Generation of an Alzheimer's Disease Model Using Human iPSC-derived Neurons and Astrocytes Produced by a Transcription Factor-Based Differentiation Protocol

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Abstract

There is an urgent need for new technologies and models to aid in the elucidation of the pathology and biological mechanisms of neurodegenerative diseases such as Alzheimer's Disease (AD) and in the development of effective therapies and potential cures to treat them. Human induced pluripotent stem cell (iPSC)-derived disease models can provide researchers with a system that closely resembles the neurons and astrocytes of the human AD brain and avoid many of the practical and ethical issues complicating other models. At Elixirgen Scientific we have established a rapid, robust transcription factor-based differentiation protocol, the Quick-Tissue™ technology, which yields reproducible results and has been successfully applied to iPSC lines derived from both healthy and diseased donors. Our human iPSC-derived excitatory neurons show robust expression of neuronal and excitatory markers just 10 days from the start of differentiation. iPSC-derived neurons from an AD patient showed higher amyloid beta (Aβ) and total tau protein expression than those from a healthy donor, as demonstrated by ELISA. The addition of beta secretase inhibitors resulted in decreased levels of Aβ proteins. Our human iPSC-derived astrocytes show similar gene expression profiles to human primary astrocytes at day 47 post differentiation. When generated from an AD donor, the cell morphology indicates that a pathological phenotype can be observed in AD patient iPSC-derived astrocyte cultures. Using our Quick-Tissue™ technology we have differentiated iPSCs to both excitatory neurons and astrocytes capable of recapitulating diseased phenotypes.

Methods

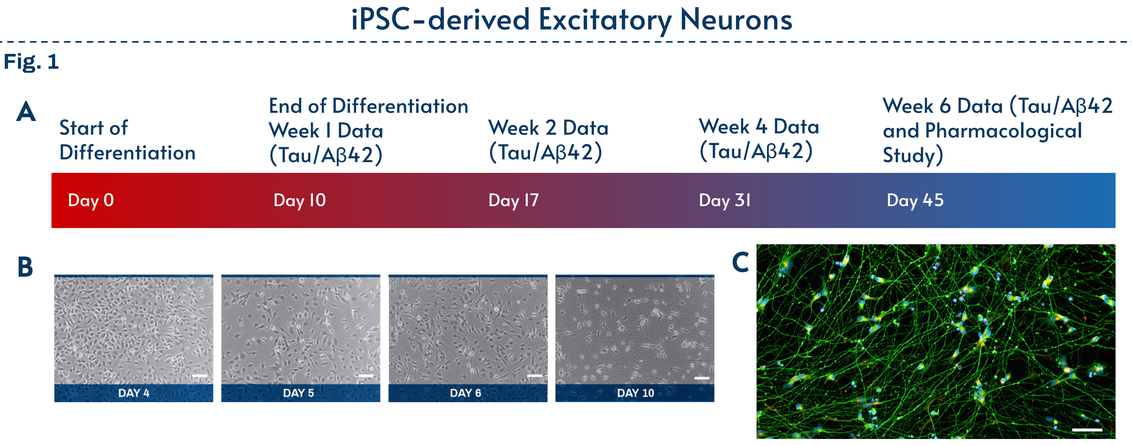
iPSCs were cultured and differentiated with our Quick-Tissue™ technology as previously described in the Quick-Neuron™ Excitatory - SeV Kit and Quick-Glia™ Astrocyte - SeV Kit protocols (https://www.elixirgensci.com/resources/?resource-type=user-guides).

Materials:

- California Institute of Regenerative Medicine iPSC lines: CW50065 (healthy control, Female, 74 y/o donor) and CW50114 (AD patient, Female, 72 y/o donor)
- Elixirgen Scientific iPSC Differentiation Kits: Quick-Neuron™ Excitatory Kit (EX-SeV) and Quick-Glia™ Astrocyte Kit (AS-SeV)

iPSC-derived Excitatory Neurons

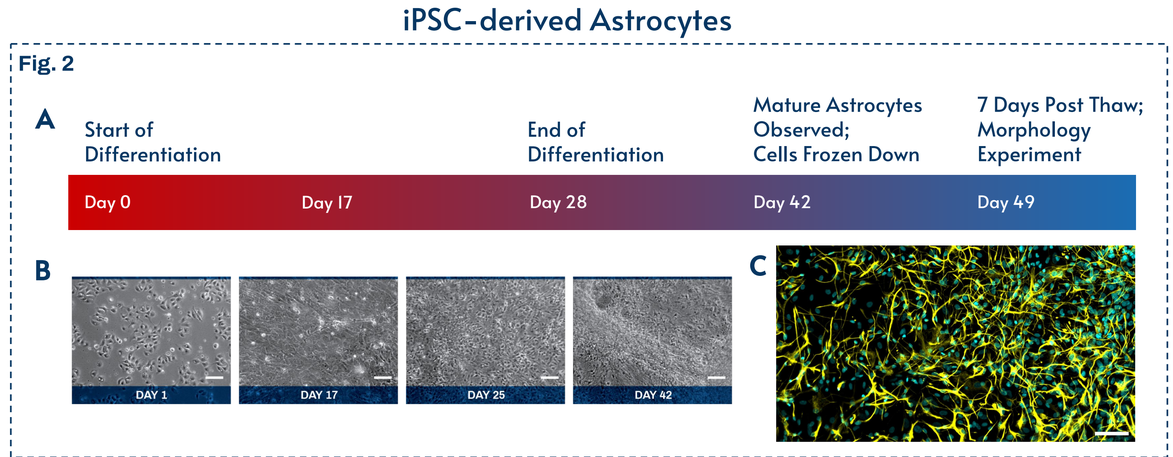
Fig. 1



Excitatory Neuron Cell Type Characterization (Fig.1): (**A**) Elixirgen Scientific's EX-SeV differentiation reagent kits provide the tools to differentiate iPSCs into excitatory neurons in just 10 days. Continued culture and further characterization and analysis were performed. (**B**) Phase contrast images show exemplary neurite outgrowth and neuronal morphology by day 10 of the differentiation process (scale bar = 100 µm). (**C**) An ICC image shows high expression of pan-neuronal marker TUBB3 (green) and excitatory neuron marker VGLUT1 (red) on day 10 of differentiation (scale bar = 100 µm).

iPSC-derived Astrocytes

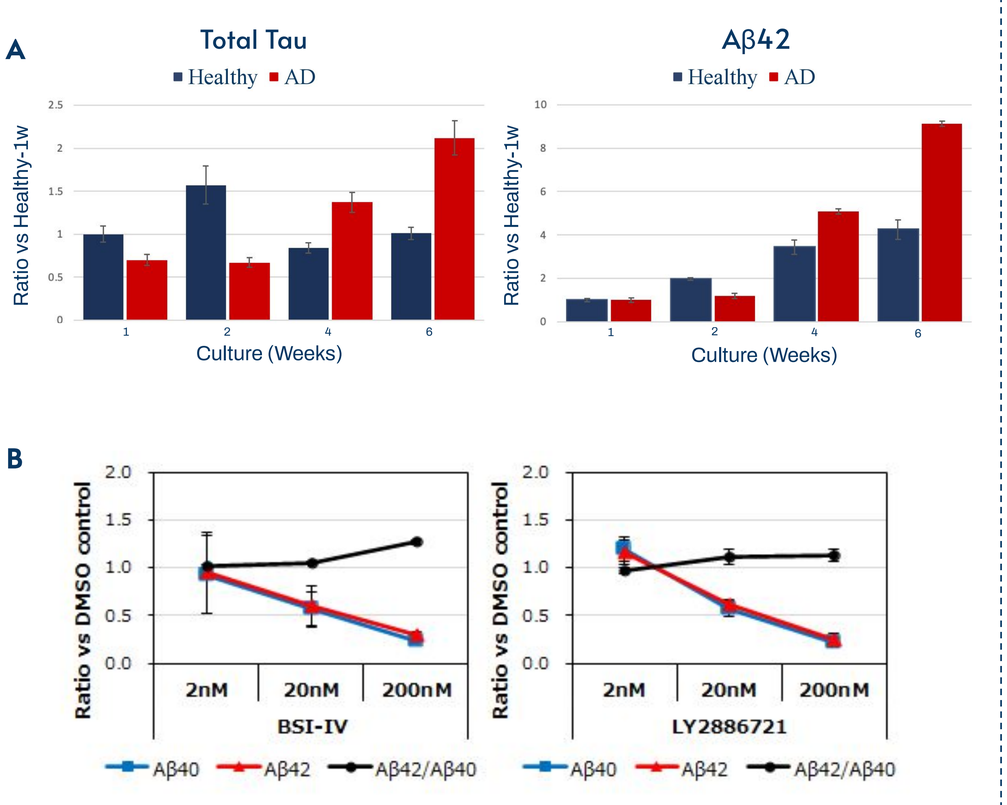
Fig. 2



Astrocyte Cell Type Characterization (Fig.2): (**A**) Elixirgen Scientific's AS-SeV differentiation reagent kits provide the tools to differentiate iPSCs into astrocytes in just 28 days. (**B**) Phase contrast images display typical astrocyte morphology by day 28 of the differentiation process (scale bar = 100 µm). (**C**) An ICC image shows high expression of astrocyte marker GFAP (yellow) on day 28 of differentiation (scale bar = 100 µm).

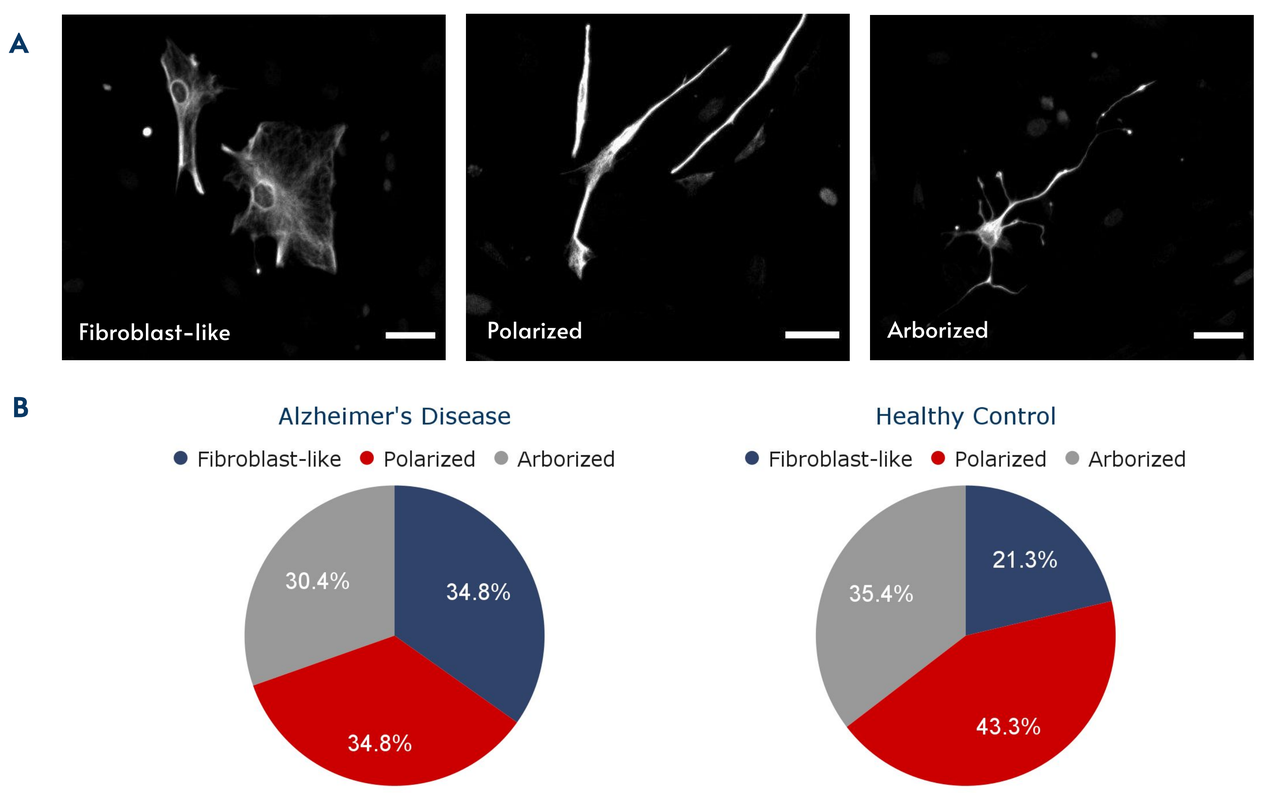
Results

Fig. 3



Generating an AD Model with iPSC-derived Excitatory Neurons: Phenotypic and Pharmacological Assays (Fig.3): When iPSCs generated from an AD patient are differentiated into excitatory neurons with Quick-Neuron™ - Excitatory, the resulting cells have a distinct phenotype that distinguishes them as a useful *in vitro* AD model, in comparison to the phenotype of cells differentiated from healthy donor iPSCs. (**A**) ELISA results show increased levels of total Tau and Aβ42 of excitatory neurons derived from an AD patient when compared with excitatory neurons derived from a healthy control after at least 4 weeks in culture. (**B**) A pharmacological assay, done after 6 weeks of culture, revealed decreased levels of Aβ40 and Aβ42 proteins with the addition of beta secretase inhibitors: BACE1 inhibitor-IV (BSI-IV; 565788, Sigma Aldrich) and BACE1 inhibitor LY2886721 (MedChemExpress).

Fig. 4



Generating an AD Model with iPSC-derived Astrocytes: Phenotypic Assays (Fig.4): When iPSCs generated from an AD patient are differentiated into astrocytes using Quick-Glia™ - Astrocyte, the resulting cells have a distinct phenotype that distinguishes them as a useful *in vitro* AD model, in comparison to the phenotype of cells differentiated from healthy donor iPSCs. (**A**) ICC images depict the different morphologies seen in a population of astrocytes derived from iPSCs at day 49 and stained with GFAP (scale bar = 20 µm). (**B**) A higher percentage of the astrocyte population had a fibroblast-like morphology when derived from iPSCs generated from an AD patient when compared with astrocytes derived from a healthy control iPSC line.

Conclusions

- A rapid, reproducible transcription factor-based iPSC differentiation method for both neurons and astrocytes has been established by Elixirgen Scientific.
- Phenotypic assays demonstrate notable differences between cells derived from a healthy control iPSC line vs an Alzheimer's disease patient iPSC line.
- Neurons differentiated from AD lines respond to inhibitory compounds similarly to the way primary cells from an AD patient would be expected to respond.
- Transcription factor-based iPSC differentiation technology allows for a relevant AD model suitable for disease modeling, proof of concept for therapy, drug discovery, pharmacological testing, and more.

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