Characterization of Microcarrier Based hiPSC Derived Neurons for Alzheimer's Disease Studies

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Introduction

- The brain's accurate function is dependent on a balance of excitatory (glutamatergic) and inhibitory (GABAergic) neurons.
- Alzheimer's disease is associated with a decrease of GABAergic activity, resulting in E/I imbalance and network hyperexcitability.
- This imbalance results in neuron death, memory loss, and cognitive impairment in Alzheimer's disease patients.
- This study generates neurons from **hiPSC** and then uses **immunofluorescence** and **calcium imaging** to investigate their identification and function.
- The goal is to develop a human-relevant model for studying AD-related synapse dysfunction and enabling treatment screening.

Methods

- **Expansion and characterization of NPC:**

NPCs were grown in NEM and characterized using immunofluorescence for SOX1, SOX2, and NESTIN markers.

- **Differentiation of NPC's:**

NPCs were seeded onto microcarriers and differentiated into neurons over a 30 to 40 day period utilizing BDNF and GDNF.

- **Neuron Dissociation:**

Mature neurons were enzymatically dissociated from microcarriers to create viable single-cell suspensions

- **Calcium Imaging:**

Neural activity was confirmed by detecting calcium transients.

Results

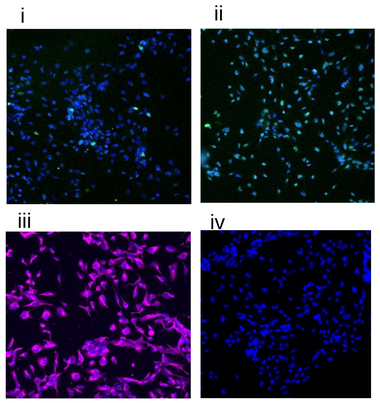

Figure1.Immunofluorescence images of NPC's:
i. The cells were stained using Rabbit SOX1, anti-rabbit and Hoechst stain which expressed SOX1 at DAPI and GFAP.
ii. The cells were stained using Rabbit-SOX2, anti-rabbit and Hoechst stain which expressed SOX2 at DAPI and GFAP.
iii. The cells were stained with NESTIN.

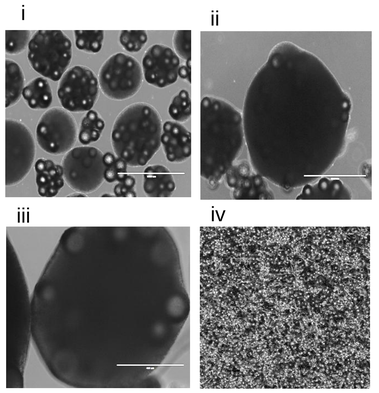

Figure 2. Microcarrier differentiation of NPC's:
i. The microcarrier-based differentiation of NPCs into neurons at day 4.
ii. The microcarrier-based differentiation of NPCs into neurons at day 20.
iii. The microcarrier-based differentiation of NPCs into neurons at day 40.
iv. Successfully generated neurons.

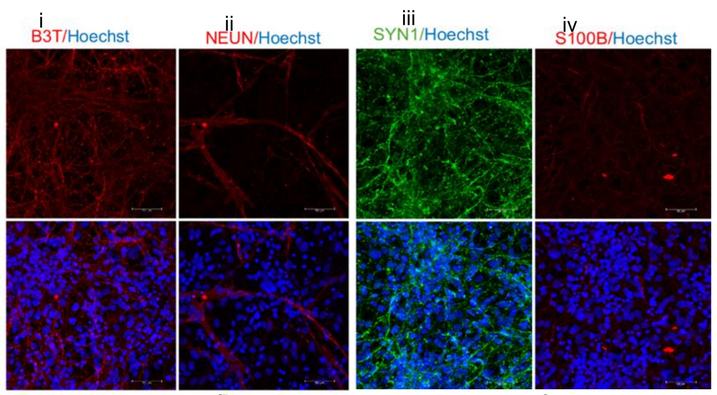

Figure 3. Immunofluorescence images of neurons at 40x magnification:The blue stain (Hoescht) is the only mature neuronal marker. i). Cells marked with βIII-tubulin (red) and hoechst (blue, nuclear stain) exhibit neuronal cytoskeleton expression. ii). Cells labelled with NEUN (red) show developed neurons. iii). Cells marked with SYN1 (green) show synapse development. iv). S100B (red) reveal the presence of a tiny astrocyte population.

Results

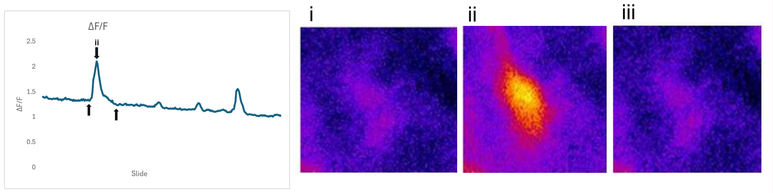

Figure 4. Representation of calcium imaging of neurons. i.) Image indicating no neural activity, ii.) image indicating a strong calcium signal indicating active firing of neuron, iii.) image indicating no firing of neuron.

Summary, Conclusions and Future Directions

This study effectively demonstrates scalable differentiation and dissociation of hiPSC-derived neurons with a microcarrier-based technology. The dissociation technique successfully produced a high- yielding single-cell suspension while preserving neuronal survival and functional integrity. Immunofluorescence and calcium imaging validated cell identity and function, indicating their suitability for AD modeling.

Acknowledgements

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References

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