Fast, reproducible and scalable generation of functional microglia from hiPSCs for neuroinflammation and neurodegenerative disease research & drug development

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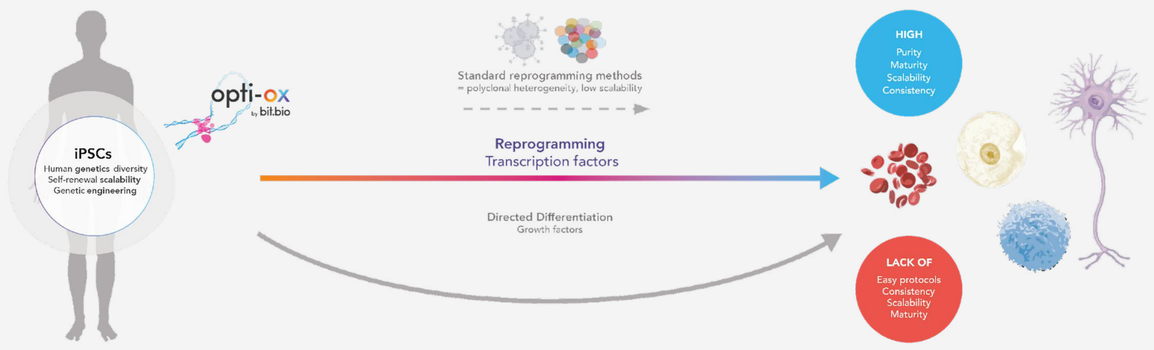
Abstract

Microglia are the tissue-resident macrophages of the brain, accounting for 10-15% of total cells within the central nervous system (CNS). These cells survey neuronal function, play roles in neurogenesis & synaptic remodelling, are the first responders to infection, and are thereby implicated in various CNS diseases. The life sciences sector relies predominantly on animal models to mimic disease states for drug discovery although they do not always recapitulate human cell and disease phenotypes. To bridge this translational gap, several in vitro human models have been developed for the study of microglia, most typically primary microglia extracted directly from either embryonic, neonatal or adult tissue. However, primary cells are limited in supply, difficult to source, and often show donor-to-donor and user variability.

There is a need for functional, consistent, & scalable disease-relevant human cells for both neuroimmune research and the development of therapeutics against neurodegenerative diseases. bit.bio's opti-ox™ (optimised inducible overexpression) technology enables the highly controlled expression of transcription factors to rapidly reprogram human iPSCs (hiPSCs) into somatic cell types, in a scalable manner. Using opti-ox precision reprogramming we have generated hiPSC-derived microglia, termed ioMicroglia, that within days are converted from hiPSCs to functional microglia. ioMicroglia, 10 days post-revival, display typical morphology and express key phenotypic markers (TMEM119, P2RY12, IBA1, CD11b, CD45, and CD14). RNA sequencing demonstrates that ioMicroglia have a transcriptomic signature similar to primary adult and foetal microglia. Functionally, ioMicroglia can perform phagocytosis, secrete proinflammatory cytokines upon stimulation, and can be co-cultured with glutamatergic neurons. Therefore, ioMicroglia provide a rapidly maturing, functional, consistent, and scalable hiPSC-based model system for neurodegenerative research and drug discovery.

bit.bio's approach to cellular reprogramming

Precise control of transcription factor expression through iPSC engineering



While the use of human iPSC derived cell models have been hindered by the lack of consistency and scalability of differentiation methods, bit.bio technologies (Discovery Platform & opti-ox) are opening new avenues by allowing controlled expression of TF combinations for optimal cellular reprogramming of human cell types from hiPSCs.

1. Human ioMicroglia are ready for experiments within 10 days

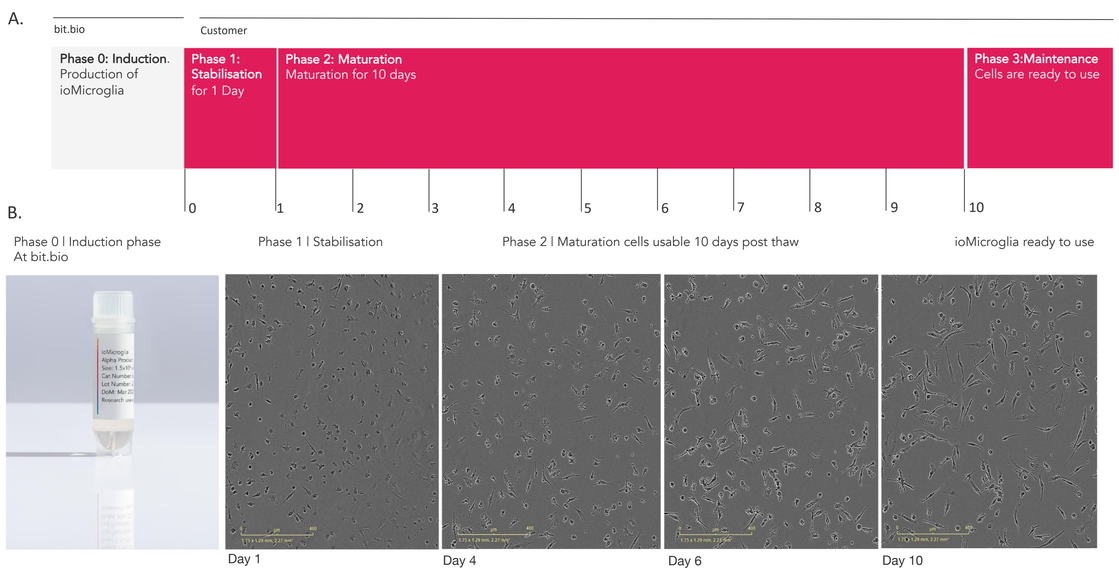


Figure 1: Generation of ioMicroglia.

(A) Cells are shipped in a cryopreserved format and are programmed to mature into microglia upon revival and cultured in the recommended media. The protocol for generation is in 4 phases. Phase 0: an induction phase carried out at bit.bio. Phase 1: stabilisation for 24 hours with doxycycline. Phase 2: maturation for a further 9 days Phase 3: the maintenance phase, from day 10 the cells are ready to use. **(B)** Images show Microglia stabilisation and maturation phases post thaw. Key microglia morphology is observed from day 4 with cells ready to use at day 10. Images acquired on the incucyte® at 10x magnification, 400µm scale bar.

2. ioMicroglia express phenotypic markers

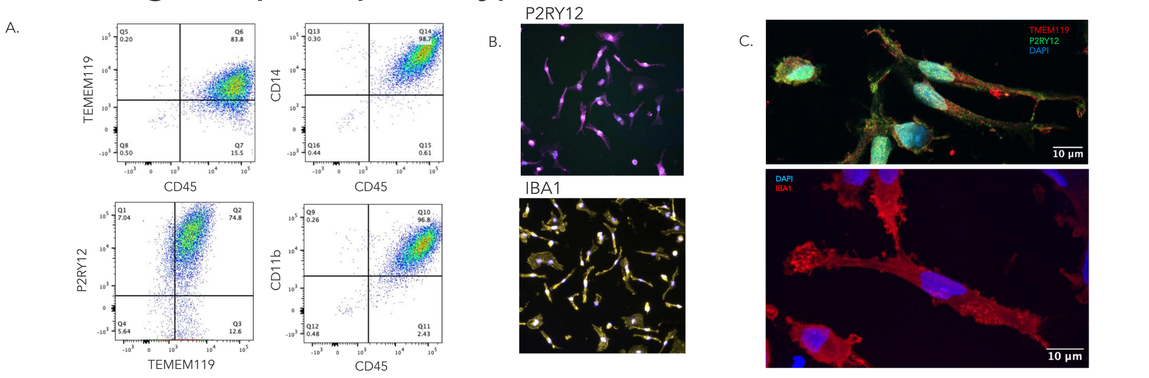


Figure 2: ioMicroglia have high purity and are homogenous for key marker expression.

(A) Flow cytometry analysis of day 10 cells shows key microglia marker expression of TMEM119, P2RY12, CD14, CD45 and CD11b with a purity of above 95% for CD45, CD11b and CD14 >80% TMEM119+ CD45+ and >70% TMEM119+ P2RY12+. **(B)** immunocytochemistry staining shows homogenous expression of P2RY12 and IBA1 image taken at 10x **(C)** immunocytochemistry images show co-staining for TMEM119 and P2RY12 along with IBA1 expression. Cells display key morphology and marker expression, images taken at 20x with 10µm scale bar.

3. ioMicroglia phagocytose *e.coli* and amyloid beta-42

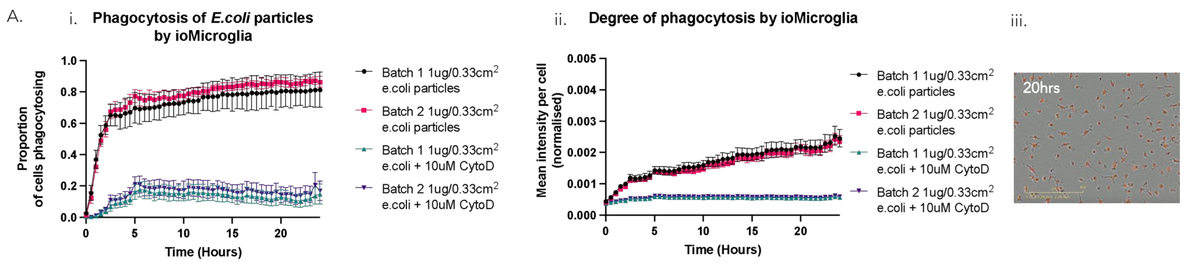


Figure 3: ioMicroglia can phagocytose *e.coli particles* and Aβ42 (A) Day 10 ioMicroglia were incubated with 1ug/0.33cm^2 pHrodo™ RED labelled *e.coli* particles for 24 hours +/- cytochalasin D control. Images were acquired every 30 mins on the incucyte® looking at red fluorescence and phase contrast (i) Proportion of cells phagocytosing *e.coli* particles over 24 hours. (ii) Fluorescence intensity per cell displaying degree of phagocytosis per cell, data from two independent batches. (iii) representative images of phagocytosis at 20 hours post treatment. **(B)** Day 10 ioMicroglia were incubated with 500nM AF488 labelled Aβ42 +/- cytochalasin D for 20 hours with images acquired every 30 mins on the incucyte and degree of phagocytosis calculated based on fluorescence.

4. ioMicroglia secrete proinflammatory cytokines upon activation

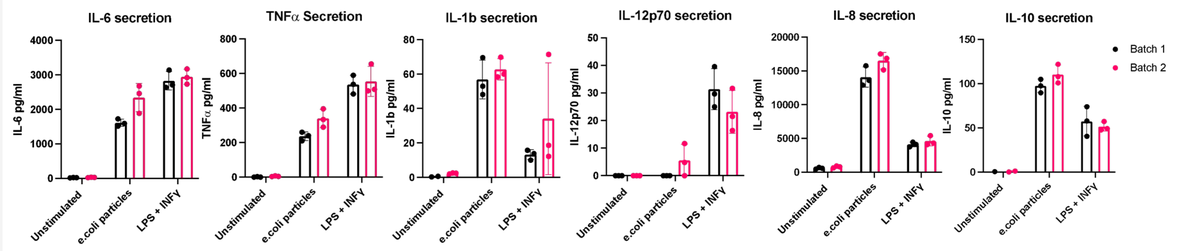
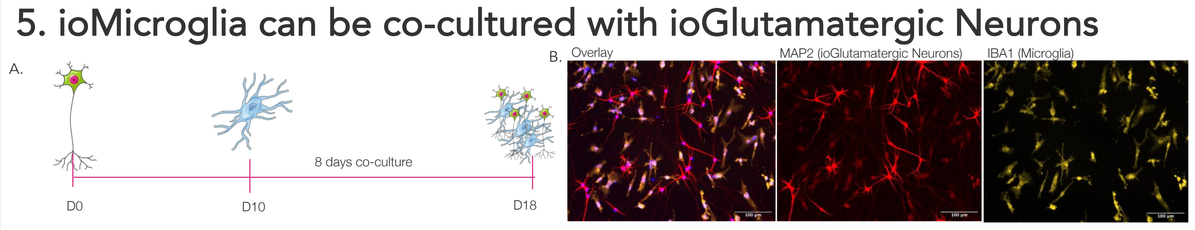


Figure 4: ioMicroglia secrete proinflammatory cytokines. ioMicroglia were stimulated with LPS 100ng/ml and IFNɣ 20ng/ml for 24 hours or pHrodo™ RED labelled *e.coli* particles. Supernatants were harvested and analysed using MSD V-plex proinflammatory kit™. ioMicroglia secrete TNFα, IL-6, IL-8, IL-1b, IL-10 and IL-12p70 in response to stimuli. Predominantly producing a proinflammatory response. This is consistent between two independent batches.

5. ioMicroglia can be co-cultured with ioGlutamatergic Neurons



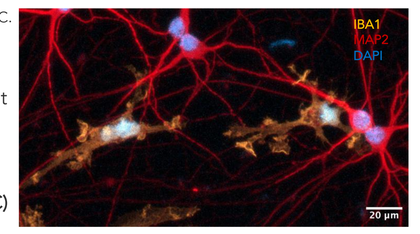


Figure 5: ioMicroglia can be co-cultured with ioGlutamatergic Neurons.

(A) Schematic describing the co-culture process ioGlutamatergic Neurons were seeded and matured from day 0. ioMicroglia were added directly to ioGlutamatergic Neurons at day 10. Morphology and marker expression was assessed at day 18. **(B)** immunocytochemistry at day 18 shows expression of IBA1 (ioMicroglia) and MAP2 (ioGlutamatergic Neurons), within the co-culture images at 10x with 100µm scale bar. **(C)** Confocal microscopy Image taken at 40x, 20µm scale bar. Microglia display a more ramified morphology and indications of interactions with neurons.

6. ioMicroglia are highly defined and cluster to primary microglia

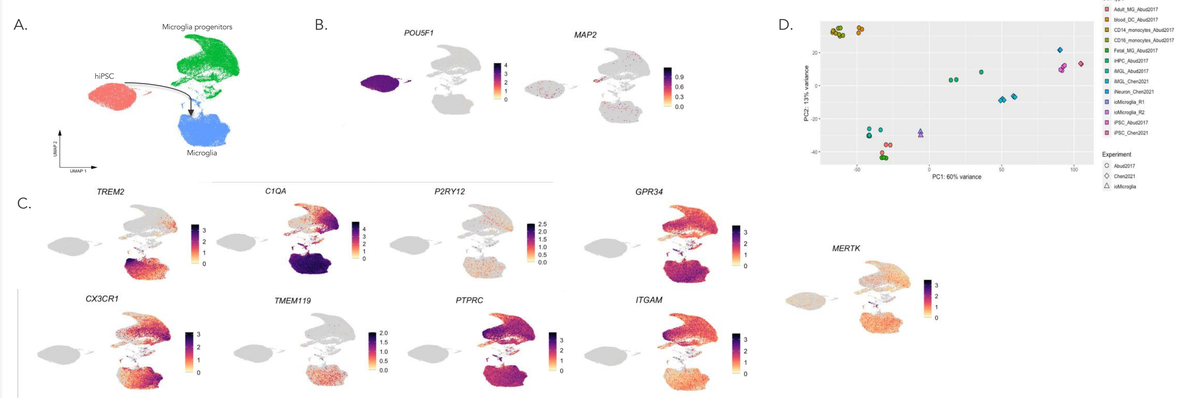


Figure 6: Expression profile of key microglia markers and comparison to primary cell data by single cell (scRNA seq) and Bulk RNA seq. (A) Displays the developmental process from iPSCs into microglia by scRNA seq. **(B)** Expression of both iPSC (POU5F1) and pan-neuronal marker (MAP2) shows microglia are not enriched for either of these populations. **(C)** Microglia show enrichment for phenotypic markers (TREM2, C1Qa, P2RY12, GPR34, CX3CR1, TMEM119, PTPRC, ITGAM, MERTK) matching protein expression in figure 2. **(D)** PCA plots of BULK RNA seq data show ioMicroglia cluster closely to primary foetal and adult microglia data sets derived from Abud et al, 2017(1).

Summary and conclusions

- opti-ox driven hiPSC-derived Microglia, ioMicroglia, are generated within 10 days post thaw.
- ioMicroglia are a defined population with phenotypic marker expression above 96% indicated by flow cytometry, immunocytochemistry and morphology. scRNA seq and BULK RNA seq data further confirms microglia cell identity showing loss of pluripotency markers during the differentiation process.
- ioMicroglia display functional traits, proinflammatory cytokine secretion and phagocytosis, with consistency across batches.
- Upon co-culture with ioGlutamatergic Neurons, ioMicroglia show a physiologically relevant ramified morphology.
- In addition, ioMicroglia have a similar transcriptomic profile to primary microglia and other hiPSC-derived microglia[1].
- ioMicroglia provide a defined, consistent and functional hiPSC-based model system for basic research and drug discovery applications in the CNS and immune fields.

References: 1) Abud E, et al., Neuron, 2018; 94(2): 278-293