INTEGRATION OF POLYCARBONATE CELL CULTURE MEMBRANES INTO A POLYMER-BASED MICROFLUIDIC PLATFORM FOR RAPID DRUG SCREENING

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ABSTRACT

A 3D hybrid microfluidic platform with integrated polycarbonate track-etched membranes has been developed. The platform enables cell culture and screening of chemotoxic reagents whilst the cell viability analysis has been carried out off-chip. Diffusive and convective modes of cross-membrane drug transfer have been established. In either mode, we successfully demonstrated on-chip incubation of HL60 myeloid leukemia cells and investigation after exposure to toxic mitomycin C (MMC) in the range of concentrations from 0 to 50 mM. The results of the viability assay on-a-chip were found to be consistent with a standard curve obtained off-chip and indicated ca. 40 % cell survival at MMC concentration of 50 mM.

KEYWORDS

Track-etched, membrane, cell culture, drug screening

INTRODUCTION

There is a dynamically growing demand for microfluidic platforms that simulate *in vivo* conditions for cell culture, handling and analysis. Such platforms should allow monitoring of various physiological processes and a large degree of freedom in the control of experimental conditions, while using reduced volumes of reagents and cells [1-3]. These devices thus bear great promise for high-throughput, automated drug screening in a point-of-care environment by relatively unskilled users. Polycarbonate track-etched membranes (PCTEMs), which assist to simulate the natural environment of cells, are commercially available and widely used in a variety of cell biology applications [4-9].

The here pursued development of the hybrid process for direct incorporation of commercial membrane inserts into poly(methylmethacrylate) (PMMA) is an attractive option for these mimetic systems due to the simplicity and low cost of the fabrication, the wide choice of available membrane materials and morphologies that can be matched to a desired application and ease of access to off-chip surface modification treatments.

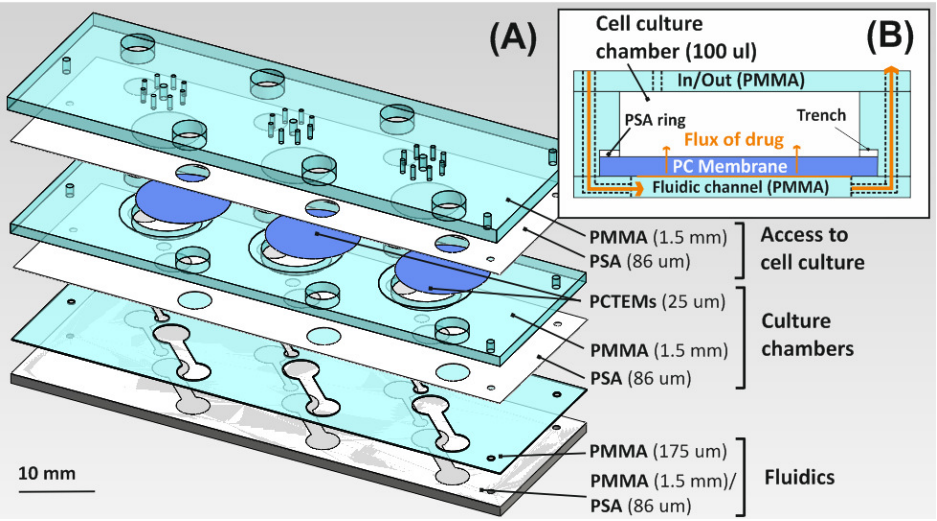

Figure 1: 3D schematic of the device assembly (A) and cross sectional view (B). The device consists of three main compartments: (i) fluidic structures defined in the bottom PMMA/PSA layer, (ii) PMMA culture chambers with integrated GE Whatman® polycarbonate track-etched membranes installed on PSA rings and (iii) a PMMA cap with inlets to introduce cell culture and gaps to supply sufficient aeration of cells.

However, integration of commercial membranes with commonly used polymeric materials has been technologically challenging. Of particular difficulty is the reproducibility in manufacturing while meeting the constraints imposed by the need for

a sterile cell culture environment and ensuring consistent flow behavior between each cell culture chamber. Additionally, the majority of demonstrated systems are based on cast-moulded PDMS structures [10-13] which limits the fabrication throughput, narrows the range of material-compatible solvents and chemical reagents, and shortens the overall life-time of the system.

In the present paper, we describe the design, fabrication and experimental techniques to operate the platform in various flow regimes. The novelty and opportunity of the presented system lies within the potential embedding of any type of biocompatible transport control into a microfluidic system, e.g. membranes with different porosities and morphologies suitable for both colorimetric or fluorometric bioassays. In combination with a specific fluidic design, this opens up new options for automated, direct and continuous on-chip analysis of cellular metabolic processes.

WORKING PRINCIPLE

Our microfluidic platform (Fig. 1) consists of a fluidic supply channel and a cell culture chamber separated by a thin, low-dead-volume porous membrane (pore size 0.015 – 0.2 µm). On the one hand, these PCTEMs serve as a physical barrier for suspended cell culture against contaminants. On the other hand, the membrane permits controlled exposure of the cells to various concentrations of a drug which is transported via a channel through the membrane to interact with the cells. The track-etched membranes feature low internal dead volume and serve as a physical barrier for suspended cell culture against contaminants whilst allowing controlled transport of a drug to the cells.

MATERIALS AND METHODS

Various polymer rapid prototyping techniques were evaluated for reproducible integration of the membrane inserts into PMMA. Figure 1A shows one of the developed schemes for assembly using a pressure sensitive adhesive (PSA) based bonding technique. The microfluidic prototypes were manufactured using 1.5-mm thick PMMA (Radionics, Ireland) and 175-µm PMMA sheets (Goodfellow, UK) , 86-µm thick pressure sensitive adhesive layers (PSA, Adhesive Research, Ireland), cyclopore and nucleopore WHATMAN® (GE Healthcare, UK) PCTEMs inserts. The following prototyping techniques were applied: a CO_2 laser ablation system (Zing 16 Laser, Epilog, USA) to structure the PMMA sheets, a standard knife plotter (ROBO Pro cutter/plotter, Graphtec, USA) for cutting of the PSA and membranes. The PCTEMs were placed on PSA rings inside a shallow trench engraved by a laser. All layers were stacked and the assembly was irreversibly bonded to create the final device. The entire assembly was carried out under UV light (254 nm) using Nova Scan Digital UV Ozone System (IA, USA) to ensure sterile conditions for cell culturing.

The HL60 suspension cells were grown in RPMI-1640 medium with 10 % FBS. The cells were incubated for the times indicated (24 hr, 48 hr) in media containing MMC at the concentrations in the range of 0–50 mM. The incubation was followed by the trypan blue exclusion viability assay.

The computational fluid dynamic (CFD) simulation was performed using the COMSOL Multiphysics 4.2a (COMSOL Group, Sweden). First the transient flow profile was calculated. Once a steady-state was reached, a concentration step at the inlet was initiated and the development of the concentration profile was obtained for both fluidic designs.

RESULTS AND DISCUSSION

Figure 2A shows the assembled device being leak tested with three colors of dyed water. The CFD simulations (Fig. 2) reveal how drug transport is controlled either by convection (Fig. 2B) or diffusion from the fluidic channel across the porous membrane (Fig. 2C). The flow-through platform is best suited for rapid exposure of cells to the drug, while the diffusion platform allows long-term experiments and ensures a more gradual change of the drug concentration in the cell chamber.

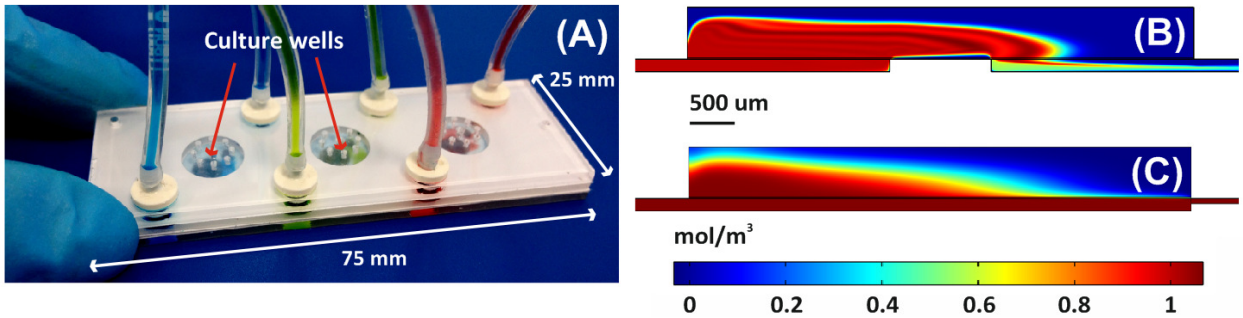

Figure 2: Picture of the fabricated device during the fluidic test (A), simulated concentration profile inside the culture chamber for the design implementing convection through the membrane after 2 sec (B) and for design enabling diffusive delivery of drug after 60 seconds (C).

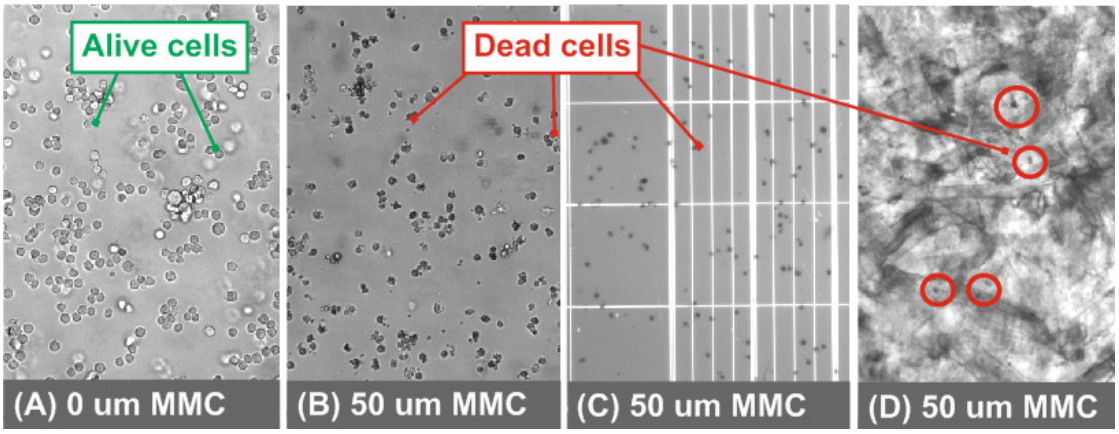

Figure 3: Results of trypan blue viability test on HL60 cancer cells after 24 hr incubation: before (A) and after treatment with 50 mM MMC in a well plate showing ca. 40% survival rate (B); after 50 mM MMC, sample taken from the membrane chip (C) and membrane surface of the opened chip (D).

As a feasibility study, the platform was used to carry out an *in vitro* cell viability assay by controlled incubation of human HL60 cells with mitomycin C [10] (MMC), a DNA-damaging chemotoxic agent. The incubation performed on-chip with the experimental endpoint (a trypan blue dye exclusion viability test) was run off-chip. Figure 3 shows images of the cells before and after the MMC treatment.

CONCLUSIONS AND OUTLOOK

In the present paper, we describe a 3D hybrid microfluidic platform with integrated GE WHATMAN® PCTEMS inserts enabling controlled cell culture and screening of chemotoxic reagents. Herein, we discuss the design, fabrication strategy and experimental techniques for operation of the analysis platform. The operation has been successfully demonstrated on both on-chip incubation of HL60 myeloid leukemia cells and screening of mitomycin C (MMC) exposure at concentrations up to 50 mM. The results of the viability assay on-a-chip were found to be consistent with a standard curve obtained off-chip and indicated ca. 40 % survival rate at MMC concentration of 50 mM. Future tests will expand the chip to allow multiplexing of chemotoxic exposures on cultured cells and rapid testing of combinations of sub-lethal drug doses of complementary agents. The objective is to minimize overall agent doses to optimize treatment. The established system may be well suited hospital settings, clinics and other remote environments where preliminary cell screening of prescribed drugs is to be performed by unskilled users.

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