A Hyaluronic Acid-Based Hydrogel Culture Platform for iPSC-derived Midbrain Neuronal Culture

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Statement of Purpose: While there is increasing recognition of the importance of cell-cell and cell-matrix interactions during development, regeneration, and disease progression in the central nervous system, *in vitro* experimental models often lack a three-dimensional (3D) microenvironment, which can have significant effects on cellular differentiation, growth, and communication. Hyaluronic acid (HA) is abundant in the extracellular matrix of the central nervous system and has diverse biological functions through direct cell signaling. We are developing an HA-based hydrogel platform for 3D culture of iPSC-derived, midbrain-fated neural cells, which include dopaminergic and GABAergic neurons. Dopamine release by cultures was detected in response to various treatments that should induce membrane depolarization and synaptic firing. These results might be significant for studying Parkinson's diseases and opioid addiction, as well as exploring potential biomaterials for improved models of neural development and regeneration.

Methods: Human iPSC lines were differentiated to midbrain-fated neural cultures based on previously published methods (1). Briefly, iPSCs were seeded onto Matrigel-coated six-well plates at 200,000 cells per cm^2 in E8 medium with 5 µM Y27632. At 24 h after plating, 50% DMEM/F12 and 50% neurobasal, N2, and B27-vitamin A (base medium) was added with stage 1 medium components (LDN-193189 and SB431542) for 3 days, switched to stage 2 medium (LDN, SB, purmorphamine, CHIR99021, Sonic hedgehog and FGF8) for 4 days, stage 3 medium (LDN, CHIR and all-trans retinoic acid (ATRA)) for 4 days, and finally to stage 4 medium (BDNF, GDNF, dibutyryl cyclic AMP (dbCAMP), l-ascorbic acid(AA), DAPT, CHIR and TGF-β3) for 3 days. On day 15, cells were dissociated and cryopreserved. Cells can then be thawed and maintained in maturation medium (BDNF, GDNF, dbCAMP, AA, DAPT and TGF-β3) plus 5 µM Y27632 and form cell aggregates (~200 µm diameter) and encapsulated in 3D hydrogels for an additional 21 days before assessment. Cells were also cultured in 2D on Matrigel-coated (40 mg/mL), 96-well plates. Hydrogels were crosslinked from thiolated HA (~750 kDa, 5-6% thiolation (2)), 4-arm, thiol-terminated polyethylene glycol (PEG) and 8-arm, norbornene-terminated PEG modified with RGD peptides. Hydrogels were crosslinked to embed cell aggregates in 3D culture using the type I photoinitiator LAP (lithium phenyl(2,4,6-trimethylbenzoyl) phosphinate) and UV exposure (365 nm, 15 s). Dopamine release was measured from supernatants using high-performance liquid chromatography electrochemical detection (HPLC-ED).

Results: 3D cultures of midbrain-fated, iPSC-derived neural cells contained both dopaminergic and GABAergic neurons, as evidenced by expression of tyrosine hydroxylase (TH) and glutamate decarboxylase (GAD67), respectively (A) and expression of the pan-neuronal marker β-III tubulin (B). The ApoLive-Glo™ assay (Promega, Madison, WI) provides a measure of cell viability for neuronal cultures cells grown in 2D layers or in 3D aggregates in hydrogels (C). The signal is quantified as live/dead per cell (N=3, error bars = standard deviation, *: $p < 0.05$). 3D culture demonstrated significantly higher live/dead signal (C). Morphine (10 µM), potassium (100 mM), and d-amphetamine (100 µM) each induced dopamine release, confirming dopaminergic function.

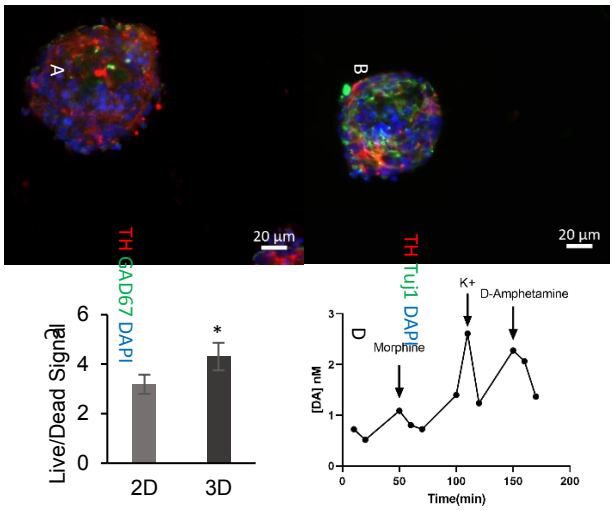

Figure 1. iPSC-derived Dopaminergic Neuron Cultured in HA-based Hydrogel. A-B) Cryosections (12µm) of of neuronal cell aggregates encapsulated in hydrogels. C) ApoLive-Glo™ assay provides a measure of cell viability for neuronal cultures cells grown in 2D layers or in 3D aggregates in hydrogels. D) Drug induce dopamine release in the 3D culture measured by HPLC.

Conclusions: After 36 days, 2D and 3D cultures of midbrain-fated iPSCs yielded dopaminergic and GABAergic neurons. Furthermore, potassium, morphine and d-amphetamine evoked dopamine release, which indicates the presence of relatively mature dopaminergic and GABAergic phenotypes. While 2D culture and animal models are the dominant experimental platform, we have developed a 3D culture platform that uses human derived iPSCs. The 3D environment could allow for the investigations of cell-cell and cell-matrix interactions. In addition, our system has a more mechanically and molecularly defined environment than *in vivo* and can enable us to understand the effects of different molecular compounds on neuron differentiation and regeneration. Finally, using human iPSCs derived neuronal culture can provide more biomimetic models for studying diseases such as Parkinson's disease (1).

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