Rapid and consistent generation of functional microglia from reprogrammed hiPSCs to study mechanisms in neurodegeneration and neuroinflammation

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Abstract

Microglia are the tissue-resident macrophages of the brain, accounting for 75-80% of leukocytes and 10-15% of total cells within the central nervous system (CNS). They 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 rodent models to mimic disease states for drug discovery. However, animal models 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 microglia cells for neuroimmune research and the development of therapeutic or preventive strategies for neurodegeneration. We used transcription factor mediated precision cellular reprogramming technology, opti-ox™, to rapidly and consistently generate mature, functional, and physiologically relevant microglia, named ioMicroglia, from hiPSCs, at scale.

ioMicroglia, 10 days post-revival, display typical morphology and express key phenotypic markers including TMEM119, TREM2, P2RY12, and IBA1. RNA sequencing demonstrates that ioMicroglia have a transcriptomic signature similar to primary adult and foetal microglia. Consistent phagocytic and cytokine secretion functionality, with various stimuli, including amyloid beta, has been demonstrated for ioMicroglia, across multiple independent laboratories within industry and academia, highlighting the experimental reproducibility of ioMicroglia. Importantly, ioMicroglia can be co-cultured with neurons to more closely mimic in vivo brain function.

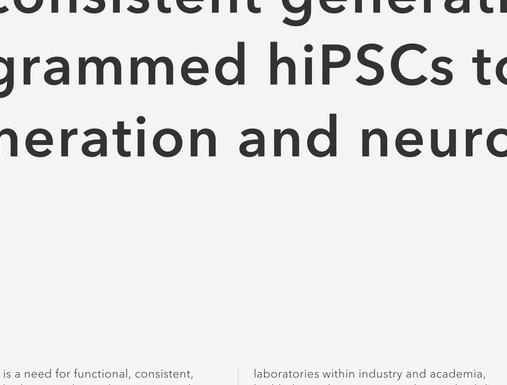
In conclusion, with opti-ox™ precision cellular reprogramming, hiPSCs are rapidly converted into functional microglia offering a robust and scalable source of human microglia which can be used as a relevant in-vitro model to investigate the role of the CNS's immune system in health and disease, and to develop novel therapies for neuroinflammation.

1. Human ioMicroglia are ready to use in 10 days and show high lot-to-lot consistency

Generation of precision reprogrammed microglia

A) Cells are shipped in a cryopreserved format and are programmed to mature into microglia upon revival and culture 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. Cells are ready to use from day 10.

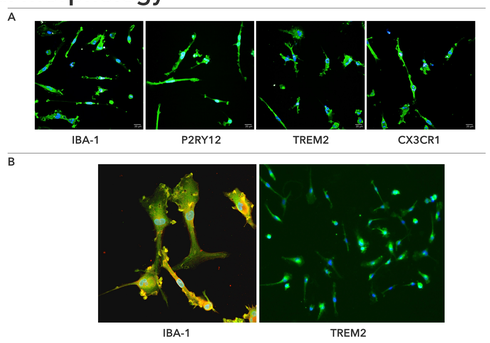
B) Bulk RNA sequencing analysis was performed on three independent lots of ioMicroglia at three different time points throughout the reprogramming protocol. Principal component analysis represents the variance in gene expression between the lots of ioMicroglia and shows high consistency across each lot at each given timepoint.



2. ioMicroglia show homogenous expression of key microglial markers and display typical morphology

A) Immunocytochemistry (ICC) was performed on day 10 ioMicroglia with antibodies against key microglia markers, IBA1, P2RY12, TREM2 and CX3CR1, and Hoechst counterstain (blue). ioMicroglia display homogenous expression of all key markers and show a typical ramified morphology. Images taken at 40x magnification on Phenix. This data was generated by Dr Milica Bulajic from Biogen (1).

B) ICC staining was performed on day 10 ioMicroglia with antibodies against key microglia markers, IBA1, TREM2, and phalloidin with DAPI counterstains (red, blue). ioMicroglia display homogenous expression of both these key markers and show a typical ramified morphology. Images taken at 20x magnification.

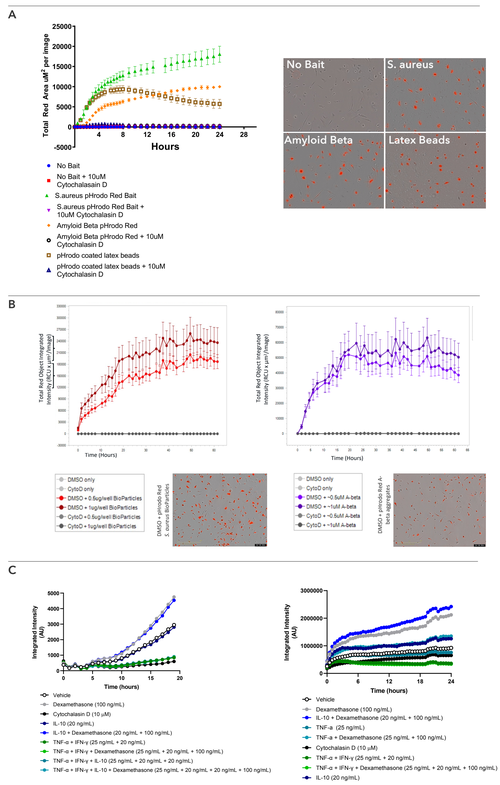


3. ioMicroglia show consistent phagocytic function with different baits including amyloid beta

A) Phagocytosis assays were performed on day 10 ioMicroglia using pHrodo RED™ *S.aureus* , pHrodo RED™ Amyloid-beta, and pHrodo RED™ latex beads +/- cytochalasin D control. Graph shows that ioMicroglia are able to phagocytose efficiently both *S.aureus* and Amyloid-beta, as expected. Representative images of phagocytosis post-incubation are also shown. Analysis was performed on the IncuCyte based on red fluorescence and phase contrast. This data was generated by Dr Helen Graves at Alchemab Therapeutics (2).

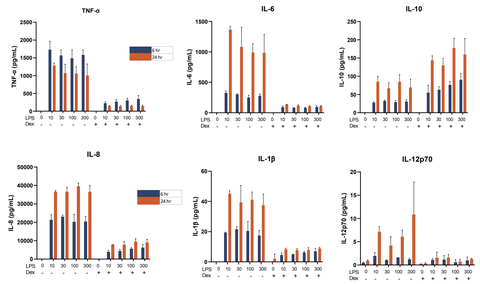
B) Phagocytosis assays were performed with day 10 ioMicroglia using pHrodo™ Red labelled *S.aureus* bioparticles and pHrodo™ RED Amyloid-beta +/- cytochalasin D control. Graphs show that ioMicroglia are able to phagocytose efficiently both *S.aureus* bioparticles and Amyloid-beta, as expected. Representative images of phagocytosis at 2 days post-incubation are also shown. Analysis was performed on the IncuCyte based on red fluorescence and phase contrast. This data was generated by Dr Milica Bulajic from Biogen (1).

C) Phagocytosis assays were performed with bacterial bioparticles with either HMC3 cells (graph on left) or day 10 ioMicroglia (graph on right), after pre-stimulation for 24 hours with IL-10; IL-10 and dexamethasone; TNF-α and IFN-γ; TNF-α, IFN-γ and dexamethasone; TNF-α, IFN-γ and IL-10; TNF-α, IFN-γ, IL-10, and dexamethasone. ioMicroglia show an overall greater phagocytic response than hMC3 cells. Effects on phagocytosis efficiency are observed with dexamethasone, IL-10 or combination (+) or TNF-α, TNF-α and IFN-γ or cytochlasin D (-) controls, as expected. This data was generated by Dr Christopher Cook from Concept Life Sciences (3).



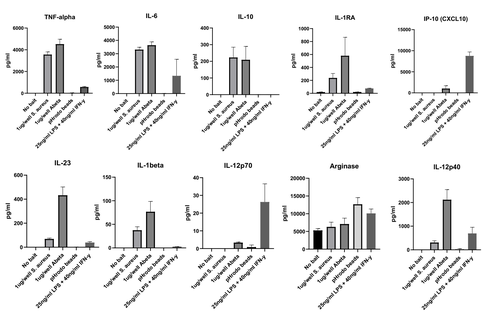
4. ioMicroglia show a proinflammatory cytokine response to LPS and dexamethasone treatment

ioMicroglia were stimulated with increasing concentrations of LPS (10, 30, 100 and 300 ng/ml) for 6-24 hours +/- dexamethasone. Supernatants were harvested and analysed using MSD V-plex proinflammatory kit. ioMicroglia secrete TNF-α, IL-6, IL-10, IL-8, IL-1β and IL-12p70 upon treatment with LPS and inhibition observed when treated with dexamethasone except for IL-10, as expected. ioMicroglia predominantly produce a proinflammatory response. This data was generated by Malika Bsibsi, Kimberly Lo, Matteo Zanella, Lieke Geerts, and Stefan Kostense from Charles River Laboratories (4).



5. ioMicroglia show functional cytokine secretion in response to various stimuli

ioMicroglia were stimulated with pHrodo RED™ *S.aureus* , pHrodo RED™ Amyloid-beta, or LPS and IFN-γ for 24 hours. Supernatants were harvested and analysed using the Biolegend Human Macrophage/Microglia Legendplex Kit. ioMicroglia predominantly produce a proinflammatory response to the different stimuli. This data was generated by Dr Helen Graves at Alchemab Therapeutics (2).

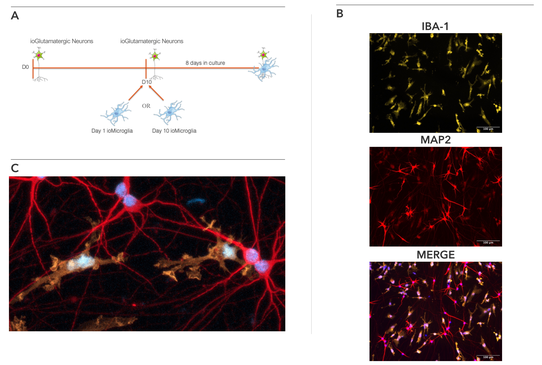


6. ioMicroglia form co-cultures with ioGlutamatergic Neurons

A) Schematic showing the co-culture protocol. ioGlutamatergic Neurons were cultured to day 10. ioMicroglia cultured to either day 1 or day 10 were added directly to day 10 ioGlutamatergic Neurons. The co-cultures were maintained for a further 8 days.

B) ICC analysis at day 8 of the co-cultures shows expression of IBA1 and MAP2. Representative images at 10x with 100µm scale bar.

C) Confocal image at 40x, 20µm scale bar. Microglia display a more ramified morphology and indications of interactions with neurons.



Summary & conclusions

ioMicroglia can be generated within 10 days post-thaw and show typical ramified morphology, and homogeneous expression of key markers, IBA1, P2RY12, TREM2 and CX3CR1.

Co-cultures containing ioMicroglia and ioGlutamatergic Neurons can be generated, where the microglia display a more ramified morphology, and show indications of interactions with the neurons.

Consistent phagocytic and cytokine secretion functionality, with various stimuli, has been demonstrated for ioMicroglia, by several independent industrial and academic laboratories, showing their experimental reproducibility.

References:
1. Dr Milica Bulajic, Translational Cell Sciences, Biogen, 225 Binney Street, Cambridge, MA 02142, USA.
2. Dr Helen Graves, Alchemab Therapeutics, Babraham Research Campus, Cambridge, UK.
3. Dr Christopher Cook, Concept Life Sciences Limited, Edinburgh BioQuarter, 9 Little France Road, Edinburgh, EH16 4UX, UK.
4. Malika Bsibsi, Kimberly Lo, Matteo Zanella, Lieke Geerts, and Stefan Kostense, Charles River Laboratories, Darwinweg 24, 2333 Leiden, The Netherlands.

May 2023