Engineering Myelination *In Vitro*

by
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Technical Summary

The brain is the powerhouse of the central nervous system (CNS)—a convoluted network wherein every piece across molecular, cellular and tissue length scales plays a crucial role. When this system is out of balance, the implications are massive: neurological diseases are the number one leading cause of disability and number two leading cause of fatalities in the world. Returning the system to its steady state parallels in complexity. Despite the challenges in drug development for CNS disorders that have contributed to shut-downs of entire neuroscience programs, the massive unmet need motivates innovation in this space: there are no cures for CNS disorders. Myelin and oligodendroglia – the myelinating cells of the CNS – play central roles in CNS homeostasis, and the pathogenesis of a myriad of neurological disorders, including Multiple Sclerosis. Academic and industrial researchers need new tools, which include new materials and procedures, to develop new strategies for myelin and oligodendroglial protection and repair.

This thesis leverages interdisciplinary technologies and concepts to address challenges and inefficiencies in the current preclinical approach to discover and develop therapies for uniquely human disorders of myelin. We sought to address the need for preclinical *in vitro* tools compatible with high content screening that can replicate key aspects of myelination and the oligodendroglial niche. Inspired by physical and mechanical properties of neuronal axons, we developed new mechanically compliant and biocompatible photopolymers and additive manufacturing methodology to create Artificial Axons. We established primary rat myelination assays and showed that Artificial Axons capture key properties of oligodendroglial and neuronal interactions, which can be imaged in real time, and quantified. We implemented induced pluripotent stem cell technology to demonstrate that some but not all aspects of oligodendrocyte mechanotransduction are conserved across rats and humans *in vitro*, further motivating the use of human cells for the study of uniquely human diseases. We also demonstrated the first quantitative axon-free human myelination assays, and explored their use in drug discovery, including for dose response and small molecule screening. Finally, we discuss the modification of these assays and manufacturing methodologies, including harnessing material properties, to scale up the fabrication of Artificial Axons with better spatial resolution, for high content screening.

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