A NEW ORGAN-ON-CHIP PLATFORM FOR PHYSIOLOGICAL RELEVANT *IN-VITRO* REPRODUCTION OF THE BLOOD–BRAIN BARRIER

H. Kiessling[1], H.Becker[2], E. Haltner[3], J. Schütte[1], I.Schulz[2], V. Rack[1], K. Topic[1], and M. Stelzle[1]

[1]NMI – Natural and Medical Sciences Institute at the University of Tübingen, GERMANY
[2]microfluidic ChipShop GmbH, GERMANY
[3]Across Barriers GmbH, GERMANY

ABSTRACT

Development of novel drugs for indication in the central nervous system often fails due to the inability of most substances to cross the blood-brain barrier. State of the art animal and cell culture models fail to predict a substance's ability to cross this barrier as they lack comparability to the complex human situation. Therefore, we are developing a model which mimics the organ environment including the specific 3D arrangement of different cell types, extracellular matrix, and perfusion by combining biology, biochemistry and microfluidic technology. In this paper we present a microfluidic device modelling the blood-brain barrier, using dielectrophoretic forces for cell assembly.

KEYWORDS

Organ-on-a-chip, dielectrophoresis, polymer microfabrication.

INTRODUCTION

Enabled by increasingly sophisticated microfluidic systems the interest in use of organ-on-chip technologies to mimic organ-like functions has risen sharply. Numerous microfluidic-mediated solutions developed over the last recent years have demonstrated enormous potential of organ-on-chip systems spread over all research areas including oncology, angiogenesis, tissue engineering and drug development [1-3]. In case of neurodegenerative diseases like Alzheimer's or epilepsy an efficient treatment remains a major challenge as most available drugs cannot pass the blood–brain barrier (BBB). Therefore innovative in-vitro BBB models are especially of great interest in order to characterize the permeability of new central nervous system (CNS) drug candidates and putatively to get insights into disease-related processes tightly connected with BBB dysfunction. The development of organ-on-chip-based BBB models is strongly directed towards comparability to the human in vivo situation to the maximum possible extent. This includes strategies which guarantee physiological relevant microenvironment conditions such as fluidic shear stress and a thin cell layer interface. To address these requirements we are developing a novel in vitro model of the BBB.

EXPERIMENTAL SET-UP

The operating principle of the microfluidic device presented is the dielectrophoresis (DEP)-driven assembling of BBB-relevant cell types such as endothelial cells on an *in-situ* polymerized polyamide membrane which is vertically formed between micro pillar structures (Fig. 1) in the middle of a microchannel. As shown in Fig. 1B, the electrical field strength reaches a maximum in the areas between the pillars, thus generating a force acting on the cells which drives them into the area between the pillars. A significant advantage of the assembly using dielectrophoresis is the fact that live and dead cells have different electrical permittivity, thus the method allows to select only live cells to be steered towards the assembly zone.

The main component of this novel system is the organ-on-chip platform "TransBBbarrier", representing the core unit which contains 10 microfluidic modules for artificial BBB formation (Fig. 2). The platform was realized by mass-production compatible injection molding technique using cyclo-oelfin-polymer (COP, tradename Zeonor). The overall dimension of the device is identical to a

standard SBS-titerplate (127.76 mm × 85.48 mm), with integrated fluidic interfaces for the connection of silicone tubing. Each single functional module represents a microchannel with insulating micropillars arranged in the middle of the channel structure over a length of 16 mm. The pillars with a height of 170 µm were arranged with two spacings, either 19 pillars with 810 µm period in a 270 µm wide channel or 21 pillars with 730 µm period in a 170 µm wide channel. In order to get fully functional modules suitable for DEP-based cell layer formation, each microchannel is equipped with a pair of opposing gold electrodes. For automated fluidic operation, the microchannels merge into two inlet and outlet interfaces each.

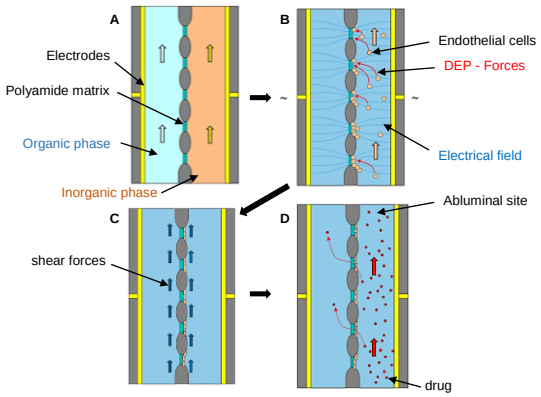

Figure 1. Assembly concept of the artificial microBBB: (A) generation of a membrane between the micro pillars by polymerization of a monomer and crosslinker; (B) accumulation of endothelial cells at the micro pillars by dielectrophoretic forces; (C) laminar flow during the cell cultivation provides for shear forces thus inducing the formation of an in vivo-like cell structure. Afterwards the permeation of drug molecules from the luminal to the abluminal channel via the blood-brain-barrier can be tested (D).

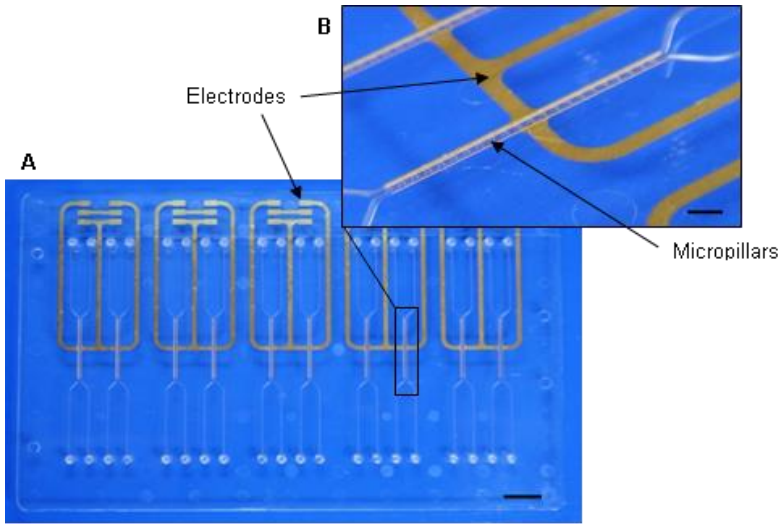

Figure 2: The TransBBbarrier chip consists of 10 separate microchannel modules and gold electrodes (A) containing a row of micropillars each designed for artificial BBB formation (B). Scale bar: (A) 5 mm, (B) 1 mm.

EXPERIMENTAL RESULTS

The cells are actively arranged by dielectrophoresis (DEP) to facilitate the formation of a dense 3D cell structure in the microfluidic system. We devised a two-phase in situ polymerization procedure to form a vertical porous polyamide membrane of approximately 10 µm thickness between the micropillars to provide a mechanical scaffold for cell attachment. The two phases of monomer solutions were driven through the chip by hydrostatic pressure in order to avoid pulsation of the flow during polymerization. Once the membrane had formed, it was dynamically coated with extra-cellular matrix protein to promote cell adhesion. We have successfully demonstrated the assembly of viable Caco-2 epithelial cells in the chips´ microstructures. During cell cultivation the chip will be held at 37 °C under constant perfusion. By providing stimuli of shear forces and cell-cell interactions we expect cells to form a barrier with behavior that closely mimics the *in-vivo* situation. The design of the microfluidic system will enable the collection of small sample volumes and optical accessibility of the cells to closely study the transport of drugs or nanoparticles across the cell layer.

An important aspect of a microfluidic system with its large surface-to-volume ratio for the proposed application in drug research is the possible substance loss due to adsorption of the molecules to the channel walls. Figure 4 shows recovery rates of three substances which are typically used as standards to test the hermeticity of barrier-function cell cultures measured in the TransBBbarrier system without cells. After washing with three to four times the volume of the system the complete amount of the substances are eluted.

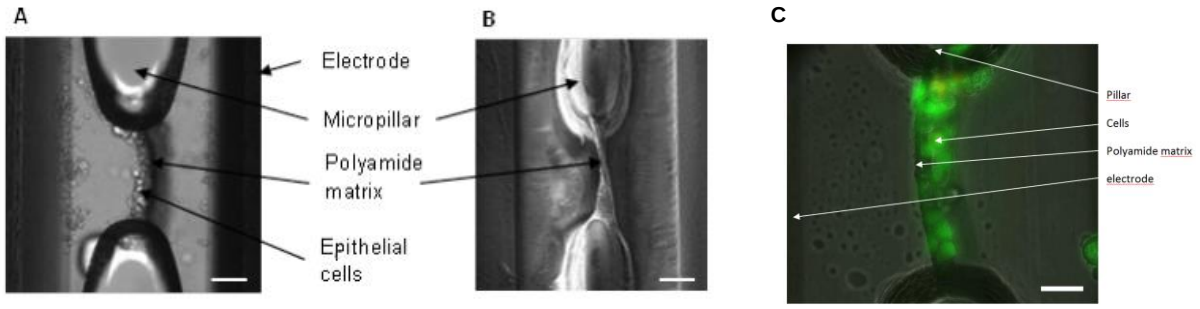

Figure 3. Microscopy image of epithelial cells (A) assembled between the micropillars by dielectrophoresis on an in situ polymerized polyamide membrane (B). Scale bars: (A) 150 µm, (B) 200 µm. (C) Microscopy image of CaCo2 cells assembled between the micro pillars; green staining with calcein indicates live cells; red staining with propidium iodide indicates dead cells; scale bar 50 µm.

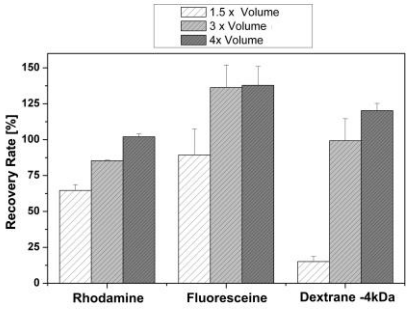

Figure 4: Recovery rates for three molecules used as standards for hermeticity tests in barrier-function cell cultures. A sample of known concentration with a volume of a multiple of the inner system volumes (chip+tubing) is pumped through the chip and collected at the output, where the concentration is measured vs. the input concentration.

CONCLUSIONS

In this paper, we present a platform concept for a blood-brain-barrier model in a microfluidic chip. We have successfully assembled live epithelial cells in these devices on an in-situ polymerized membrane between pillar structures. As soon as cell culture of these cells will be established and cells will form a barrier structure, this novel platform will represent a promising organ-on-chip system for BBB-dependent permeability studies.

ACKNOWLEDGEMENTS

This work was financially supported by the German Ministry of Education and Research (BMBF), project "trans-BBBarrier", grant agreement number 16SV5948K. We thank all project partners for the collaboration.

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CONTACT

Holger Becker, +49 3641 347050, hb@microfluidic-chipshop.com