OVERFLOW MICROFLUIDIC NETWORKS

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ABSTRACT

Living primary cells represent the best *in vitro* system for studying the origin and mechanism of complex diseases. Yet these cells can be very hard to obtain, culture and study using microfluidic systems. We present here overflow microfluidic networks (oMFNs) that are particularly appropriate for studying primary cells and cellular pathways: cells are deposited on a transparent oMFN and after an appropriate culture duration in a standard cell incubator, the oMFN is sealed with a lid and excess culture medium is accommodated by capillary structures in the periphery of the chip.

KEYWORDS: Cells, Microfluidics, Brain Disease

INTRODUCTION

Single cell analysis and investigations of cellular pathways have been reported and the potential of microfluidics for biological applications demonstrated [1,2]. Often, cells are introduced into a closed microfluidic network and experiments performed the same day. We recently reported such a system for studying pathways between cell lines from the central nervous system (CNS), wherein two cell populations were deposited in distinct areas of a poly(dimethylsiloxane) (PDMS) microfluidic network [3]. This method works well with cell lines, which are easy to grow and need short incubation times. Such studies are however best done using primary cells, but these cells are very fragile and need long incubation times (up to 10 days) before experiments can be performed. Closed microfluidic systems tend to be complex, application-specific and difficult to use by biologists.

Here, we report microfluidic chips that allow culturing primary cell populations in an open state for several days under standard conditions; the chips are closed afterwards for experiments. The chips are used to study interactions between primary cells of the CNS. The cell interactions and an experimental setup are shown in figure 1.

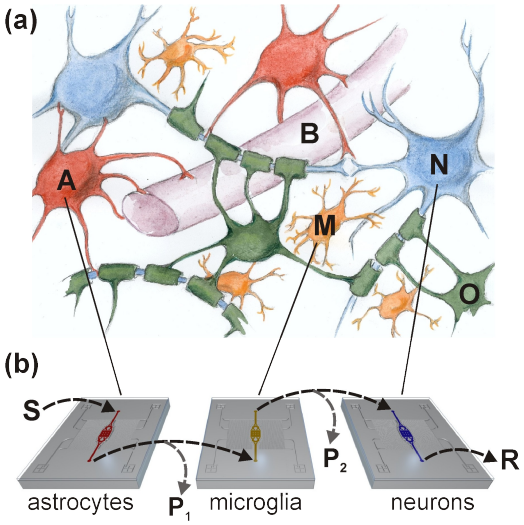

Figure 1: (a) Simplified illustration of the complex interactions between some of the cell populations involved in neurodegenerative diseases. (N: neurons, A: astrocytes, M: microglia, O: oligodendrocytes, C: blood capillary). (b) Possible setup for cellular pathway investigations using primary cells of the CNS (S: stimulus, P1,2: biochemicals involved in pathway, R: neuronal response).

EXPERIMENTAL

For optimal growth conditions of the primary cells in the microfluidic chips, PDMS oMFNs were developed that allow seeding of the cells in an open cell chamber. The oMFNs are molded from lithographically-prepared silicon masters and treated with a solution of fibronectin or poly-L-lysine to promote cellular adhesion onto the PDMS surface. Seven µL of cell suspension is placed onto the cell chamber (4 mm^2, 150 µm deep) of an oMFN which is placed in an incubator as long as needed for the cells to establish their phenotype. Afterward, a Si lid comprising ports for fluidic connection is placed onto the oMFN. Excess liquid is removed by capillary structures in the overflow zone. The weight of the lid and the capillary pressure in the overflow zone ensure sealing between the PDMS oMFN and the Si lid, figure 2.

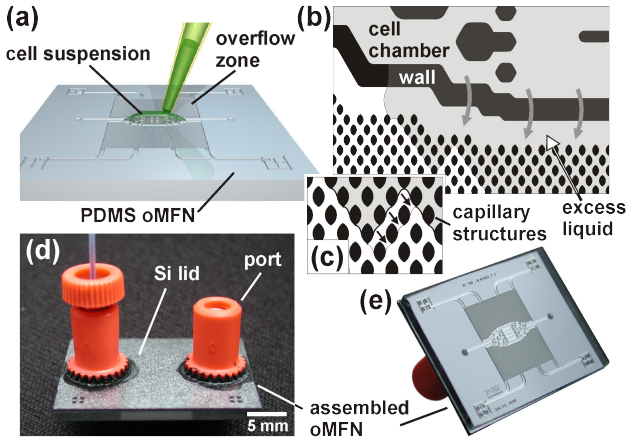

Figure 2: Design and photographs of a PDMS oMFN. (a) A small drop of cell suspension is placed on the cell chamber of an oMFN. (b) Excess liquid overflows the wall of the cell chamber during assembly and is absorbed by capillary structures (c). (d, e) Photographs of a oMFN after assembly, showing the Si lid and the ports.

RESULTS

A number of cell types were seeded and grown on oMFNs, among which primary cells such as rodent glia, astrocytes and neurons. Fitness of primary cells grown in oMFNs for up to 6 days was verified using microscopy and staining procedures in the chip after closing, figure 3. Pathways between cell populations can be studied either by connecting two or more oMFNs with each other, or by implementing multiple cell chambers on a single oMFN. A design, having two chambers on a chip, is shown in figure 4a. There, chambers are seeded individually with cells and the oMFN is closed without leakage. A biochemical pathway was performed between primary astrocytes and microglia. The astrocytes were perfused at 1 µL min^{-1} with 50 µM glutamate and consequently released ATP, which was directed to the microglia chamber. ATP triggers the P2X7 receptor of microglia, which was indicated by the uptake of propidium iodide (PI), figure 4(b, c). In a control experiment, the receptor was inhibited using 100 µg mL^{-1} oxidized ATP (oATP). A significant reduction of PI uptake was observed, figure 4(d, e).

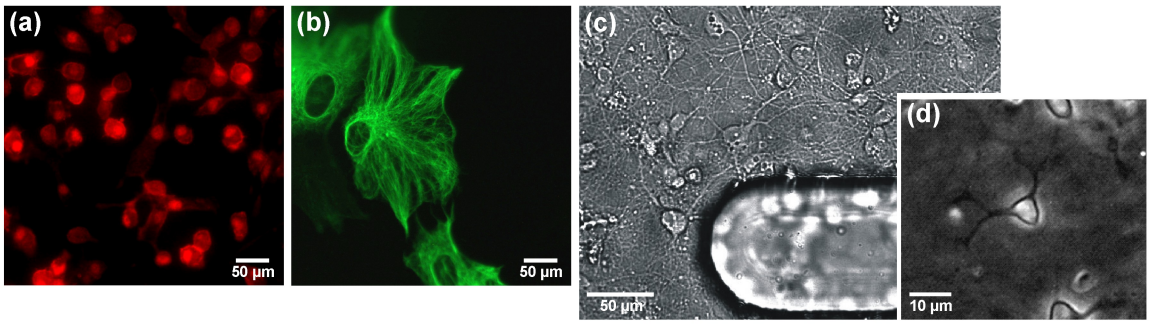

Figure 3: Fluorescence microscope images of primary cells grown in oMFNs for up to 6 DIV. (a) microglia, (b) astrocytes and (c, d) neurons from the hippocampus.

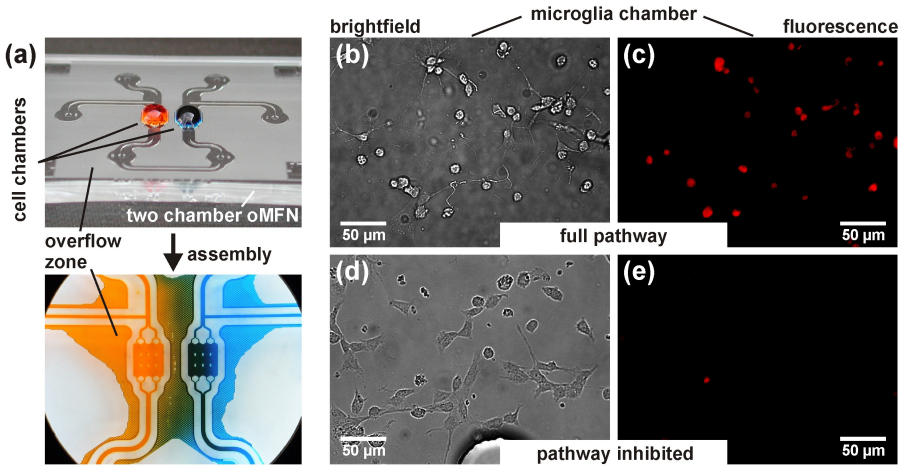

Figure 4. Biochemical pathway between two primary cell populations. (a) A two-chamber-oMFN is used to cultivate two primary cell populations independently and is then sealed. (b, c) PI uptake of microglia in the second chamber due to an ATP release of glutamate-stimulated astrocytes in the first chamber. (d, e) Inhibition of an ATP receptor (P2X7) in microglia using oATP results in a significant reduction of dye uptake.

CONCLUSION

oMFNs provide a simple and flexible method for cellular studies involving primary cells. Not only can cells be grown and used to reproduce pathways, but they can also be fixed at the end of experiments and analyzed using confocal fluorescence microscopy after removal of the lid. Investigating pathways between cells of the CNS, using oMFNs, may help to identify key (bio)chemicals involved in brain diseases that can not be discerned using classic cell biological methods.

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