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Yan Li
Florida State University, yli4@fsu.edu

Yuanwei Yan
Florida State University

NEURAL PATTERNING OF HUMAN INDUCED PLURIPOTENT STEM CELLS FOR STUDYING NEUROTOXICITY

Yuanwei Yan, Department of Chemical and Biomedical Engineering, Florida State University
Julie Bejoy, Department of Chemical and Biomedical Engineering, Florida State University
Junfei Xia, Department of Chemical and Biomedical Engineering, Florida State University
Jingjiao Guan, Department of Chemical and Biomedical Engineering, Florida State University
Yi Zhou, Department of Biomedical Sciences, Florida State University
Yan Li, Department of Chemical and Biomedical Engineering, Florida State University
Yli4@fsu.edu

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Existing models using adult human neural stem cells have the restricted access. Human induced pluripotent stem cells (hiPSCs) can generate allogeneic or patient-specific neural cells/tissues and even mini-brains to provide robust in vitro models for applications in drug discovery, neurological disease modeling, and cell therapy. Toward this goal, the objective of this study is to construct 3-D neural models from hiPSCs through the scalable embryoid body-based suspension culture which can generate cortical glutamatergic neurons and motor neurons by tuning the sonic hedgehog (SHH) signaling. The differentiation of human iPSK3 cells was induced using dual inhibition of SMAD signaling with LDN193189 and SB431542. Then the neural tissue patterning was tuned through the treatment with cyclopamine (the SHH antagonist) or purmorphamine (the SHH agonist) along with other factors and further maturation. The neural cells were characterized at day 20, day 35, and day 55. Abundant glutamatergic neurons (>60%) was observed with the cyclopamine treatment, while the cells were more enriched with motor neurons expressing Islet-1 and HB9 (>40%) with the purmorphamine treatment. The cells also expressed pre- and post-synaptic markers (Synapsin I and PSD95), and generated action potentials in response to depolarizing current injections and spontaneous excitatory post-synaptic currents after maturation. To assess the cellular responses, three classes of small molecules/drugs were investigated: (1) N-methyl-D-aspartate to induce general neural toxicity; (2) matrix metalloproteinases inhibitors to affect matrix remodeling; (3) amyloid β (1-42) oligomers to induce disease-specific neural toxicity. Differential responses to various treatments were observed for different neuronal subtypes. Overall, this study can provide a transformative approach to establish 3-D neural models for neurological disease modeling (e.g., Alzheimer's disease), drug discovery, and cell therapy.

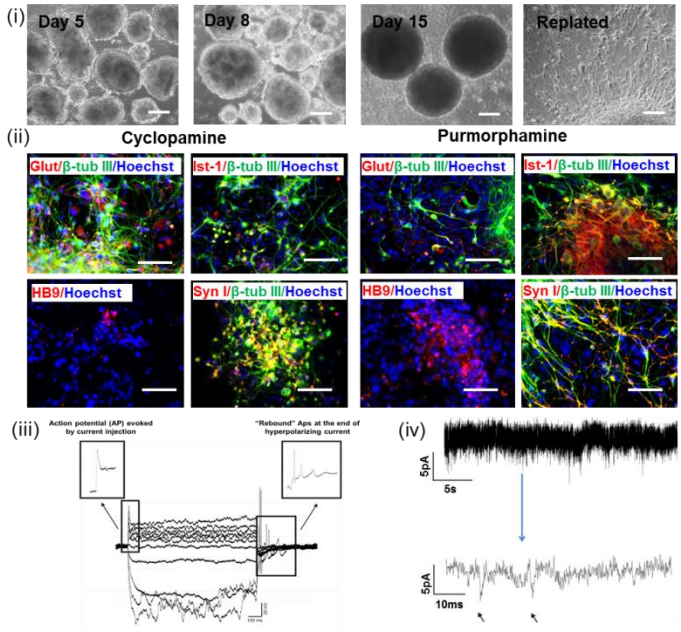

Figure 1 – Characterizations of neural spheres derived from human iPSK3 cells. (i) Neural spheres derived from iPSK3 cells based on embryoid body (EB) formation; (ii) cyclopamine-treated cells (day 35) and purmorphamine-treated cells (day 35). At day 35, cyclopamine-treated cells have more cortical glutamatergic (Glut) neurons while purmorphamine-treated cells have more motor neurons expressing Islet-1 (Ist-1) and HB9. β-tub III indicates β-tubulin III. Both populations express pre-synaptic marker synapsin I (Syn I). Scale bar: 100 μm. (iii) Action potentials in response to depolarizing current injections and at the end of hyperpolarizing current injections ("rebound" action potentials) (day 30 cells); (iv) spontaneous excitatory post-synaptic currents (day 30 cells).