INVESTIGATING NEUROPROTECTIVE EFFECTS OF PRIMARY GLIAL CELLS USING OVERFLOW MICROFLUIDIC NETWORKS

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ABSTRACT

The complex intercellular processes within neuroinflammatory events, taking place during brain diseases, are still insufficiently understood. Intercellular pathways between isolated populations of different brain cells need to be studied to unravel the complexity of such events. Here we show how overflow microfluidic networks (oMFNs) can be employed to study the neuroprotective potential of primary astrocytes under the conditions of an ischemic stroke. Astrocytes and neurons were cultivated in two individual chambers of an oMFN and stressed using an oxygen glucose deprivation chamber (OGD). Up to 45% of the neurons died under these conditions. In contrast, neuronal death was significantly reduced to ~5% when the neurons received fluid from the chamber with the astrocytes culture.

KEYWORDS: Microfluidics, Neurodegenerative Diseases, Cellular Pathways

INTRODUCTION

The inflammatory processes occurring in neurodegenerative diseases with high social and medical impact such as ischemia, Alzheimer's or Parkinson's disease are still poorly understood, despite intense research efforts over the years. A significant obstacle to a better understanding of the role of neuroinflammation in these diseases is the complexity of the mechanisms of intercellular communication occurring between different brain cells; the unraveling of this complexity is still a major research challenge. We earlier developed overflow microfluidic networks (oMFNs) for the culture of different cell types [1]. In this study, we show how to use oMFNs for studying intercellular pathways within the neuroinflammatory context of ischemic stroke: by loading different primary cell populations on a chip and exposing the cells independently or jointly to various (stress) conditions, a new method for efficiently studying neuroprotective effects of glial cells is achieved.

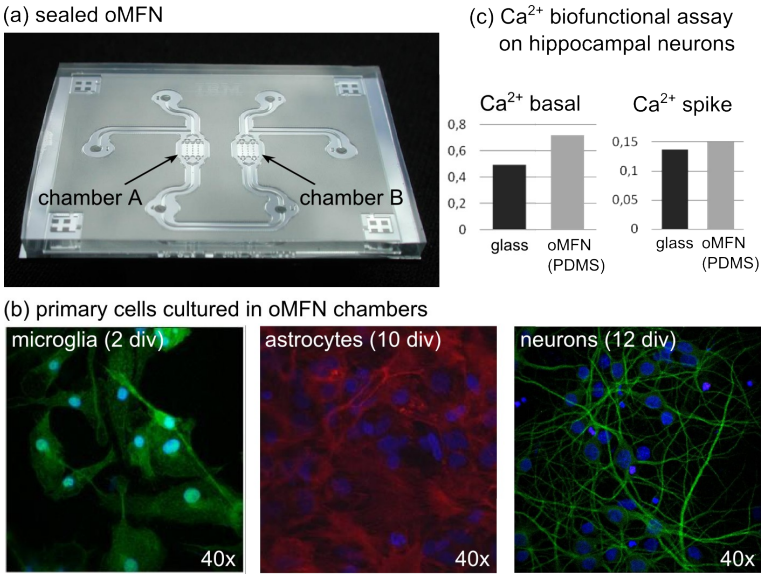

Figure 1: oMFNs for studying intercellular pathways. (a) Photograph of a sealed oMFN having 2 cell chambers and various input/output ports for liquids. (b) Example of primary cells (from rat) deposited and cultured in cell chambers. (c) Calcium imaging on neurons in an open oMFN shows no significant difference compared to neurons grown on standard substrates (glass).

EXPERIMENTAL

Figure 1 shows an oMFN having 2 cell chambers. Cells are plated in each chamber and the oMFN chip is placed in a cell incubator. Cells can be regularly inspected and assessed in terms of morphological/biofunctional properties using optical, fluorescence and electrophysiological characterization methods. Before experiments, the oMFN is closed with a lid having various ports. Here, by addressing cells in each chamber independently or sequentially, and by performing control experiments or stimulating cells, a basic set of 8 experiments can be performed. As an example, cells in both chambers can be perfused with medium independently (experiment A1) or serially (A2). Experiments involving the stimulation of 2 cell types in disconnected (B3) and connected (B4) chambers are described below, Fig. 2.

(a) nomenclature

		Chamber B			
		control		stimulated	
Chamber A	control	A1	A2	A3	A4
	stimulated	B1	B2	B3	B4

independent chambers

connected chambers

(b) scenarios

	A1	A2	B3	B4
Chamber A	hippocampal astrocytes (HA)			
Chamber B	hippocampal neurons (HN)			
Stimulus	oxygen glucose deprivation (OGD)			

Figure 2: Nomenclature of experiments and performed experiment scenarios addressing pathways between two cell types in oMFNs. (a) In practical terms, each experiment is based on drawing various media with and without stimulating factors through either one cell chamber or both chambers while monitoring cellular response using optical and fluorescence microscopy. After the experiments, the sealed oMFNs can be opened for cell fixation and/or confocal microscopy. (b) HA and HN cells have been cultured in an oMFN and exposed to different conditions.

RESULTS

Figure 3 shows the result of culturing hippocampal astrocytes (HAs) and hippocampal neurons (HNs) under oxygen and glucose deprivation (OGD) protocol (a widely known in vitro method for mimicking ischemia [2]). When chambers containing HAs and HNs are not in microfluidic communication, death of HAs is marginal while up to 45% of HNs become significantly permeable to propidium iodide (PI, a cell death dye) within the first 24 h. After transient OGD, the recovery of HNs is markedly higher (only ~20% of the neurons stain with PI after 24 h and ~5% after 48 h) when medium circulates from the HA chamber to the HN chamber. This illustrates the neuroprotective action of HAs on HNs, which is presumably due to neurotrophic factors contained in the HA culture.

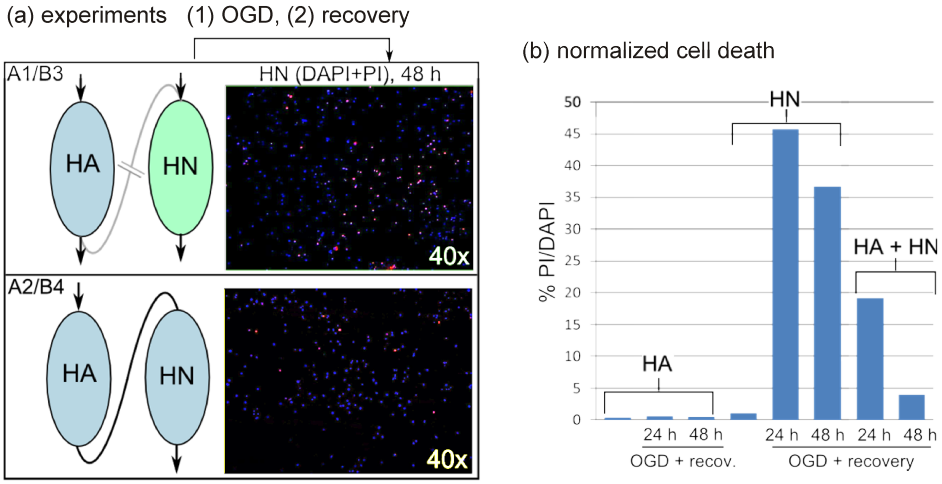

Figure 3. Demonstration of the neuroprotective action of HAs on HNs during OGD experiments. (a) and (b) Neuronal death is significant 24 and 48 h after OGD except when HA are in biochemical communication with the neurons, illustration the neuroprotective capability of HAs.

CONCLUSION

By varying cell combinations and stress parameters, the method shown here can be broadly applied to the investigation of specific cell type contribution to the neuroinflammatory events leading to neuronal degeneration. Currently, the system is being used to also investigate neuronal viability following exposure of neurons from various parts of the brain to conditioned medium exiting a chamber containing astrocytes, which are previously exposed to inflammatory challenge using β-amyloid and IL1 beta (data not shown).

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