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High-Throughput 3D Neural Cell Culture Analysis Facilitated by Aqueous Two-Phase Systems (G)

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Introduction: The three-dimensional (3D) culture of neural cells in extracellular matrix (ECM) gels holds promise for modeling neurodegenerative diseases. However, air-liquid interfacial tension and evaporation can result in inconsistent 3D cultures at low volumes. Thick-layer hydrogels can counter these factors, but large diffusion distances, high cost, and incompatibility with standard imaging tools, plate readers and assays limit their use. To address these limitations, we have developed a thin-layer, 3D culture technique using a commonly used self-assembling ECM hydrogel (Matrigel) combined with an aqueous two-phase system (ATPS).

Methods: A dextran T10 (D10) and hydroxypropyl methylcellulose 4000 cPs (HPMC) ATPS was used to confine small volumes of Matrigel containing the model neural cell line, SH-SY5Y, into thin layers in a 96-well plate format. SH-SY5Y cells were differentiated and cell viability and morphology were observed under epifluorescence microscopy. The ATPS-Matrigel 3D culture method was characterized by monitoring the distribution of 3.0 µm microbeads within gel constructs without cells.

Results: Matrigel evaporation was eliminated in the ATPS-Matrigel 3D culture method, and small volumes (20 µl and lower) formed evenly thin gels. SH-SY5Y cells were observed to extend neurite-like processes in three-dimensions when differentiated, and cell viability remained high, suggesting minimal negative impact of the protocol on cell growth.

Conclusion: We demonstrate a low cost, simple, high-throughput, 3D neuronal cell culture system that is compatible with well-established equipment and commercially available materials.

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