Evaluation of hiPSC-derived tri-culture as in-vitro model for neurotoxicity and neuroinflammation

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Background

Human induced pluripotent stem cell (hiPSC)-derived models offer an opportunity to improve the translational assessment of central nervous system (CNS) liabilities associated with neuroinflammation and neurotoxicity. However, conventional in vitro systems often lack the cellular complexity required to capture interactions between neurons, astrocytes, and microglia that contribute to adverse CNS pharmacodynamic responses and may limit translation of nonclinical findings. The objective of this study was to evaluate an hiPSC-derived tri-culture model as a physiologically relevant in vitro system for characterizing neuroinflammatory and neurotoxicity phenotypes relevant to CNS safety pharmacology.

Methods

Human iPSC-derived neurons, astrocytes, and microglia were combined to establish tri-cultures. Neuroinflammatory responses were induced using lipopolysaccharide (LPS), while reference neurotoxicants were used to model neuronal injury. Cellular responses were assessed using live-cell imaging, high-content imaging, and cytokine analysis of culture supernatants. Quantitative endpoints included neuronal morphology, glial activation, inflammatory mediator release, neurite length, and neurofilament-light chain (NF-L) levels.

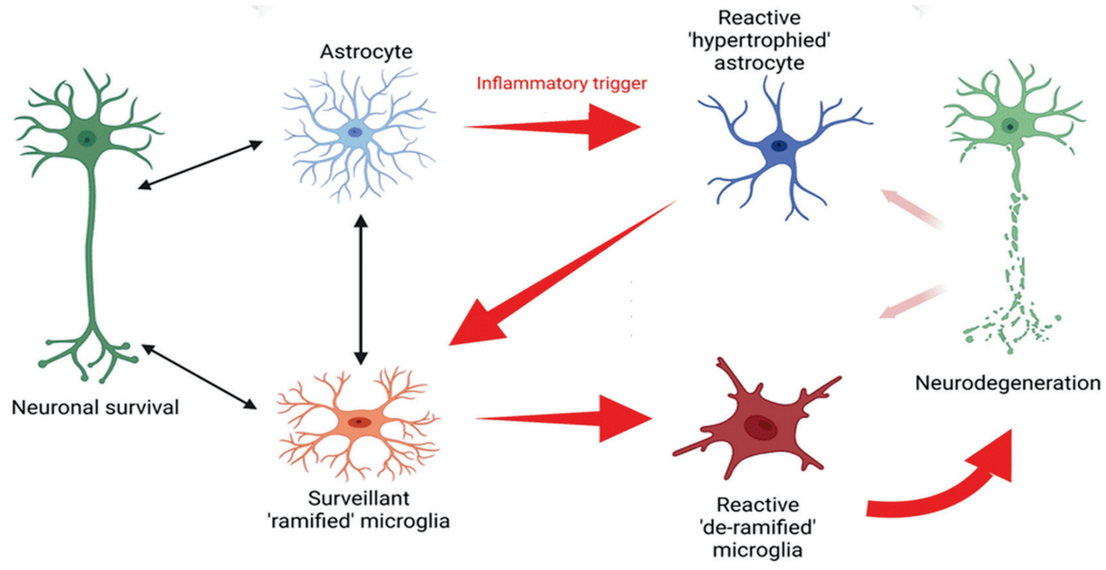

Figure 1. Cross-talk between neurodegeneration and neuroinflammation
Neuronal homeostasis relies on astrocytes and microglia activation status, that is disturbed in pathogenic conditions as neurodegeneration.
Diseased neurons secrete factors that activate microglia and astrocytes, initiating a cascade of inflammatory triggers that induce further neurodegeneration and neuroinflammation.
Adapted from: Ullah, F., Gamage, R., Sen, M.K. et al. The Effects of Modified Curcumin Preparations on Glial Morphology in Aging and Neuroinflammation. Neurochem Res 47, 813–824 (2022). https://doi.org/10.1007/s11064-021-03499-4

Ncyte® Microglia – Cytokine release and phagocytic activity

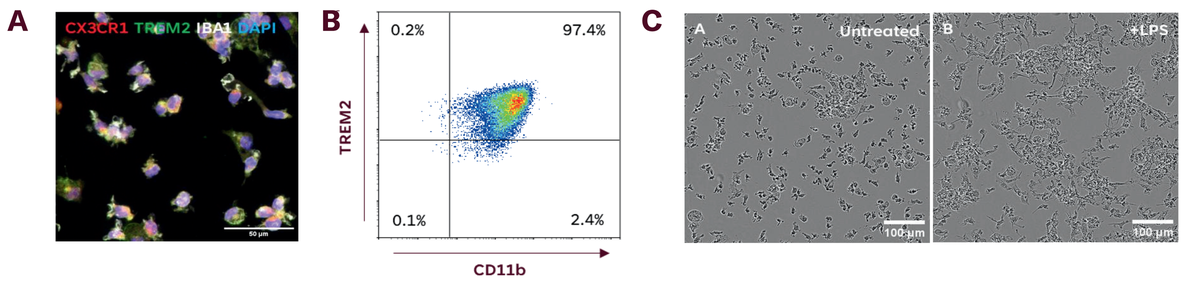

Figure A. Immunofluorescent staining of Ncyte® Microglia with markers IBA, TREM2 and CX3CR1, co-stained with DNA marker DAPI, on day 7 post-thaw.
Figure B. Flow cytometry analysis of Ncyte® Microglia showing TREM2/CD11b double positive cells (97.4%).
Figure C. Brightfield images of Ncyte® Microglia. A) Untreated microglia and B) Microglia treated for 18hrs with LPS

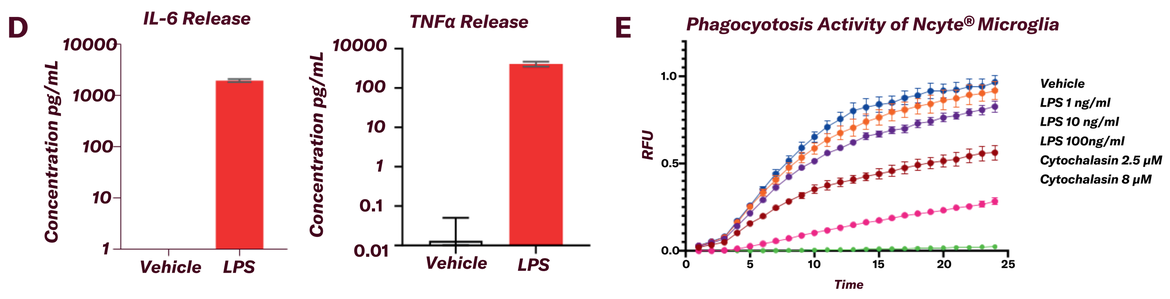

Figure D. Release of cytokines IL-6, TNFα on day 7 post-thaw in Ncyte® Microglia treated for 18hrs with LPS as measured by Mesoscale Discovery.
Figure E. Day 7 Ncyte Microglia were incubated with pHrodo Green labelled E. coli particles for 24 hours +/- cytochalasin D or +/- LPS control. Images were acquired every 30 mins on the Incucyte® looking at green fluorescence and phase contrast. The graph displays the fluorescence intensity per cell displaying degree of phagocytosis per cell.

Ncyte® Neural Mix – Mature neurons and astrocytes co-culture expressing SYN and PSD-95

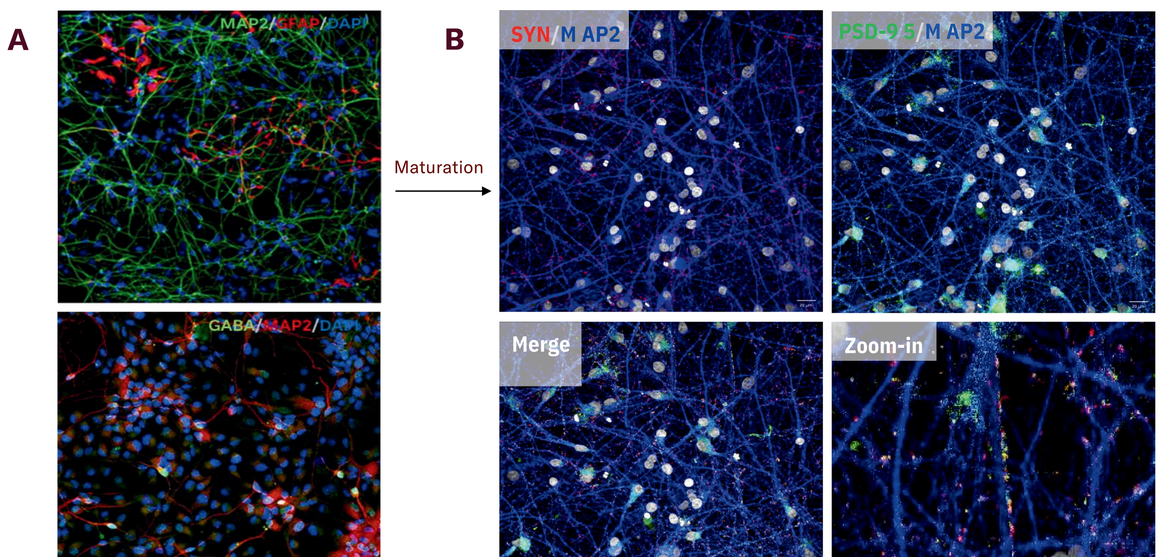

Figure A. Fluorescent images of Ncyte Neural Mix with >70% of βIII-Tubulin+ neurons, ≥ 50% of s100β+ astrocytes in non-neuronal population. Presence of glutamatergic and GABAergic neurons
Figure B. Ncyte neural mix can be matured and maintained in culture and exhibit synaptic maturation as evident by the colocalization of pre-synaptic marker synaptophysin-SYN (in red), post-synaptic marker PSD-95 (in green) and neuronal marker MAP2 (in blue). Nuclei were stained with DAPI (in grey).

Miniaturized tauopathy assay based on Ncardia's human triculture system

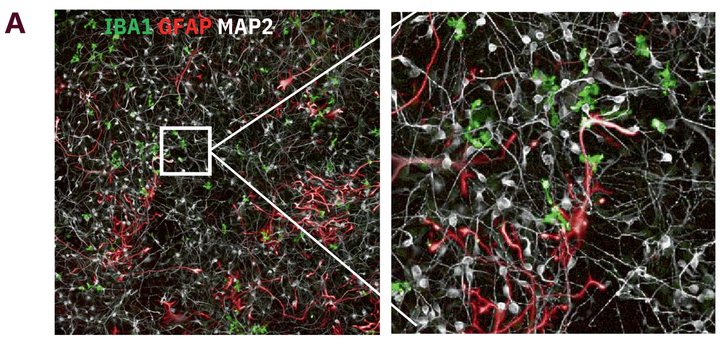

Figure A. Fluorescent images from Ncyte tri-cultures stained for microglia (IBA1 in green), astrocytes (GFAP in red) and neurons (MAP2 in white). Nuclei were stained with DAPI (in grey).

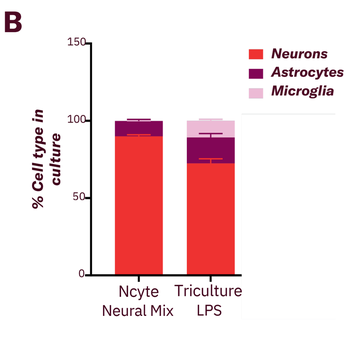

Figure B. Tri-culture is composed of neurons (~70%), astrocytes (~17%) and microglia (~11%)

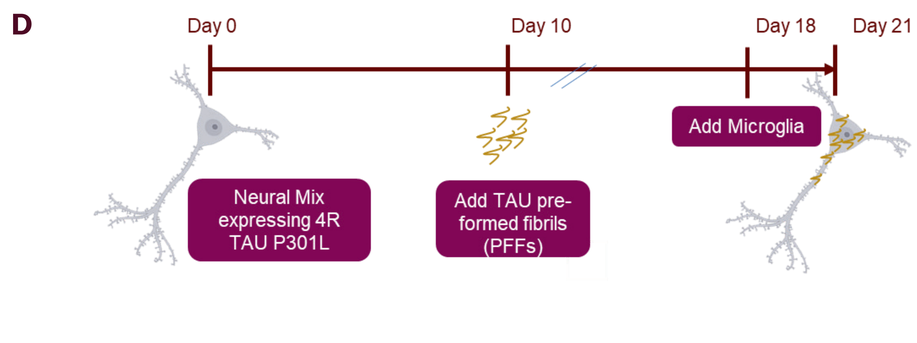

Figure D. Schematic of the AD model using iPSCs derived neurons, astrocytes and microglia treated with TAU pre-formed fibrils (PFFs). Day 10 post-thaw co-cultures expressing 4R TAU P301L were treated with TAU PFFs. On day 18, Ncyte microglia were added. Co-culture of neurons, astrocytes and microglia was kept for 21 days for endpoint analysis of phospho-TAU (pTAU), MC-1 positive TAU in neurons (by ICC-IF).

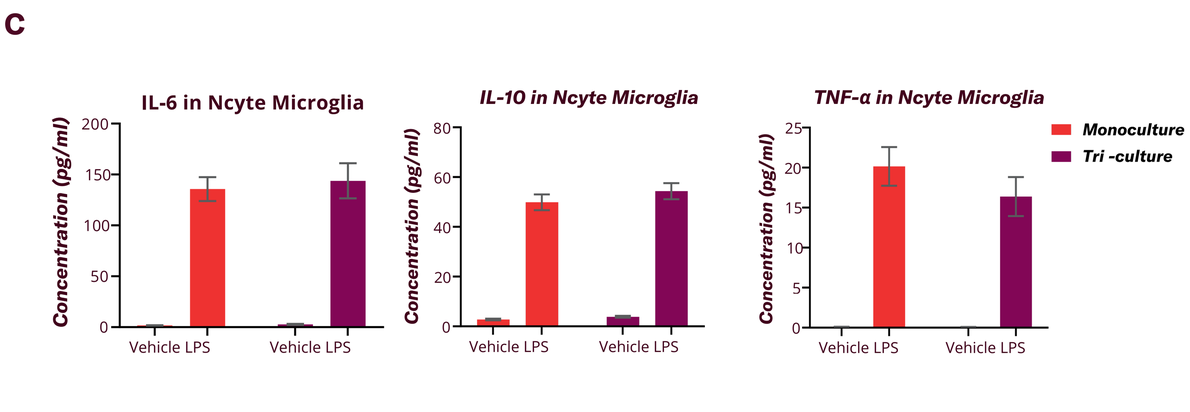

Figure C. Under basal condition, Ncyte microglia do not secrete key pro-inflammatory cytokines either in mono or tri-culture systems. Upon stimulation with LPS, Ncyte® Microglia exhibit a strong pro-inflammatory reaction both in monoculture as well as in a tri-culture system comprising of neurons, astrocytes, and microglia. LPS treatment significantly increases the production of the key inflammatory mediators IL-6, IL-10 and TNF-α in both models, as measured by Mesoscale Discovery.

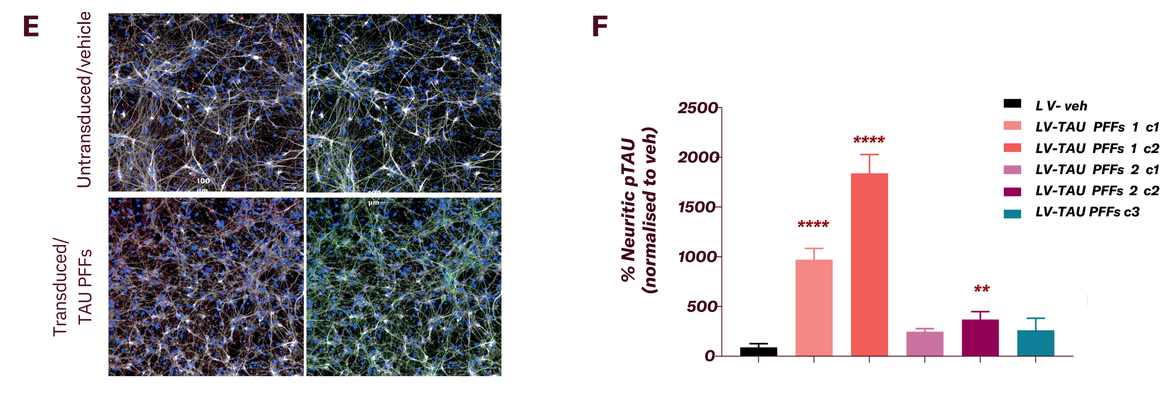

Figure E. Fluorescent images of untransduced and transduced day 21 post-thaw tri-cultures stained for phospho-TAU (red), MC-1 (green), TAU (grey) and DAPI (blue). Transduced/TAU PFFs treated tricultures show increased levels of TAU, pTAU and MC-1 TAU.

Figure F. High content-based quantification of the normalized parameters (pTAU puncta counts) of transduced tricultures (in black) compared to transduced tricultures treated with TAU PFFs.

Conclusion

In conclusion, the hiPSC-derived tri-culture model enables quantitative assessment of neuroinflammatory and neurotoxicity-related endpoints within a human-relevant cellular environment. The model provides a platform for investigating neuron–glia interactions and detecting adverse CNS pharmacodynamic effects and may support nonclinical evaluation of potential CNS safety liabilities and their translation to clinical risk assessment.

Screen your therapeutics' efficacy in reducing neuroinflammation and neurodegeneration with Ncardia's human *in vitro* tri-culture model

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