

DEVELOPMENT OF A 3D PHARMACOLOGICAL SH-SY5Y NEUROSPHERE MODEL TO STUDY PATHOLOGICAL MECHANISMS UNDERLYING COGNITIVE IMPAIRMENT AND ITS APPLICATION IN DRUG SCREENING

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Cognitive impairment represents one of the most disabling symptoms of schizophrenia and remains poorly responsive to current pharmacological treatments. While two-dimensional (2D) neuronal models have provided valuable insights into cellular mechanisms, they are limited in capturing certain aspects of the spatial organization and intercellular communication relevant to neural network function. To complement these approaches, we developed a pharmacological three-dimensional (3D) neurosphere model based on SH-SY5Y neuroblastoma cells, designed to reproduce cellular and functional alterations associated with cognitive deficits while remaining compatible with high-throughput drug screening workflows.

Neurospheres were generated by culturing SH-SY5Y cells in a methylcellulose-based matrix to promote uniform aggregation. After formation, a two-step differentiation protocol was applied: 5 days with 10 μ M retinoic acid (RA) followed by 7 days with either 80 nM phorbol 12-myristate 13-acetate (PMA) or 1 μ M glucagon-like peptide-1 (GLP-1). Morphological parameters including sphericity, border definition, and compactness were quantified to assess maturation. Functional responses were evaluated through intracellular calcium mobilization assays using the Calcium 6 dye upon depolarization with KCl.

Both PMA- and GLP-1-differentiated neurospheres exhibited compact, rounded morphologies with smooth borders and high circularity, reflecting neuronal differentiation and improved structural organization compared to non-differentiated aggregates, which appeared irregular and unstable. Functionally, GLP-1-differentiated neurospheres showed the strongest intracellular calcium response to KCl stimulation, indicating enhanced excitability and neuronal functionality.

In summary, this 3D neurosphere model represents a robust and reproducible pharmacological platform that bridges the gap between simple 2D assays and more complex neural systems or *in vivo* approaches. Its reproducibility and compatibility with imaging-based readouts make it suitable for high-throughput applications aimed at investigating the cellular impact of pharmacological insults such as MK-801 and dopamine, thus contributing to the study of cognitive dysfunction in neuropsychiatric disorders.