The Effects of Hyaluronic Acid Molecular Weight on the Differentiation Potential of Human Neural Stem Cells in 3D Contexts

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Statement of Purpose: Neurological disorders impact many people around the world and are the leading cause of disability and death. Nearly one billion people around the world suffer from one of many neurological disorders such as Alzheimer's Disease (AD), Parkinson's Disease (PD), migraines, and others. The use of induced pluripotent stem cells has facilitated the *in vitro* study of neurological disorders through the controlled differentiation of these cells into neural stem cells and finally, astrocytes, oligodendrocytes and neurons. However, most *in vitro* models investigating neuropathologies have been traditionally developed and implemented using 2D microenvironments such as coated or uncoated plastic or glass surfaces. These surfaces exhibit rigidity values orders of magnitude higher than central nervous system (CNS) tissue and are therefore not representative of the natural milieu for these cells. In addition, CNS cells experience a 3D environment which cannot be provided by cell culture surfaces. In this research work, we aimed to design, fabricate, and evaluate microenvironments to support the *in vitro* culture of human neural stem cells in 3D contexts and to regulate their differentiation potential. Towards this end, we used multi-interpenetrating polymer networks (mIPNs) as our bioinspired scaffolds. The effects of hyaluronic acid molecular weight, pre-differentiation and culture conditions on the differentiation of human neuronal stem cell were evaluated.

Methods: In this study we used human neural stem cells (iHNSCs, Cell Applications) as the cell source. The cells were expanded according to the provider's protocol using growth media supplemented with B27, basic fibroblast growth factor (bFGF), endothelial growth factor (EGF) and heparin. Cells were expanded and then divided into two groups: one group was encapsulated in the mIPNs and the other group was pre-differentiated for 5 days in neural differentiation media on plates coated with collagen I before being encapsulated. The mIPNs were composed of varying levels of hyaluronic acid (HA) high and low molecular weight (HA-HMW and HA, LMW), collagen type I (col I), and poly(ethylene glycol) diacrylate (PEGDA). The cells were collected and encapsulated based on a procedure previously developed by our laboratory[1]. The cells in the mIPNs were cultured at 37°C and 5.0% CO_2 for 14 days in different differentiation media. Constructs were mechanically characterized using dynamic mechanical analysis (DMA) on a TA Instruments 3100 mechanical tester.

Results: Mechanical data demonstrated that different mIPN formulations containing HA-HMW or HA-LMW were fabricated to closely match the complex modulus of human brain cortex tissue (Figure 1A). Initial qualitative assessment of cell morphology using Laser Confocal microscopy reveled than cell morphology was modulated by both pre-differentiation conditions and the presence of HA of different chain lengths. It appears that long HA chain length limits the spreading of cells in 3D contexts (Figure 1B). Gene and protein expression profiles are being analyzed to characterize the differentiation stage for each experimental group.

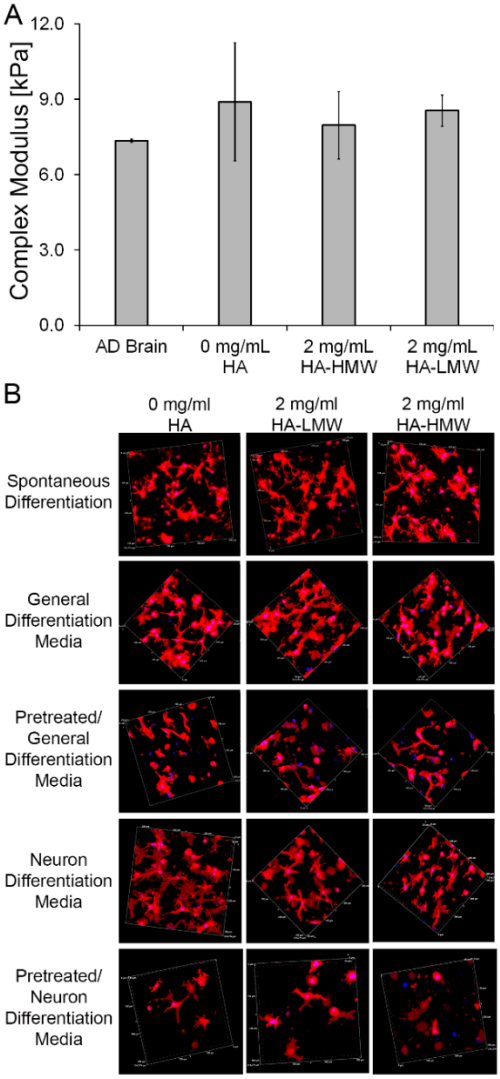

Figure 1. Complex modulus of selected mIPNs (A). Representatives images of cell morphology (B)

Conclusions: The proposed bioinspired mIPNs displayed complex modulus within the range of mechanical performance of human cortex tissue while displaying distinct chemical composition. The spreading of iHNSCs in our 3D scaffolds appears to be a function of HA content, HA molecular weight and pre-differentiation conditions.

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References: 1. Jimenez-Vergara AC. Sci Rep. 2020;16