Generation and functional characterisation of motor neurons derived through transcription factor mediated programming of human pluripotent stem cells

Authors

L. Foulser
T. Brown
G. Mastrogiovanni
H. Garnett
J. Conde-Vancells
K. Firth
M. Dermit
M. Herrera-Vaquero
M. Kotter
M. Metzakopain
P. Balfour
S. Milde
T. Oosterveen
V. Yianni
W. Bernard

*Poster presenter (luke.foulser@bit.bio)

bit.bio
The Dorothy Hodgkin Building
Babraham Research Campus
Cambridge CB22 3FH
United Kingdom
info@bit.bio I www.bit.bio

Abstract

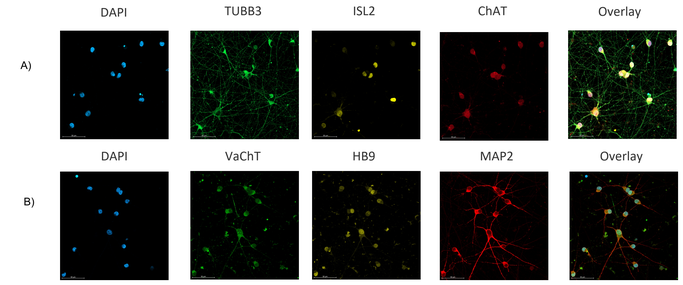
High-density microelectrode arrays (HD-MEAs) are a powerful tool to measure the electrophysiological properties of human neurons in vitro and are a cornerstone method in the development of new therapeutics for neurological diseases. High-density electrophysiology experiments that produce publishable, translatable data rely on high-quality, functional, and physiologically relevant cells as input.

Our proprietary opti-ox™ (optimised inducible overexpression) iPSC reprogramming technology enables highly controlled expression of transcription factors, which can rapidly reprogram hiPSCs into specific cell types of interest, to provide a robust, consistent, reliable and scalable cell source for in vitro applications.

In this poster, we demonstrate how the MaxWell MaxTwo HD-MEA system has been used for functional characterisation of opti-ox™ precision reprogrammed ioMotor Neurons™, in addition to protein expression and transcriptomic analysis. Electrophysiological properties were assessed by a MaxTwo 'activity scan' confirming electrical activity as early as day 14 post-thaw, which further increased over a period of 42 days. Furthermore, we show that ioMotor Neurons™ are responsive to electrical stimulation using the MaxTwo 'electrical stimulation' module.

In conclusion, opti-ox™ technology can be utilised for the scalable and consistent production of functional hiPSC-derived motor neurons. These cells, termed ioMotor Neurons™, have the potential to advance the development of new therapeutics for MNDs and to further our understanding of motor neuron development and maturation in vitro.

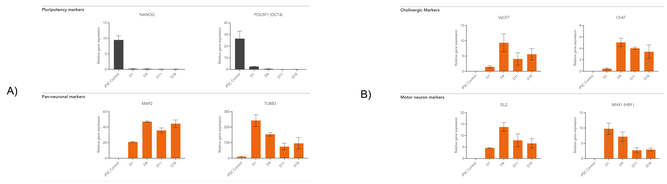
1. ioMotor Neurons homogeneously express key motor neuron markers



A) Immunofluorescent staining on day 11 of reprogramming demonstrates that ioMotor Neurons are positive for TUBB3 (green), ISL2 (yellow) and ChAT (red). DAPI was used as counterstain (blue). MAP2 positive neurons co-localize with the motor neuron markers ISL2 and ChAT indicating that cells have a motor neuron identity. Scale bar is 50um.

B) Immunofluorescent staining on day 11 of reprogramming showing that ioMotor Neurons are all positive for LHX3 (green), HB9 (yellow) and MAP2 (red). DAPI was used as counterstain (blue). Scale bar is 50um.

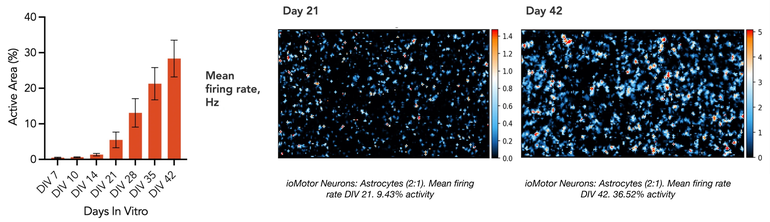
2. RT-qPCR – Gene expression of key neuronal & lower motor neuron markers



A) RT-qPCR with ioMotor Neurons at 5 timepoints day 0 (iPSCs), 1, 4, 11, and 18. A swift downregulation of pluripotency markers (NANOG and OCT4) can be seen by Day 1. From day 1, ioMotor neurons show consistent expression of the pan-neuronal markers MAP2 and TUBB3.

B) From day 1, ioMotor neurons show upregulation of cholinergic markers (VaChT, ChAT). For motor neuron specific markers, ioMotor Neurons show upregulation of markers ISL2 and MNX1 (HB9) as early as Day 1.

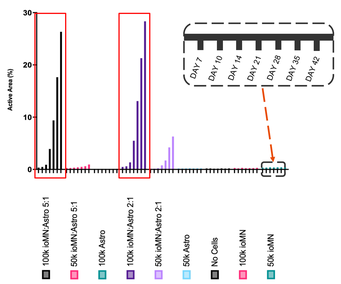
3. ioMotor Neurons rapidly gain functional activity over time.



A) ioMotor Neurons were plated with astrocytes at a 2:1 ratio to perform Multi-Electrode Array (MEA) analysis over a period of 42 days. ioMotor Neurons show electrical activity as early as 14 days post-thaw, with % of active area increasing throughout the length of the experiment.

B) Heatmap showing increase in % active area from day 21 (9.43%) to day 42 (36.52%) in culture.

4. Active area comparison between different co-culture and mono-culture conditions.



Bar graph showing active area percentage for multiple experiment conditions. Conditions include variations of densities and mono- and or co-cultures.

Areas highlighted in red show significant increase in active area over time demonstrating astrocytes accelerate maturation and support synaptic formation; thus; robust and abundant signal bursts resulting from microcircuit assembly in cocultures.

Mono-culture active areas are negligible, some detection – but significantly lower than co-culture scenarios. This indicates that ioMotor Neurons™ are of high purity and unable to form synapses without the presence of other cell types (see 100/50k Astro & 100/50k ioMN).

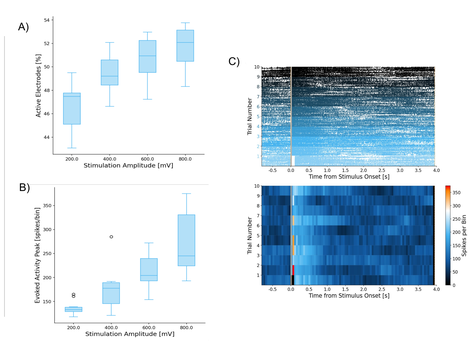
5. ioMotor neurons respond to electrical stimulation

Electrical stimulation was performed at Day 42 for four different voltages (200mV, 400mV, 600mV, and 800mV) over 10 different trials. ioMotor Neurons were plated with astrocytes at a 2:1 ratio.

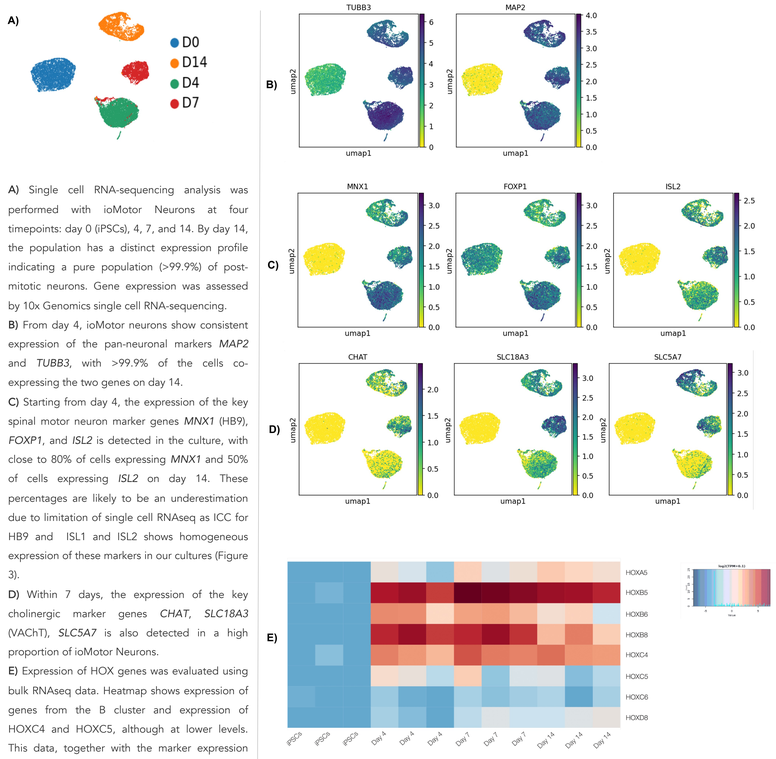
A) ioMotor Neurons become more active as the intensity of the stimulus increases.

B) ioMotor Neurons evoke a higher peak activity as the stimulus increases.

C) Plots showing immediate response to the stimulus before returning to baseline.



6. Single cell RNA-sequencing show ioMotor Neurons are a pure population of neurons with lower motor neuron identity



A) Single cell RNA-sequencing analysis was performed with ioMotor Neurons at four timepoints: day 0 (iPSCs), 4, 7, and 14. By day 14, the population has a distinct expression profile indicating a pure population (>99.9%) of post-mitotic neurons. Gene expression was assessed by 10x Genomics single cell RNA-sequencing.

B) From day 4, ioMotor neurons show consistent expression of the pan-neuronal markers *MAP2* and *TUBB3*, with >99.9% of the cells co-expressing the two genes on day 14.

C) Starting from day 4, the expression of the key spinal motor neuron marker genes *MNX1* (HB9), *FOXP1*, and *ISL2* is detected in the culture, with close to 80% of cells expressing *MNX1* and 50% of cells expressing *ISL2* on day 14. These percentages are likely to be an underestimation due to limitation of single cell RNAseq as ICC for HB9 and ISL1 and ISL2 shows homogeneous expression of these markers in our cultures (Figure 3).

D) Within 7 days, the expression of the key cholinergic marker genes *CHAT*, *SLC18A3* (VAChT), *SLC5A7* is also detected in a high proportion of ioMotor Neurons.

E) Expression of HOX genes was evaluated using bulk RNAseq data. Heatmap shows expression of genes from the B cluster and expression of HOXC4 and HOXC5, although at lower levels. This data, together with the marker expression from single cell RNAseq, suggests that ioMotor Neurons have a posterior hindbrain or spinal cord (cervical region) identity.

Note, this data is from cells in continuous culture, so minor variations may exist between this data and data from cryopreserved cells.

ioMotor Neurons are a pure population of neuronal cells with homogeneous expression of key lower motor neuron markers including MNX1 (HB9), FOXP1, ISL2 as characterized by single cell RNA sequencing and ICC.

ioMotor Neurons have a defined cholinergic identity as shown by the expression of key cholinergic genes CHAT, SLC18A3 and SLC5A7.

ioMotor Neurons offer rapid functionality – spontaneous activity is demonstrated by HD-MEA.

Mono-cultures are devoid of activity due to high purity resulting in a lack of synapse formation.

Electrical stimulation of ioMotor Neurons shows response to stimulus. Further, as voltage increases, as does the activity and intensity of the culture.

ioMotor Neurons can be generated with high consistency, as shown by bulk RNA-sequencing.

Rapidly maturing motor neurons that express key motor neuron already from day 4 post-revival.

Cells are easy to culture using a simple, 3-step protocol – ideal for researchers without iPSC expertise.

References:
Pawlowski, et al Stem Cell Reports. 2017 Apr 11;8(4):803-812.

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