ioASTROCYTES ADVANCE THE DEVELOPMENT OF RELIABLE CO-CULTURE SYSTEMS FOR CNS RELATED DRUG DISCOVERY PIPELINES

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Abstract

Astrocytes are essential for maintaining homeostasis and defence within the central nervous system (CNS). They also play a key role in immune responses, making them crucial contributors to CNS disorders and neurodegenerative diseases. Therefore, accurate in vitro models that incorporate astrocytes are essential to advance our understanding of the cellular and molecular mechanisms underlying these diseases.

ioAstrocytes are functional human astrocytes derived from iPSCs, generated through deterministic cell programming using opti-ox™ technology. An optimised inducible system that tightly controls the expression of transcription factors to convert iPSCs consistently into astrocytes within days. ioAstrocytes exhibit a stellate morphology, express key astrocytic markers, can be co-cultured with other CNS cell types and recapitulate key human astrocytic functions. In particular, when exposed to pro-inflammatory stimuli, ioAstrocytes appropriately release cytokines such as IFN-β, IP-10, IL-6, and IL-8, which are central to the astrocyte-driven neuroinflammation in neurodegenerative conditions.

Astrocytes also contribute to neuroimmune responses by phagocytosing tissue debris and abnormal protein aggregates. ioAstrocytes effectively phagocytose pHrodo bioparticles, demonstrating their potential as a model to understand the role of astrocytes in clearing disease-specific protein aggregates, such as fibrillar amyloid aggregates in Alzheimer's disease. Moreover, the interaction between astrocytes and neurons is critical for various neuronal functions including survival, energy metabolism, ion homeostasis, and neuroprotection. ioAstrocytes support co-cultures with neurons, significantly influencing the neuronal network activity of ioGlutamatergic Neurons. This capacity highlights the potential of ioAstrocytes to be used in optimised co-culture models to identify compounds that protect from damaging insults within the CNS.

In conclusion, ioAstrocytes offer a powerful, scalable platform that bridges translational gaps in neurodegenerative disease research. By advancing our understanding of neuroinflammation and neurodegeneration, ioAstrocytes can support the development of targeted therapies for CNS disorders, making them an invaluable asset in drug discovery and therapeutic development.

1. Precise deterministic programming of hiPSCs into ioAstrocytes

ioAstrocytes rapidly acquire a consistent astrocyte phenotype

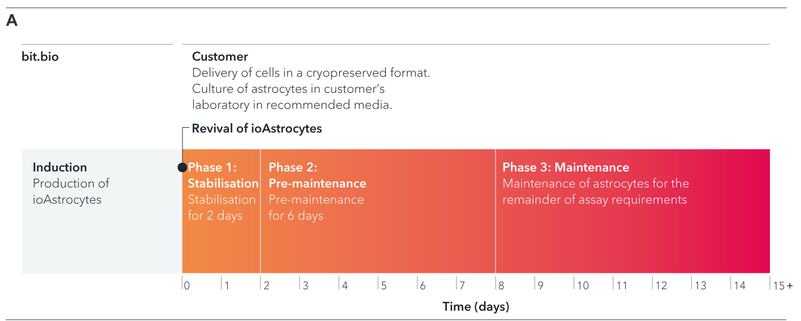
(A) Protocol for the generation of ioAstrocytes is a three-phase process: 1. Stabilisation. 2. Pre-maintenance. 3. From day 8 onwards, maintenance for the duration of assay requirement.

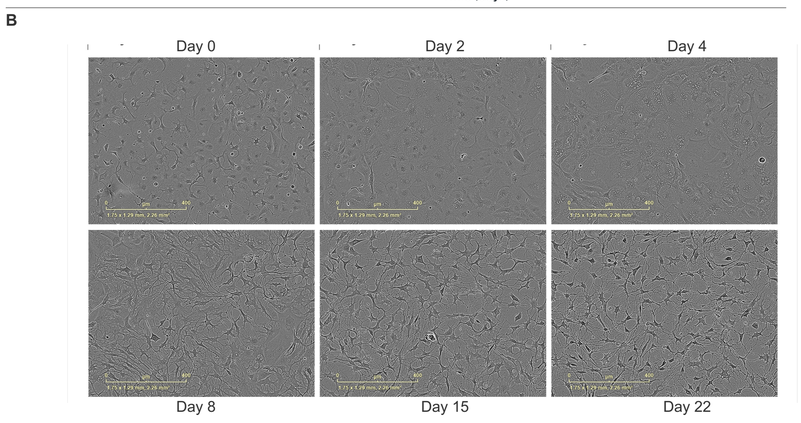
(B) ioAstrocytes consistently acquire a stellate astrocyte morphology from day 8 with branched, elongating processes that continue to intensify.

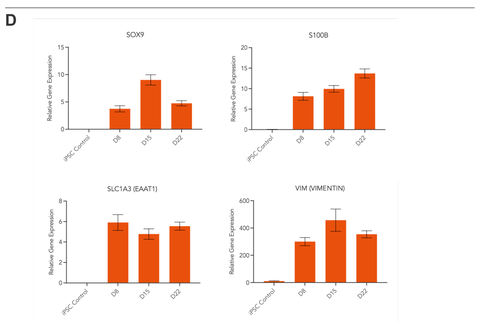
(C) Immunocytochemistry shows protein expression of key astrocyte markers at D22. S100B plays a key role in activation, neuroprotection, calcium homeostasis and astrocyte-neuron communication. SOX9 is critical for the differentiation of astrocytes. Vimentin is a cytoskeletal protein enriched in astrocytes.

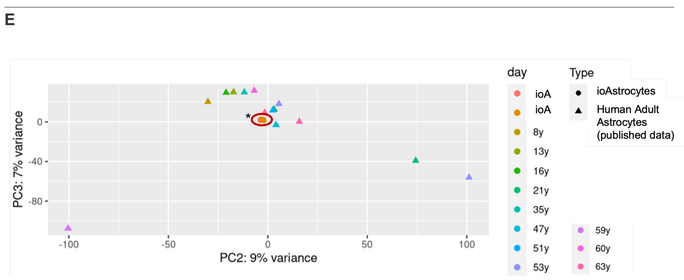
(D) Expression of key astrocyte markers EAAT1, SOX9, S100B and Vimentin (VIM) at D8, D15, and D22. ioAstrocytes show expression of key markers from as early as day 8. EAAT1 protein plays crucial roles in the regulation of glutamate neurotransmission, maintaining neuronal health and protecting against excitotoxicity.

(E) Gene expression profiles of ioAstrocytes at D15 and D22 cluster tightly together within human primary astrocytes samples obtained from adults (35 – 63 years), suggesting that ioAstrocytes are a suitable system for modelling human disorders and in biological studies.





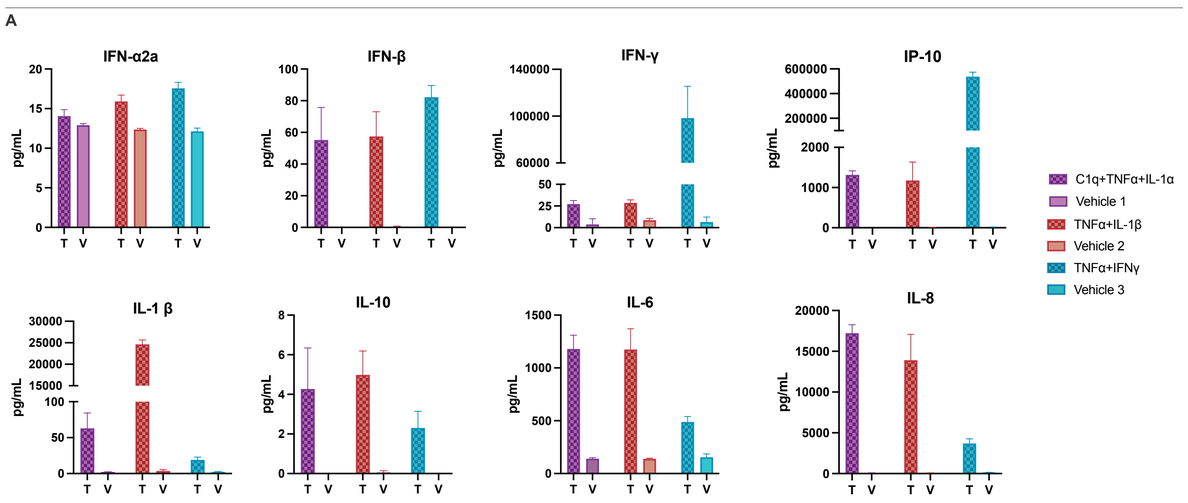




2. ioAstrocytes respond to pro-inflammatory cytokines

ioAstrocytes secrete cytokines in response to stimulation

(A) ioAstrocytes cytokine response after 24 hours of treatment with 3 different pro-inflammatory cocktails (T) or vehicle (V). MSD multiplex immunoassay demonstrates the ability of ioAstrocytes to secrete a range of cytokines upon treatment with various proinflammatory stimuli. ioAstrocytes display the expected responses to the three distinct cocktails, including a strong increase in Interleukin 6 (IL-6) secretion, known to be involved in neuroinflammation.



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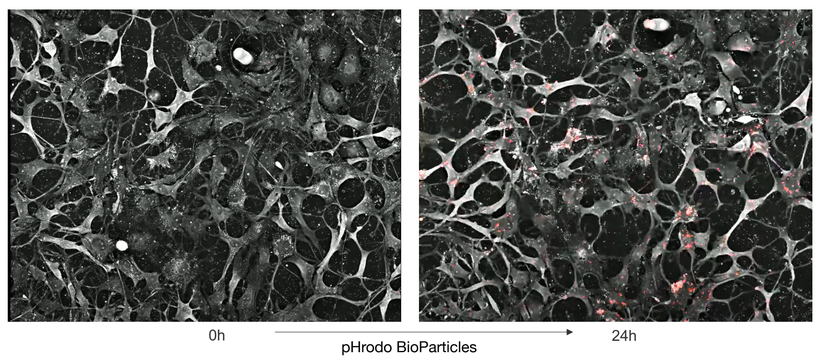
3. ioAstrocytes exhibit phagocytic functions

ioAstrocytes are capable of phagocytosing *S. aureus* bioparticles

(A) Images taken from a 24-hour NanoLive video showing the ability of the ioAstrocytes to phagocytose pHrodo® Red *S. aureus* Bioparticles®. Bioparticles were added to cultures of ioAstrocytes at D15 post-thaw. At the start (0h), the particles are located outside the cells and due to the neutral pH of the media are non-fluorescent, but when phagocytosed they are exposed to the acidic environments of intracellular organelles and fluoresce bright red, as they accumulate within the cell (24h).



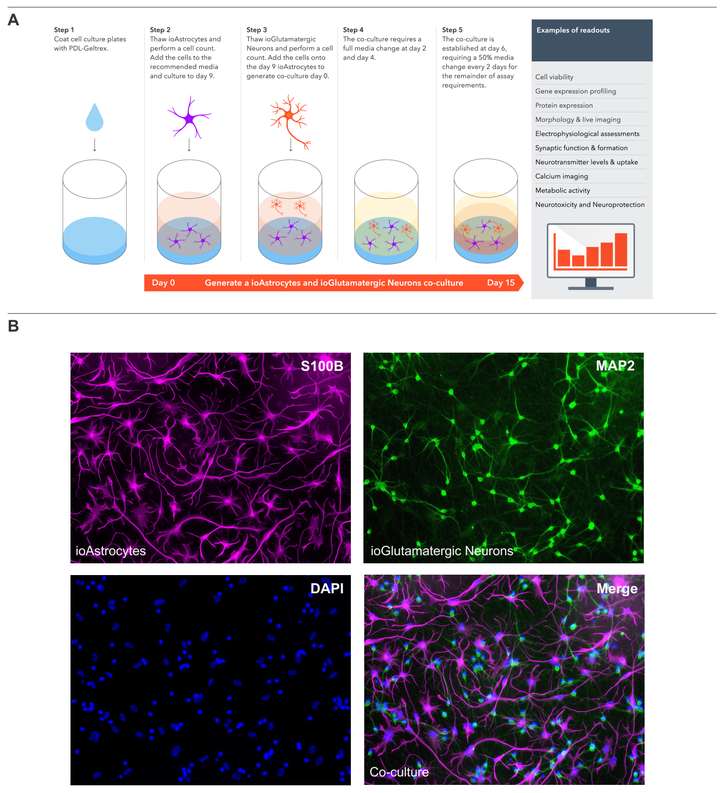
4. Co-culture of ioAstrocytes and ioGlutamatergic Neurons

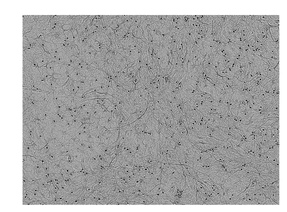
Co-culturing ioAstrocytes with ioGlutamatergic Neurons and associated disease models to facilitate research into complex neuroglial interactions

(A) Easy-to-use co-culture protocol for ioAstrocytes with ioGlutamatergic Neurons.

(B) Immunocytochemistry image of ioAstrocytes and ioGlutamatergic Neurons in co-culture; staining shows expression of pan-neuronal marker MAP2 (green), astrocyte marker S100B (purple) and DAPI nuclear staining (blue).

(C) Brightfield image of ioAstrocytes and ioGlutamatergic Neurons co-culture. Astrocyte morphology and mature ioGlutamatergic Neurons are observed when cells are co-cultured.



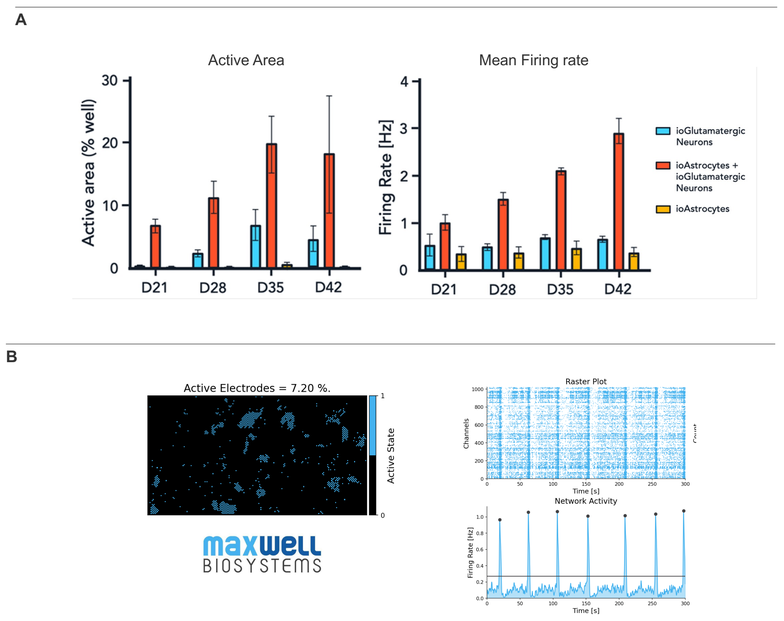


5. Modulation of neuronal activity in co-culture

ioAstrocytes support neuronal co-cultures and contribute towards network activity

(A) High-density multi electrode measurements of the neuronal activity of mono- and co-cultures of ioGlutamatergic Neurons and ioAstrocytes, showing the active area (% of well) and mean firing rate (Hz) at different time points. ioAstrocytes were directly derived from iPSC after a 10-day programming protocol (D10), and then harvested to seed with Day 0 (D0) ioGlutamatergic Neurons to establish their co-cultures. D10 ioAstrocytes and D0 ioGlutamatergic Neurons were also used to generate the respective mono-cultures.

(B) Representative image of the active area, raster plot and network activity of a well where D10 ioAstrocytes and D0 ioGlutamatergic Neurons were co-cultured for 35 days.



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Summary & conclusions

Co-culture ready – ioAstrocytes support functional neuronal networks within co-culture settings, enabling in-vitro modelling of complex CNS biology.

Functional – ioAstrocytes display key phagocytic and cytokine secretion functions as well as a demonstrable influence on neuronal network activity.

Consistent - Get reproducible results from every vial with lot-to-lot consistency of highly characterised & defined hiPSC-derived cells.

ioAstrocytes are functional human iPSC-derived astrocytes, deterministically programmed using opti-ox technology, converting pluripotent cells into defined, consistent, astrocytes, within days.

ioAstrocytes provide a unique opportunity to model human astrocyte behavior more accurately, allowing researchers to explore the complex interactions between astrocytes, neurons, and other CNS components.

By mimicking essential astrocyte functions such as inflammatory responses, protein clearance, and neuronal support, these models enable a deeper understanding of the cellular processes that drive neurodegeneration.
ioAstrocytes have the potential to not only advance basic research but also accelerate the identification of prospective therapeutic targets and the development of new treatments, making them a vital tool in combating CNS disorders.