.

References:

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Acknowledgements: NIH Pharmaceutical Biotechnology Training Program (5 T32 GM 8732), NSF (DMR 1006711), and HHMI.