A MICROFLUIDICS PLATFORM FOR HUMAN TAU MUTATION NEURONS

Emma Torrance[1], Graham Robertson[1], Selina Wray[2], John Hardy[2], Trevor Bushell[1] and Michele Zagnoni[1*]

[1]University of Strathclyde, UK, and [2]University College London, UK

ABSTRACT

This paper reports a microfluidic approach for assessing network formation in human induced pluripotent stem cell (iPSC)-derived neurons. For the first time, we show the ability of iPSC-derived neurons to form functional synaptically driven networks within a controlled microfluidic environment, thus providing an *in vitro* stem cell based model for studying dementia.

KEY WORDS: induced pluripotent stem cell derived neurons, frontotemporal dementia, tau mutation

INTRODUCTION

Neurological disorders are an increasing problem upon the health care system. As reported by the World Health Organization, dementia affects an estimated 35.6 million people worldwide, with this number tripling by 2050 [1]. One of the main pathophysiological features of dementia is neuronal cell death and subsequent destruction of neuronal networks and synaptic connectivity within the brain. The most common cause of dementia, Alzheimer's Disease, is characterized by intraneuronal aggregates of the microtubule-associated protein tau. Coding and splice-site mutations in the tau gene (*MAPT*) have also been shown to be a cause of frontotemporal dementia (FTD) with tau pathology. Whilst the use of current translational animal models has shown limited success, miniaturized *in vitro* models are a promising alternative for mimicking disease conditions. Indeed, combining microfluidic and neuroscience techniques enables the spatiotemporal control of the extracellular environment and network formation to be obtained with microscale precision [2]. Here, we present a microfluidic approach to study the functional communication between co-cultures of iPSC-derived cortical neurons obtained from control and FTD patients as a model for human neurodegenerative disease.

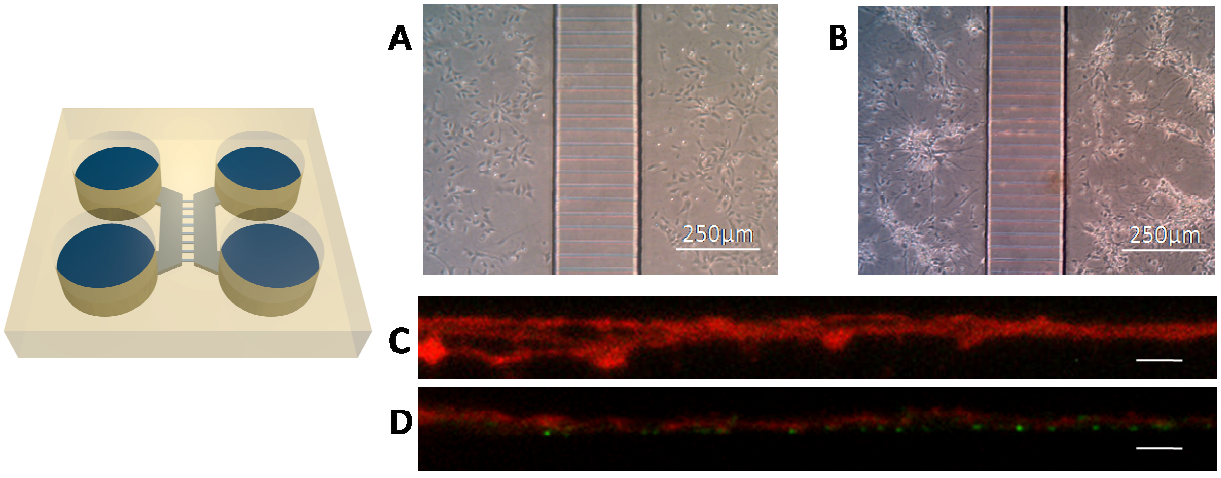

Figure 1: iPSC-derived neurons formed functional synapses within microchannels at DIV84. iPSC-derived neurons were seeded into devices after 40 days post-differentiation and cultured for up to 100 days. iPSCs imaged using brightfield microscopy at (A) DIV41 and (B) DIV46. Neurite growth within the microchannel stained for neuronal marker, β-III tubulin (red) and synaptophysin (green) for synaptic vesicles at (C) DIV50 and (D) DIV84. Scale bar is 10µm unless otherwise stated.

EXPERIMENTAL

The device, based upon a structure originally employed for axonal damage and repair [3], has two environmentally isolated chambers to permit cellular adhesion and growth (Figure1A&B). Between the chambers are a series of microchannels, which allow for axonal projections to pass through and build synaptic connections between the two populations, ultimately forming a synaptically connected neuronal network. iPSC were differentiated into cortical glutamatergic neurons *in vitro* by dual SMAD inhibition followed by an extended period of neurogenesis [4], before transferring into the microfluidics at day 40 post-differentiation. Neurite growth and synapse formation in the microchannels was confirmed by β-III tubulin and synaptophysin immunofluoroscent staining at various time points. For these stages, synaptic activity between the two populations was also tested using indirect and direct applications of glutamate following a recently developed microfluidic protocol [5].

RESULTS AND DISCUSSION

Initially, at 50 days *in vitro* (DIV50), neurite growth was confirmed within the microchannels, however, the absence of synaptophysin staining indicated no synapse formation (Figure1C). Conversely, at DIV84, synaptophysin staining revealed clear synapse formation at this later time point (Figure1D).

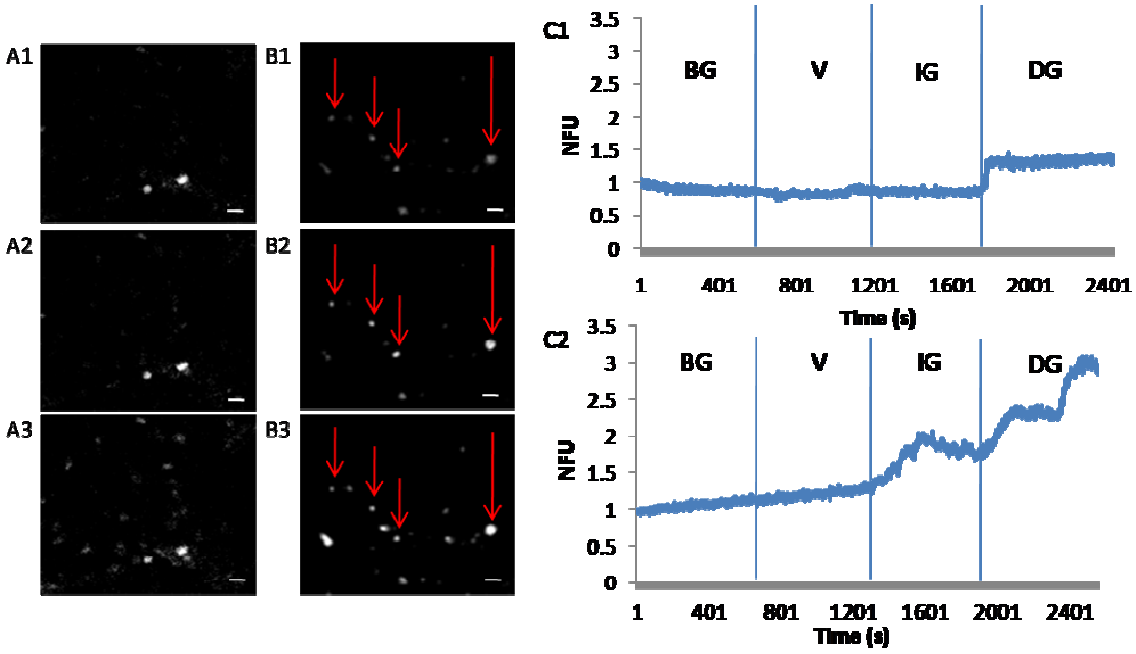

Figure 2: iPSC-derived neurons formed functional synaptically driven networks. iPSC-derived neurons were loaded with Flou4 (5µM, 1h) and the intracellular calcium levels were monitored in the presence and absence of glutamate application. Representative image illustrating the intracellular Ca^{2+} levels at DIV50 in A1) Vehicle control, A2) Indirect glutamate application, A3) Direct glutamate application and at DIV84, B1) Vehicle control, B2) Indirect glutamate application, B3) Direct glutamate application. Representative normalized traces of calcium fluxes from cells during an experiment illustrating the increases in intracellular Ca^{2+} in DIV84 (C2) neurons but not DIV50 (C1) after indirect glutamate stimulation (100µM) via the opposing culture. Scale bar is 10µm. BG, background; V, vehicle; IG, indirect glutamate; DG, direct glutamate.

To ascertain the functional cell connectivity between the two environmentally isolated chambers, calcium imaging was used to monitor neuronal activity in response to the direct and indirect application of glutamate (100µM) [5]. At DIV50, cells responded to direct application of glutamate but not its indirect application, confirming the lack of synapse formation within these cultures. At DIV84 and

DIV91, cells responded to both direct and indirect glutamate application revealing matured synaptic connectivity between the two isolated neuronal networks (Figure2B2).

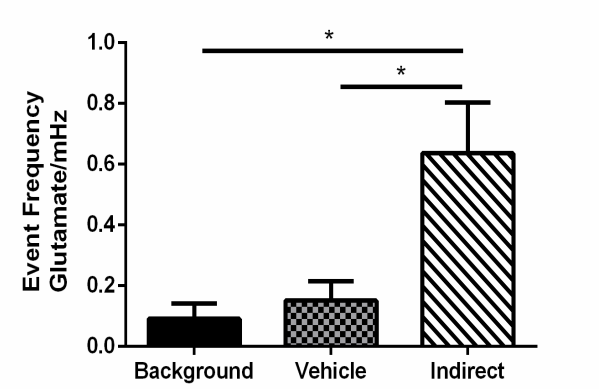

Figure 3: The number of calcium events increases in response to indirect glutamate application. Calcium imaging analysis revealed that the event frequency of iPSC neurons at DIV91 under indirect glutamate stimulation increased significantly with respect to indirect vehicle stimulation (n=33, p<0.05).

Typically, more than 300 data points were obtained from each Ca^{2+} imaging analysis. This showed the potential of the system for simultaneous high-throughput screening of functional communications between the two environmentally isolated neuronal networks. After synapse maturation, a significant increase in Ca^{2+} events, in the indirectly stimulated chamber (Figure 3), occurred in response to an injection of glutamate in the opposite chamber.

CONCLUSION

We successfully developed a microfluidic assay for assessing the functional connectivity of human iPSC-derived neuronal networks. Further development of an *in vitro* human model for the spread of tau mutations has the potential for novel assessment of the mechanisms underlying Alzheimer's disease. The system and techniques used here are also suitable for modeling other central nervous system diseases as well as for miniaturized drug screening using patient samples.

ACKNOWLEDGEMENTS

This work has been supported by the National Centre for Replacement Refinement & Reduction of Animals in Research (NC3Rs) Crack It Challenge Untangle award.

REFERENCES

[1] World Health Organization and Alzheimer's Disease International 2012. Dementia: a public health priority.
[2] J.W. Park, H.J. Kim, M.W. Kang, N.L. Jeon, *Lab Chip*, 13, 509-21, 2013.
[3] A.M. Taylor, M. Blurton-Jones, S.W. Rhee, D.H. Cribbs, C.W. Cotman, and N.L. Jeon, *Nature Methods,* 2, 599-601, 2005.
[4] Y. Shi, P. Kirwan, J. Smith, H.P. Robinson, F.J. Livesey, *Nat Neurosci,* 15, 477-86, 2012.
[5] G. Robertson, T.J. Bushell, and M. Zagnoni, *Integr Biol*, 6, 636-644, 2014.

CONTACT

* Michele Zagnoni, tel: +44-141-548-5985; michele.zagnoni@strath.ac.uk