MICROFLUIDIC CO-CULTURE PLATFORM FOR CNS AXON MYELINATION
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ABSTRACT
A microfluidic compartmentalized co-culture platform for central nervous system (CNS) axon myelination research has been developed. The platform is composed of two compartments connected via arrays of shallow microfluidic channels that function as fluidic and physical isolation barrier between the two compartments. Neurons from E 16-18 rats and oligodendrocytes (OLs) from P1-2 rats were co-cultured inside the device and showed successful development into mature cells as well as isolation of neuronal cell bodies from axonal layer formed in the axon/glia compartment.

KEYWORDS: Neuron culture, myelination, co-culture

INTRODUCTION
Myelination of the central nervous system (CNS) is a sequential, multi-step process that requires reciprocal signaling between axons and myelin-producing cells – oligodendrocytes (OLs) [1]. Dysfunction of neuron/OL interaction and/or loss of myelin underline many neurological disorders including multiple sclerosis, Alzheimer’s disease and psychological disorders such as schizophrenia. However, the signals that regulate myelination in the mammalian CNS remain largely unknown. This is due not only to the complexity of the myelination process but also to the lack of suitable in vitro models of CNS myelination to unravel the cellular and molecular basis. In this paper, we present a novel microfluidic compartmentalized co-culture platform for in vitro CNS axon myelination research.

EXPERIMENTAL
The microfluidic co-culture platform is fabricated through a poly (dimethyl siloxane) (PDMS) soft-lithography process. It is composed of one circular open access compartment (i.e., the soma compartment) for neurons and one closed co-centric ring compartment (i.e., the axon/glia compartment) for OLs. The two compartments are connected by arrays of 2.5 μm high and 200-800 μm long axon-guiding microchannels (Fig. 1), which function as a physical barrier between the two compartments and allow only axons, but not dendrites, to grow across into the neighboring axon/glia compartment. Therefore, OLs loaded into the axon/glia compartment interact only with axons but not with neuronal soma or dendrites. In addition, fluidic isolation between the two compartments can be achieved if the culture medium in each compartment is kept at different levels. The small hydrostatic pressure generated by the different levels of the medium counteracts diffusion, thereby creating a separate environment for each compartment. The open-compartment design minimizes the mechanical stress to neurons cultured inside the
device during both cell loading and feeding. Also, this open-compartment design eliminates possible negative effects associated with low CO₂ exchange of a closed compartment design.

RESULTS AND DISCUSSION

The efficiency of fluidic isolation in the microdevice was tested by applying 30 µL volume difference to create a hydrostatic pressure difference between the compartments using either different color dyes in the two compartments or a fluorescent dye (FITC) in the axon/glia compartment. A sharp boundary was evident between the axon/glia compartment and the axon-guiding microchannels (Fig. 2). Such fluidic isolation was maintained for over 70 hours.

Primary cortical neurons from E16-18 rat embryos were cultured inside the soma compartment for two weeks, at which time extensive axonal networks were formed in the axon/glia compartment. Ols from P1-2 rats were then loaded into the axon/glia compartment and were co-cultured with neurons for two more weeks. Compared to previously suggested square shaped neuron culture platform [2], the current circular design allows neurons to be positioned directly at the inlets of axon-guiding channels during cell loading. This results in higher probability for axons to cross through these microchannels and thus form a denser axonal layer in the axon/glia compartment. The efficiency of axon growth was determined by the percentage of the area covered with

Fig. 1. Schematic illustrations of the microfluidic compartmentalized CNS neuron co-culture platform. (a) 3D-view of the circular culture platform; (b) cross-sectional view showing a minute difference in fluidic levels for fluidic isolation; (c) scanning electron micrographs (SEMs) of a PDMS device showing the bottom side of the axon-guiding channels before bonding (Inset: Close up view of one axon-guiding microchannel bonded onto the substrate. Scale bar, 10 μm).

Fig. 2. Fluidic isolation in a circular shaped co-culture platform using color dyes and a fluorescent dye (FITC).
Axons in the axon/glia compartment. Axon coverage was found to be 79.7% in the microdevice after four weeks of neuron culture. Immunocytochemistry was used to demonstrate the successful isolation of cell soma and dendrites from the axonal network inside the axon/glia compartment (Fig. 3 (a), (b)). After one-two weeks of co-culture, OLs loaded into the axon/glia compartment differentiated into mature cells expressing myelin basic protein (MBP) (Fig. 3 (c)). The microfluidic co-culture platform could be used to study axon-glia interactions.

CONCLUSIONS

We have developed a microfluidic compartmentalized co-culture platform for studying CNS axon-glia signaling and demonstrated successful co-culturing of CNS neurons and OLs in isolated environment. We expect that this system will provide a powerful tool for future mechanistic dissection of CNS axon-glia signaling networks in vitro and offer a platform for high-throughput screening for potential drug candidates that promote myelination and myelin repair.

REFERENCES
