GENERATING CELL CO-CULTURES BY RAPID CELL ADHESION ON OPPOSITE SIDES OF POLYESTER MEMBRANES

Conni Hanke[1,2], Petra S. Dittrich[2] and Darwin R. Reyes[1]*

[1]*National Institute of Standards and Technology, USA*

[2]*Department of Chemistry and Applied Biosciences ETH Zurich, SWITZERLAND*

ABSTRACT

A cell co-culture was generated on opposite sides of a permeable polyester (PET) membrane by combining dielectrophoretic (DEP) and electrostatic forces to hold cells against gravity in a multilayer microfluidic device (Figure 1). HepG2 cells were trapped underneath the PET membrane (bottom channel) when DEP attractive forces were applied. Cells immediately adhered on the PET membrane by the electrostatic forces from a hybrid cell adhesive material (hCAM) [1] deposited on the surface of the electrodes. NIH-3T3 cells adhered on the top channel via the hCAM deposited on that side of the membrane. Both cell lines remained attached and viable at least 24 h after generating the co-culture.

KEYWORDS: Permeable polyester (polyethylene terephthalate, PET) membrane, dielectrophoresis (DEP), cell co-cultures, cell capture, multilayer microfluidic device

INTRODUCTION

The use of permeable membranes (e.g. transwell/Boyden chamber) as cell attachment substrates has become prevalent for studies such as cell migration, cell-cell communication and drug transport. Cells cultured on permeable membranes can be exposed to different culture environments on their basal and apical sides. This allows for the exchange of molecules from both sides of the cell membrane, which promotes metabolic function similar to cells *in vivo*. Microfluidic multilayer permeable devices have the combined advantages of Boyden chambers and microfluidic systems. These devices represent a platform where cells could be interrogated independently of each other while maintaining control over their cell microenvironment. While others have demonstrated that cells can be assembled in distinct cell layers with [2] or without [3] a membrane to separate them, none of these approaches have taken advantage of the cell loading capacity of DEP. The combination of DEP with these multilayer systems offer the advantage of decreasing the total time of experiment due to the increased cell loading capacity added by the DEP trapping forces. Cells can be trapped and concentrated on the sides of the membrane where the DEP electrodes are fabricated, thereby decreasing the waiting period needed for cells, in some assays, to be confluent. The attachment of cells on opposite sides of a membrane in microfluidic devices using DEP on both sides of the membrane has yet to be demonstrated. Here we present our efforts on cell attachment on opposite sides of PET membranes in a multilayer microfluidic device using DEP and hCAM. On the top side of the membrane cells attach and grow on the hCAM, whereas at the bottom side of the membrane DEP and the hCAM trap and anchor the cells while at the same time cells are prevented from being dislodged off the membrane by gravity and shear forces.

EXPERIMENTAL

Our device consists of two PDMS channels assembled perpendicularly to each other and separated by a PET membrane (Figure 1). A gold microelectrode array was patterned on the PET membrane using standard photolithography [4]. Briefly, for the gold lift-off process the membrane was coated with two different photoresists (LOR 3A and S1813). The photoresists were covered with a photomask and exposed to UV-light to transfer the microelectrodes pattern. The pattern was then developed and the substrates were dried overnight. Gold was deposited (approx. 45 nm) and the excess was lifted-off to obtain the final microelectrodes pattern.

The device was assembled with the gold microelectrodes facing the ITO substrate that closed the device at the bottom. The gold electrodes and the ITO were connected to an arbitrary waveform generator by bonding silver/copper wires to gold pads and ITO utilizing an electrically conductive adhesive. The hCAM was deposited on both sides of the membrane to promote immediate cell attachment (Figure 2). A layer-by-layer deposition process was used to deposit poly(allylamine hydrochloride) (PAH), poly(styrenesulfonic acid) (PSS) and fibronectin on the PET membrane in the order shown in figure 2.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

Cells were suspended in sucrose (approx. 0.15 mol/L), and DEP was performed in the bottom channel by applying a sine wave of up to 10 V_{p-p} at a frequency of 13 MHz between the gold microelectrode on the PET membrane and the ITO at the bottom of the channel. A viability dye, Calcein AM, was used to stain the cytoplasm of HepG2 cells. A second dye, CellMask, was used to stain the cell membranes of the NIH-3T3 cells.

RESULTS AND DISCUSSION

The DEP experiments showed that the attractive forces pulled the HepG2 cells towards the PET membrane in the bottom channel and the hCAM held the cells in place even after the DEP forces were turned off and fluid flow continued. The hCAM held in place the NIH-3T3 cells on the opposite side of the membrane (top channel). Figure 3 shows HepG2 and NIH-3T3 cells trapped on the PET membrane in the bottom and top channel, respectively. Both cell types spread throughout the membrane and were held in place by the hCAM.

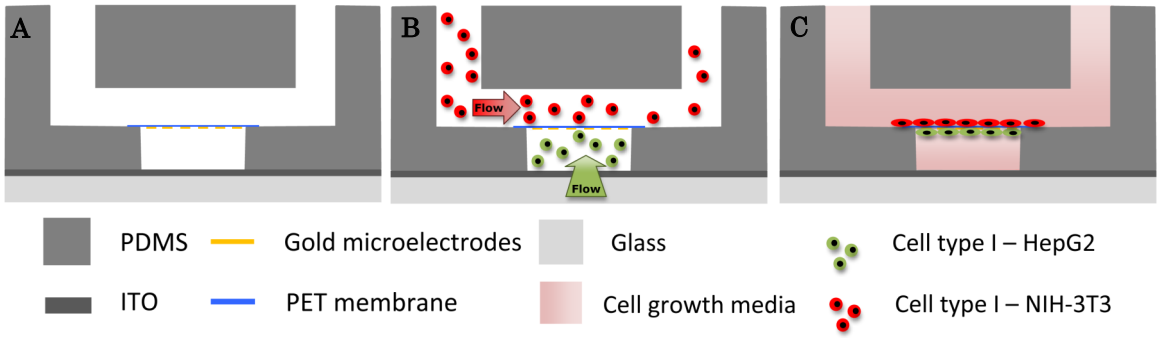

Figure 1. Sketch of a cross-section of a multilayer microfluidic device for the generation of cell co-cultures. A) The device is comprised of two PDMS channels assembled perpendicular to each other. A PET membrane with patterned microelectrodes is sandwiched between the PDMS channels. B) Cells are introduced into the channels in sequential fashion. C) Cells are trapped and anchored with DEP and hCAM at the bottom and with hCAM at the top.

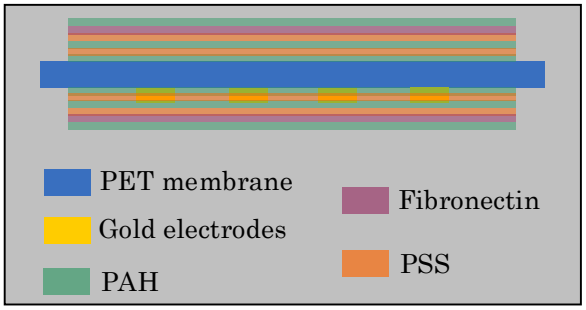

Figure 2. Diagram of the hCAM layers deposited on both sides of the PET membrane.

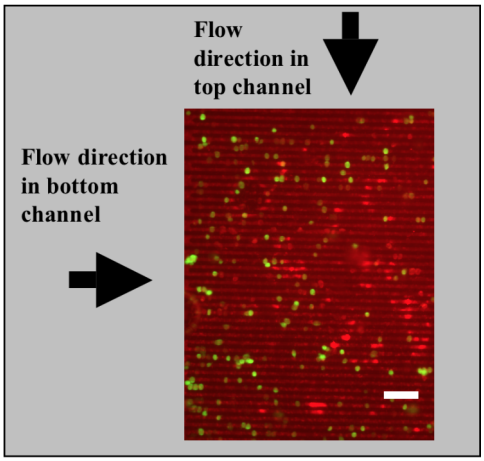

Figure 3. Micrograph of HepG2 (green) and NIH-3T3 (red) cells while being trapped in a multilayer device. Scale bar: 100 µm.

The cells were still viable 24 h after the DEP trapping experiment. Figure 4 shows HepG2 cells (green) and NIH-3T3 cells (red) attached on opposite sides of the PET membrane. The insert shows a 2D image taken from 3D stack images of two cells separated by the PET membrane. This new approach demonstrates an easy and rapid way of cell enrichment and attachment on the bottom side of a PET membrane and cell attachment via hCAM on the top channel of a multilayer microfluidic system.

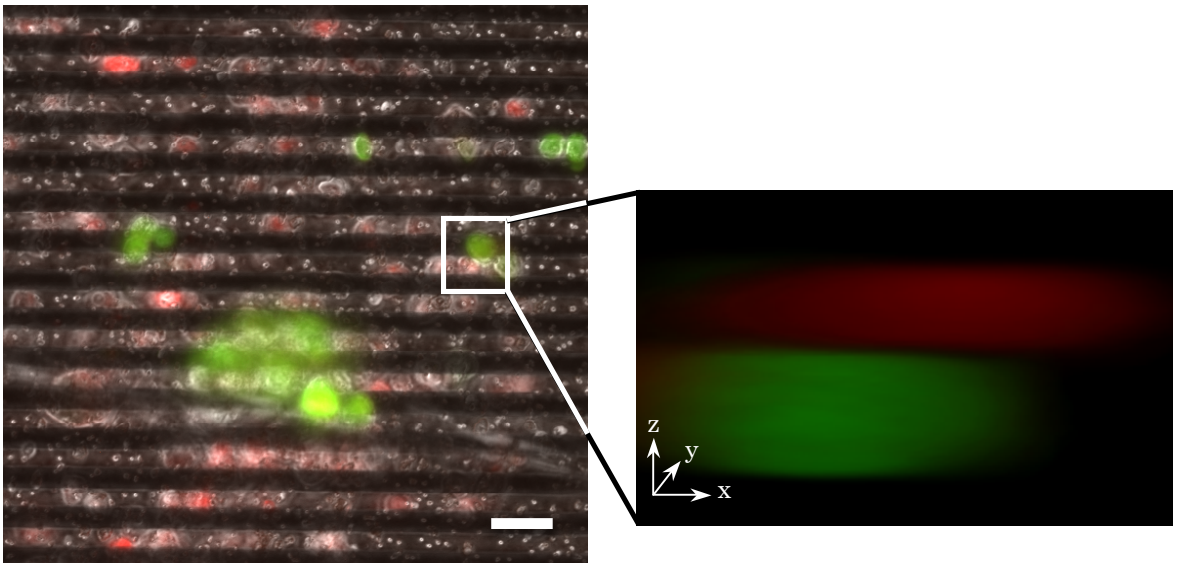

Figure 4. Micrograph of NIH-3T3 (red) and HepG2 (green) cells 24 h after seeded. The cells were still attached on both sides of the PET membrane. The black horizontal lines are the gold microelectrodes deposited on the PET membrane. The insert shows a 2D image from 3D image stacks (after deconvolution processing) of the spatial arrangement of one HepG2 and one NIH-3T3. The black space between the cells indicates the position of the PET membrane. Scale bar: 30 μm.

CONCLUSIONS

The patterning of gold microelectrodes for DEP trapping on permeable PET membranes and assembly of the membrane in a multilayer microfluidic system was accomplished. The trapping capabilities of DEP forces and the hCAM layer were demonstrated by the anchorage of HepG2 cells to the bottom of the membrane, despite gravity and fluid flow field. NIH-3T3 cells were anchored onto the top side of the membrane via the hCAM to generate a co-culture separated by the PET membrane. Cells were viable 24 h after cells were DEP trapped and spread across the membrane on their respective sides. This is a first effort in obtaining a co-culture system in a microfluidic device where cells are attached on the PET membrane using DEP forces. This system could potentially become a powerful tool to study cell-cell interactions in cell co-cultures (e.g. cell-cell communication and cell migration), where conditions can be tuned independently to accommodate the best growth and function conditions for each cell line. These devices represent a unique platform to interrogate cells in ways that have yet to be envisioned.

ACKNOWLEGMENTS

C.H. acknowledges the Swiss National Science Foundation for financial support. This research was performed in part at the NIST Center for Nanoscale Science and Technology.

REFERENCES

[1] D. R. Reyes, J. S. Hong, J. T. Elliott, M. Gaitan *Langmuir* **2011**, *27*, 10027-10034.
[2] D. Huh, et al. *Science* **2010**, *328*, 1662-1668.
[3] W. Tan and T. A. Desai *J. Biomed. Mater. Res.* **2005**, *72A*, 146–160.
[4] C. Hanke, P. S. Dittrich, D. R. Reyes *ACS Appl. Mater. Interfaces* **2012**, *4*, 1878-1882.

CONTACT

*Darwin R. Reyes, tel: +1-301-975-5466; darwin.reyes@nist.gov