Serotonergic neurons in 3D-hydrogels: Tunable environments to study axon dynamics

J.H. HAIMAN, M. HINGORANI, G. DUNN, S. JANUSONIS

Department of Psychological & Brain Sci., Univ. of California, Santa Barbara, CA

Serotonergic axons (fibers) have a ubiquitous distribution in vertebrate brains, where they form meshworks with well-defined, regionally-specific densities. In humans, perturbations of these densities have been associated with abnormal neural processes, including neuropsychiatric conditions. The self-organization of serotonergic meshworks depends on the cumulative behavior of many serotonergic axons, each one of which has a virtually unpredictable trajectory. In order to bridge the high stochasticity at the microscopic level and the regional stability at the mesoscopic level, we are developing tunable hydrogel systems that can support causal modeling of these processes. These same systems can support future restorative efforts in neural tissue because serotonergic axons are nearly unique in their ability to robustly regenerate in the adult brain. In the study, we extended our research in 2D-primary brainstem cultures (Hingorani et al., 2022) to 3D-hydrogels. Tunable hydrogel scaffolds can closely mimic the mechanical and biochemical properties of actual neural tissue in all three dimensions and are therefore qualitatively different from 2D-environments. However, the integration of these scaffolds with highly sensitive neurons poses unique challenges. As the first step in building a hydrogel-based platform for the bioengineering of serotonergic axons, we studied primary brainstem neurons in several commercially available hydrogel platforms. The viability and dynamics of serotonergic somata and neurites were analyzed at different days *in vitro* with immunocytochemistry and high-resolution confocal microscopy. In addition, live imaging of neuron growth cones was performed, and the observed dynamics was compared to our extensive database of holotomographic (refractive index-based) recordings in 2D-cultures. The progress and key problems will be discussed.

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