PUMP-FREE MEMBRANE-CONTROLLED PERFUSION MICROFLUIDIC PLATFORM

V.N. Goral, Elizabeth Tran, and P.K. Yuen
Corning Incorporated, Corning, NY, USA and

ABSTRACT

We present an extension of the perfusion microplate technology and incorporate it into a microfluidic platform for passive pump-free perfusion cell culture and cell-based assays. By incorporating the perfusion microplate technology into microfluidic devices, all the benefits of microfluidic technologies, such as small sample volumes, fast and efficient fluidics exchanges and fluid properties at the micro-scale, can be fully taken advantage of with this passive pump-free perfusion-based microfluidic platform.

KEYWORDS: Passive Pumping, Pump-Free, Perfusion, Microfluidic Platform, Membrane

INTRODUCTION

Currently, most of the perfusion-based cell culture systems for mimicking *in vivo*-like cell environment were based on microfluidic devices and media were perfused by external pumps [1-4]. Also, these devices typically require a long period of hands-on learning because of external pumping accessories required for fluid pumping until they can be used effectively. Therefore, it is advantageous to develop a pump-free perfusion-based cell culture system or/and method that will require little to no hands-on learning but yet is effective in providing an *in vivo*-like cell environment for improving cellular morphology, cell viability, life-span and cell-specific functionality. In this study, we extend and incorporate the perfusion microplate technology into a microfluidic platform for passive pump-free perfusion cell culture and cell-based assays.

EXPERIMENTAL

Based on the perfusion microplate technology [5], which utilizes a combination of hydrostatic pressure generated by different liquid levels in the wells and fluid wicking through narrow strips of a porous membrane connecting the wells to generate fluid flow, a series of pump-free membrane-controlled perfusion microfluidic devices was developed. Each pump-free membrane-controlled perfusion microfluidic device comprises at least three basic components: an open well, a micron-sized deep chamber/channel and a wettable porous membrane (Fig. 1). Each component is fluidly connected either by the porous membrane or by the micron-sized deep chamber/channel.

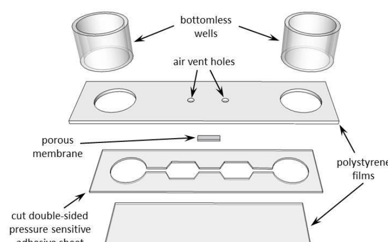

Figure 1: Schematic diagram depicting the assembly of a pump-free membrane-controlled perfusion microfluidic device with two open wells and two micron-sized deep chambers. A porous membrane is used to connect the two micron-sized deep chambers together while each open well and each chamber are connected by a micron-sized deep channel. An air vent hole in each chamber is used to let air escape during device preparation, such as priming, extracellular matrix (ECM) coating and/or cell seeding into the chambers.

RESULTS AND DISCUSSION

In addition to introducing continuous perfusion flow (Fig. 2), continuous side-by-side fluid flow could be introduced to generate spatially and temporally stable concentration gradient (Fig. 3), and to "pattern" and culture cells in the micron-sized deep cell culture channel such that only half of the channel would be seeded and cultured with cells (Fig. 4 and 5). We combined this side-by-side cell seeding with the concentration gradient generation and used them to perform an end-point cell migration assay using MDA-

MB-231 cells. We first seeded MDA-MB-231 cells into half of the micron-sized deep cell culture channel and after cell attachment, we generated an EGF concentration gradient across the cell culture channel width. After 16 hours of culture in serum-free medium, there were very few cells (approximately 25 cells) migrated towards the other half of the cell culture channel when only media were perfused into the cell culture channel (Fig. 6). On the other hand, when EFG and medium were perfused side-by-side into the cell culture channel generating an EGF concentration gradient, more MDA-MB-231 cells (approximately 65 cells) migrated towards the other half of the cell culture channel where the EGF concentration was higher. The experiments demonstrated the feasibility of performing an end-point migration assay using the pump-free membrane-controlled perfusion microfluidic device.

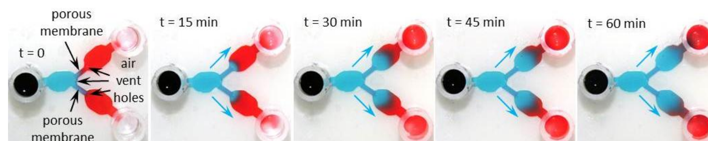

Figure 2: Time lapse images during a continuous fluid perfusion flow demonstration in a pump-free membrane-controlled perfusion microfluidic device with three open wells (6 mm diameter) and three micron-sized deep chambers (8 mm long × 5 mm wide × 137 μm deep). Two porous membranes (3 mm long × 1.2 mm wide 5 μm pore size cellulose acetate membranes) were used to connect the three chambers together while each open well and each chamber were connected by a micron-sized deep channel (3 mm long × 2.5 mm wide × 137 μm deep). An air vent hole (0.8 mm diameter) in each chamber was used to let air escape during device priming. The blue arrow indicates flow direction. Initial fluid volumes in the left and the two right open wells were 120 μl and 20 μl, respectively.

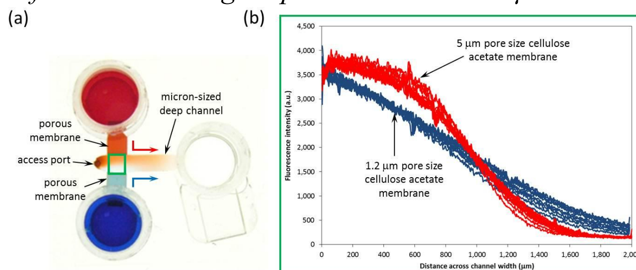

Figure 3: (a) Red and blue colored food dye solutions were used to demonstrate the continuous side-by-side fluid perfusion flow in a pump-free membrane-controlled perfusion microfluidic device with three open wells (6 mm diameter) and one micron-sized deep channel (10 mm long × 2 mm wide × 137 μm deep). The device was capable of generating a stable concentration gradient (green box area) under the continuous side-by-side fluid perfusion flow. Two porous membranes (3 mm long × 2 mm wide 5 μm pore size cellulose acetate membranes) were used to connect the two left open wells and the channel together in a cross-shaped configuration while the right open well was connected to the other end of the channel. An access port (0.8 mm diameter) was used for device priming. The red and blue arrows indicate flow direction. (b) Concentration gradient of FITC fluorescent dye solution across the channel width (green box area depicted in (a)) with either two 1.2 μm or 5 μm pore size cellulose acetate membranes under the continuous side-by-side fluid perfusion flow. Each curve represents a measurement at a 15 minute interval with a total time of 3 hours.

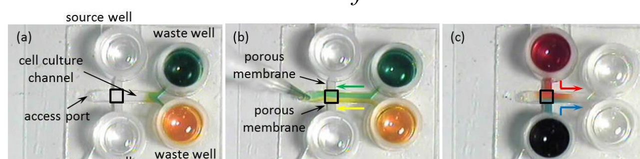

Figure 4: Various colored food dye solutions were used to demonstrate (a) device preparation, such as priming, ECM coating and samples loading, (b) side-by-side sample loading by withdrawing liquid through an access port (0.8 mm diameter) using a pipette and (c) continuous side-by-side fluid perfusion

flow in a pump-free membrane-controlled perfusion microfluidic device with four open wells (6 mm diameter), one wide (13 mm long × 2 mm wide × 137 μm deep) and two narrow (2 mm long × 0.2 mm wide × 137 μm deep) micron-sized deep channels. The device was also capable of generating a stable concentration gradient (black box area in (c)) under the continuous side-by-side fluid perfusion flow. Two porous membranes (3 mm long × 2 mm wide 5 μm pore size cellulose acetate membranes) were used to connect the two left (source) open wells and the wide (cell culture) channel together in a cross-shaped configuration while the two right (waste) open wells were connected to the other end of the wide channel by the two narrow channels. The green, yellow, red and blue arrows indicate flow direction.

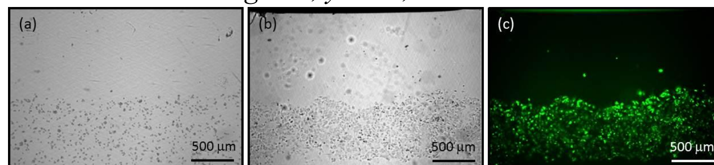

Figure 5: Bright field images (black box area depicted in Fig. 4c) (a) after seeding C3A cells in half of the cell culture channel of the pump-free membrane-controlled perfusion microfluidic device using the side-by-side sample loading method depicted in Fig. 4b and (b) after 42 hours of perfusion cell culture. (c) The corresponding live/dead cell staining image depicted in (b).

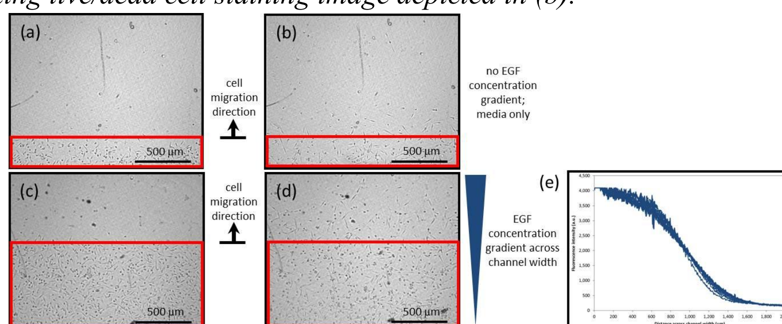

Figure 6: Bright field images (black box area depicted in Fig. 4c) of an end-point MDA-MB-231 cell migration (a) and (b) without and (c) and (d) with a stable EGF concentration gradient under continuous side-by-side fluid perfusion flow using the pump-free membrane-controlled perfusion microfluidic device depicted in Fig. 4. (a) and (c), and (b) and (d) 2 hours and 16 hours after seeding MDA-MB-231 cells in half of the cell culture channel using the side-by-side sample loading method depicted in Fig. 4b, respectively. (e) Concentration gradient of FITC fluorescent dye solution across the cell culture channel width (black box area depicted in Fig. 4c). Each curve represents a measurement at a 30 minute interval with a total time of 6 hours.

CONCLUSION

Based on the perfusion microplate technology, we developed a pump-free membrane-controlled perfusion microfluidic platform and demonstrated their use for pump-free perfusion cell culture and cell-based assays. By incorporating the perfusion microplate technology into microfluidic devices, all the benefits of microfluidic technologies can be fully taken advantage of with this perfusion-based microfluidic platform.

REFERENCES

[1] V. N. Goral *et al. Lab Chip*, 2010, **10**, 3380-3386.
[2] Y. C. Toh *et al.*, *Lab Chip*, 2009, **9**, 2026-2035.
[3] Y. Nakao *et al.*, *Biomicrofluidics*, 2011, **5**, 022212.
[4] D. Choudhury *et al. Biomicrofluidics*, 2011, **5**, 022203.
[5] V. N. Goral *et al.*, *Lab Chip*, 2013, **13**, 1039-1043.

CONTACTS

* V.N. Goral; phone: +1-607-974-1843; goralvn@corning.com
* P.K. Yuen; phone: +1-607-974-9680; yuenp@corning.com