Digital light processing of 3D structures for improved connectivity of hIPSC derived neurons

Dominic Mosses[1], Eric Hill[2], Edik Rafailov[3] and Rhein Parri[1]

[1] *College of Health & Life Sciences, School of Biosciences, Aston University, Birmingham, U.K*

[2] *Department of Chemistry, Loughborough University, Loughborough, U.K*

[3] *College of Engineering and Physical Sciences, Institute of Photonic technologies, Aston University, Birmingham, U.K*

Digital light processing, Biomaterials, 3D cell culture, Neuroscience

INTRODUCTION

The annual cost of treating brain conditions in the EU is estimated at ~798 billion Euros, with multiple brain disorders affecting ~165 million Europeans or 1 in 3 people [1]. These disorders are often therapeutically managed rather than curative with the number of treatment modalities stalling in recent years. This lack of progress can be attributed to the availability of relevant drug screening models. The screening of novel neuro chemotherapeutics often utilize *in vivo* rat/mouse models or isolated cells from the same for *in vitro* studies. Not only do these models poorly replicate the environment of the human brain but they carry ethical burdens. Recently, human induced pluripotent stem cells (hIPSCs) have been used to differentiate several cell types of the human brain [2]. However, despite their promise these cells lack the connectivity of a mature adult brain. This is due to 2D cell culture systems which fail to recapitulate developmental events leading to neuronal network connectivity [3]. We show an ability to manufacture 3D structures to improve the connectivity of hIPSC neurons thus offering a potential improvement on existing neural drug screening and experimental models.

MATERIALS AND METHODS

Three commercially available monomers were screened for their compatibility with the Cellink Bionova X digital light processor. Fourier transform infrared spectroscopy was used to quantify degree of monomer conversion (DoC). The three polymers were also used to culture hIPSC derived neural progenitor cells (hIPSC-NPCs). Viability assays were performed in order to examine the adhesion and health of cultured cells.

One polymer performed well during printing and biocompatibility studies. This polymer was therefore used to print complex multi-layered 3D structures. These structures were used to support attachment and differentiation of hIPSC-NPCs to mature cortical neurons. Astrocytes were added in co-culture to improve the connectivity and viability of neurons. A cutting-edge light-sheet microscope and calcium sensitive dye were used to assess connectivity of these co-cultures.

RESULTS AND DISCUSSION

All three polymers showed good DoC and printability in the Bionova X. Of these one was directly cytotoxic to hIPSC-NPCs, one was not cytotoxic but did not support cell attachment and one supported the attachment of hIPSC-NPCs. This polymer was utilised to fabricate high resolution structures which closely matched the dimensions of the input file. Subsequently cultured cells showed viability and electrical connectivity across multiple layers.

CONCLUSIONS

These findings present a novel material for the 3D culture of hIPSC derived neuronal cells. We show a high throughput method for DLP of complex brain cell culture substrates which is not reported in the literature as of writing. Previous study has shown an ability to support connectivity in three dimensions. However, none show this extent of cell-cell connections across all three dimensions in a multi-layered fashion. This work will form the basis for a new and improved experimental model for human neuroscience.

REFERENCES

1. Matthews, F.E., et al., *A two decade dementia incidence comparison from the Cognitive Function and Ageing Studies I and II.* Nature Communications, 2016. **7**(1): p. 11398.
2. Kirwan, P., et al., *Development and function of human cerebral cortex neural networks from pluripotent stem cells in vitro.* Development, 2015. **142**(18): p. 3178-3187.
3. Kang, S., et al., *Characteristic analyses of a neural differentiation model from iPSC-derived neuron according to morphology, physiology, and global gene expression pattern.* Scientific Reports, 2017. **7**(1): p. 12233.

ACKNOWLEDGEMENTS

We thank the Institute of Photonic Sciences, Barcelona Institute of Science and Technology, Barcelona, Spain particularly Gustavo Castro and Pablo Loza. This work was funded by the EPSRC, grant ID; EP/X018385/1.