ABSTRACT

A microfluidic-based neural stem/progenitor cell (NSPC) aggregate culture platform that can be used as an in vitro model system for studying myelino genesis has been developed. NSPC aggregates of uniform size were obtained using a microfabricated microwell array and were cultured in the microfluidic culture platform at controlled inter-aggregate distances (400, 800, 1600 µm). Formation of robust myelin sheaths was observed after 31 days of culture and the microsystem revealed that the expression of myelin sheaths shows dependency on inter-aggregate distances. Also, consistency of the myelin expression in the microsystem enabled investigation of biomolecular treatment effect on myelin expression in vitro.

KEYWORDS: Organ-on-a-Chip, Brain-on-a-Chip, Myelination, Aggregate neural culture.

INTRODUCTION

Formation of myelin sheaths by oligodendrocytes (OLs) critically contributes to proper signal transfer in central nervous system (CNS) [1, 2]. Although the loss of myelin sheaths results in many neurological disorders, detailed mechanism of myelination process remain largely unknown. This is partially due to lack of in vitro model systems that allow easy experimental manipulations. Koito et al. introduced a model where CNS myelin could be formed in vitro by culturing neural stem/progenitor cells (NSPCs) in an aggregate form [3]. Myelin sheaths were successfully formed; however, aggregate size and inter-aggregate distances could not be controlled by conventional method of culturing aggregates on multi-well plates. We present a brain-on-a-chip microsystem capable of culturing size-controlled NSPC aggregates in a spatially controlled microenvironment as an in vitro model system for studying myelination. With the developed microsystem, we have investigated the effects of inter-aggregate distances and retinoic acid (RA) treatment on CNS myelin sheath formation.

EXPERIMENTAL

The developed model is composed of two microsystem modules; a microwell array and a microfluidic culture platform (i.e. brain-on-a-chip) (Figure 1). The microwell array, made of poly(dimethylsiloxane) (PDMS), is where primary NSPCs are initially plated for generating aggregates of controlled size (Figure 1A). NSPCs were prepared from forebrains of embryonic day 16 rats and were plated on the microwell array (depth: 150 µm, opening diameter: 150 µm) at an areal density of $7 \times 10^4$ cell/mm$^2$. Abundant cells
outside the microwells were rinsed away and microwell-confined NSPCs were cultured in a 37°C, 5% CO₂ humidified incubator for 3 days *in vitro* (DIV 3) to form aggregates. The microfluidic culture platform is composed of one ring-shaped PDMS layer and a poly-d-lysine (PDL)/Matrigel™ coated glass coverslip (Figure 1B). The PDMS layer has four radially positioned culture chambers each having 10 NSPC aggregate trapping sites (Figure 1B-inset). Size-controlled NSPC aggregates retrieved from the microwell array were loaded into microfluidic culture platforms having different trap-to-trap distances and were cultured in a spatially controlled microenvironments (inter-aggregate distances: 400, 800, and 1600 µm) for 31 days. Culture medium was exchanged every 2-3 days and RA (500 nM) treatment to NSPC aggregates was applied from DIV 10 to DIV 31 at 400 µm inter-aggregate distance.

**RESULTS AND DISCUSSION**

NSPCs plated into the microwell array generated aggregates of uniform size with an average diameter being 144.8 ± 13.6 µm after 3 days (Figure 2A-B). Size-controlled NSPC aggregates added into the microfluidic culture platform via the loading port were effectively captured at the trapping sites inside four culture chambers and were cultured at controlled inter-aggregate distances. NSPC aggregates formed dense axonal network inside the culture chamber and many glia cells migrated out from the trapped NSPC aggregates starting from DIV 5, as can be seen in Figure 2C. After 31 days of culture, oligodendrocyte progenitor cells (OPCs) successfully differentiated into myelinating OLs and wrapped around the axon fibers to form robust myelin sheaths inside the microsystem (Figure 3A). In addition, formation of myelin sheaths indeed showed dependency on inter-aggregate distances. Approximately 96% of NSPC aggregates cultured at 400 µm distance showed formation of myelin sheaths, while only 73% and 60% of aggregates showed myelin sheaths formation for 800 µm and 1600 µm distance, respectively (Figure 3B). To further analyze this effect, average length of myelin sheaths per aggregate was quantified. More than two-fold increase in myelin sheath formation was observed at 400 µm distance (coefficient of variation (C.V.) < 0.5) compared to 800 µm. The C.V. increased with increasing inter-aggregate distances, from 0.80 for 800 µm to 1.03 for 1600 µm (Figure 3C). These results clearly indicate that more myelin sheaths with higher consistency can be obtained by controlling the inter-aggregate distances. This is a critical factor when comparing effects of various biomolecular treatments on myelination and cannot be controlled by previously introduced conventional method. Lastly, the effect of RA treatment (500 nM) on myelination was investigated at 400 µm inter-aggregate distance. As can be seen in Figure 3D, RA treatment significantly promoted the myelination process with approximately 60% enhancement in myelin sheaths formation.
CONCLUSION

A microsystem capable of robust cortical neuron myelin formation with high consistency has been developed for the first time. The microsystem revealed that the formation of myelin sheaths shows dependency on inter-aggregate distances and reliability of the system allowed the investigation of the effect of RA treatment on myelin expression. We believe that this brain-on-a-chip microsystem will be a powerful tool for studying CNS myelination as well as for screening potential drug candidates that promote myelination.

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REFERENCES


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