A pump-free membrane-controlled perfusion microfluidic platform

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(Received 29 April 2015; accepted 24 August 2015; published online 2 September 2015)

In this article, we present a microfluidic platform for passive fluid pumping for pump-free perfusion cell culture, cell-based assay, and chemical applications. By adapting the passive membrane-controlled pumping principle from the previously developed perfusion microplate, 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 and their use for pump-free perfusion cell culture and cell-based assays was demonstrated. Each pump-free membrane-controlled perfusion microfluidic device comprises at least three basic components: an open well for generating fluid flow, a micron-sized deep chamber/channel for cell culture or for fluid connection, and a wettable porous membrane for controlling the fluid flow. Each component is fluidically connected either by the porous membrane or by the micron-sized deep chamber/channel. By adapting and incorporating the passive membrane-controlled pumping principle into microfluidic devices, all the benefits of microfluidic technologies, such as small sample volumes, fast and efficient fluid exchanges, and fluid properties at the micro-scale, can be fully taken advantage of with this pump-free membrane-controlled perfusion microfluidic platform.
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I. INTRODUCTION

Currently, most of the perfusion-based cell culture systems for mimicking *in vivo*-like cell environment were based on microfluidic devices, and cell culture media were perfused by external pumps.^{1–10} Also, these microfluidic 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 for perfusion cell culture and cell-based assay applications 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 recognizing this advantage, various passive pumping technologies for microfluidic perfusion cell culture have been developed and demonstrated over the past years. For example, microfluidic devices based on surface tension driven passive pumping were developed for cell culture, cell-based assays, and *in vitro* (cell-free) protein expression applications.^{11–13} Also, gravity and surface tension force generated perfusion was utilized for three-dimensional (3D) perfusion culture of human mammary epithelial cells and *in vitro* anti-cancer drug screening.¹⁴ In addition, using a rocker platform, a gravity driven, periodically changing bidirectional medium flow was demonstrated in a multi-layer microfluidic device for multi-cellular 3D human primary liver cell culture.¹⁵

Recently, we have also developed and demonstrated a passive membrane-controlled pumping technology in a microplate format, the perfusion microplate.¹⁶ In this perfusion microplate,

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fluidically connected wells enabled automatic continuous fluid perfusion between the wells without the need for external pumping. Fluid flow was achieved using a combination of hydrostatic pressure generated by different liquid levels in the wells and fluid wicking through narrow strips of a cellulose membrane/filter paper connecting the wells (Fig. 1(a)). However, the perfusion microplate has its shortcomings when compared with microfluidic devices (see supplementary material (SM) in Ref. 21 for details). Briefly, since the middle cell culture well of the three-well unit in the perfusion microplate was based on an open well format, fluid exchange efficiency in this cell culture well is very poor. Also, a relatively larger fluid volume in the cell culture well limits the amount of fluid that can be exchanged in the cell culture well without resetting the fluid volumes in both the source and the waste wells. In addition, because of the open wells, each three-well unit requires two porous membranes, which increases the complexity of the perfusion microplate assembly, and limits the scale-up and design flexibility of the perfusion microplate. Thus, in this article, we present a series of pump-free perfusion microfluidic devices developed by adapting the passive membrane-controlled pumping principle from the perfusion microplate and incorporating it into a microfluidic platform for cell culture, cell-based assay, and chemical applications. By adapting and incorporating the passive membrane-controlled pumping principle into microfluidic devices, all the benefits of microfluidic technologies, such as small sample volumes, fast and efficient fluid exchanges, and fluid properties at the micro-scale (for example, two laminar fluid flows flowing side-by-side to create a long-term stable concentration gradient, which cannot be achieved with the perfusion microplate), can be fully taken advantage of with this pump-free membrane-controlled perfusion microfluidic platform. These microfluidic technologies benefits were demonstrated using some exemplary pump-free membrane-controlled perfusion microfluidic devices and the applications for pump-free perfusion cell culture and cell-based assays were also demonstrated. Other than cell culture and cell-based assay applications, we envision that the pump-free membrane-controlled perfusion microfluidic platform can also be used in chemical applications where perfusion flow is desired such as membraneless microfuel cell applications.¹⁷

II. EXPERIMENTAL DETAILS

A. Microfluidic device assembly and sterilization

First, we cut the device design in an approximately $137\text{ }\mu\text{m}$ thick double-sided pressure sensitive adhesive (PSA) sheet (ARcare[®] 90106, Adhesives Research, Inc., Glen Rock, PA, USA) using a desktop digital craft cutter¹⁸ (Fig. 1(b)). Next, we cut strips of porous membrane, such as cellulose membranes with either $1.2\text{ }\mu\text{m}$ (Whatman[®] ST 69, Catalog No. 10403012,

FIG. 1. (a) Schematic diagram depicting the cross-sectional view of the three connected wells in a perfusion microplate. Reproduced with permission from Goral *et al.*, Lab Chip **13**, 1039–1043 (2013). Copyright 2013 The Royal Society of Chemistry. (b) 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.

Whatman GmbH, Dassel, Germany), 5 μm (Order No. 12342-47-K, Sartorius Stedim Biotech GmbH, Göttingen, Germany), or 8 μm (Whatman[®] AE 99, Catalog No. 10400112, Whatman) pore size, using an X-Acto[®] precision knife (Elmer's Products, Inc., Westerville, OH, USA). We started the assembly process by peeling off the first protective layer exposing the first PSA surface of the cut double-sided PSA sheet before attaching it to the first polystyrene film, which was taken from a Corning[®] HYPERStack[™] Cell Culture Vessel (Corning Incorporated, Corning, NY, USA). After that, we aligned and inserted the porous membrane(s) into the cut channels of the cut double-sided PSA sheet. Next, we peeled off the second protective layer exposing the second PSA surface of the cut double-sided PSA sheet with the inserted porous membrane(s). We then aligned and attached the second polystyrene film, which had corresponding holes cut through the film, to the second exposed PSA surface. These holes allowed fluid to be introduced into the micron-sized deep chamber(s) or/and channel(s), and air to escape during device preparation such as priming, extracellular matrix (ECM) coating, and/or cell seeding. Care was taken to ensure that the cut double-sided PSA sheet and the polystyrene films were fully adhered to each other without any air bubbles. Also, each individual porous membrane was inspected under a microscope to ensure that there were no gaps formed between the two long edges of the porous membrane and the cut channel edges in the cut double-sided PSA sheet. After inspection, bottomless wells, which were made from strips of Corning[®] 96 well Stripwell[™] (Product No. 9102, Corning Incorporated), were aligned with the openings in the second polystyrene film and glued (Bio-PSA 7-4301 Silicone Adhesive, Dow Corning, Midland, MI, USA) to the top of the second polystyrene film. These bottomless wells were used as fluid reservoirs so that fluid could be easily pipetted into them (open wells). Finally, before each cell experiment, we sterilized each pump-free membrane-controlled perfusion microfluidic device with 300 μl of 70% ethanol in water for 30 min, washed twice with 300 μl of deionized water and 300 μl of 1 $\times$ phosphate buffered solution (PBS).

B. Fluid perfusion flow test

We experimentally tested fluid perfusion flow in each individual pump-free membrane-controlled perfusion microfluidic device using water/PBS and various colored food dye solutions (Figs. 2 and 3). We first pipetted liquid (either water/PBS or colored food dye solutions) into the micron-sized deep chamber(s)/channel(s) through the open well(s). Next, different liquid volumes were pipetted into the open wells to start the fluid perfusion flow. Fluid would start to flow from the open well(s) with a taller liquid height towards the open well with a lower liquid height until the liquid height in each open well reached the same level. For the concentration gradient characterization experiments, we used a fluorescein isothiocyanate (FITC) dye (9×10^{-6} M) (Catalog No. F2502-1G, Sigma-Aldrich, St. Louis, MO, USA) in 1 $\times$ PBS solution. The FITC fluorescent dye intensity was measured and analyzed using a Zeiss Axiovert 200 inverted fluorescence microscope equipped with an epifluorescence condenser and camera system (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA) and the AxioVision Microscope Software, respectively.

C. Side-by-side cell seeding and perfusion cell culture

For the side-by-side cell seeding and perfusion cell culture experiments, a highly proliferative liver cell line, C3A cells (CRL-10741[™], American Type Culture Collection (ATCC), Manassas, VA, USA) were first cultured in a sterile cell culture flask as previously reported.¹⁶ After priming the device with cell culture medium, 30 μl of cell culture medium was pipetted into one of the two waste wells, while 30 μl of cell suspension solution (1.4×10^6 cells/ml) was pipetted into the other waste well (Fig. 4(a)). Also, 20 μl of cell culture medium was pipetted into each of the source wells. Next, we withdrew 20 μl of liquid from the cell culture channel through the access port using a 100 μl pipette. A laminar side-by-side fluid flow along the cell culture channel flowing from the two waste wells towards the access port was then ensued (Fig. 4(b)). During this laminar side-by-side fluid flow, C3A cells in one of the waste wells were forced to flow only into half of the cell culture channel, while the other half of the cell culture

FIG. 2. (a) A pump-free membrane-controlled perfusion microfluidic device, based on the device assembly depicted in Fig. 1(b), with two 6 mm diameter open wells and two 8 mm long $\times$ 5 mm wide $\times$ 137 μ m deep chambers. A 3 mm long $\times$ 1.2 mm wide $\times$ 140 μ m thick 5 μ m pore size cellulose acetate membrane was used to connect the two chambers together while each open well and each chamber were connected by a 2 mm long $\times$ 2 mm wide $\times$ 137 μ m deep channel. A 0.8 mm diameter air vent hole in each chamber was used to let air escape during device priming. (b)–(i) Time lapse images during a continuous fluid perfusion flow demonstration. The white arrows indicate the flow direction. Initial fluid volumes in open well 1 and 2 were 120 μ l and 20 μ l, respectively. Image was taken every 15 min.

FIG. 3. (a)–(e) Time lapse images during a continuous fluid perfusion flow demonstration in a pump-free membrane-controlled perfusion microfluidic device with three 6 mm diameter open wells and three 8 mm long $\times$ 5 mm wide $\times$ 137 μ m deep chambers. Two identical 3 mm long $\times$ 1.2 mm wide $\times$ 140 μ m thick 5 μ m pore size cellulose acetate membranes were used to connect the three chambers together while each open well and each chamber were connected by a 2 mm long $\times$ 2 mm wide $\times$ 137 μ m deep channel. A 0.8 mm diameter air vent hole in each chamber was used to let air escape during device priming. The blue arrows indicate the flow direction. Initial fluid volumes in open well 1, 2, and 3 were 20 μ l, 120 μ l, and 20 μ l, respectively. Image was taken every 15 min.

FIG. 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 a 0.8 mm diameter access port using a pipette; and (c) continuous laminar side-by-side fluid perfusion flow in a pump-free membrane-controlled perfusion microfluidic device with four 6 mm diameter open wells, one 13 mm long $\times$ 2 mm wide $\times$ 137 μ m deep and two 2 mm long $\times$ 0.2 mm wide $\times$ 137 μ m deep channels. This device was also capable of generating a stable concentration gradient (black box area in (c)) under the continuous laminar side-by-side fluid perfusion flow. Two identical 3 mm long $\times$ 2 mm wide $\times$ 140 μ m thick 5 μ m pore size cellulose acetate membranes were used to connect the two open source wells and the 2 mm wide cell culture channel together in a cross-shaped configuration while the two open waste wells were connected to the other end of the 2 mm cell culture channel by the two 0.2 mm wide channels. The green, yellow, red, and blue arrows indicate the flow direction.

channel remained free of cells. After the side-by-side cell seeding, C3A cells were allowed to attach to the bottom of the cell culture channel for 2 h in a CO₂ HEPA-filtered incubator (Model 3130, Forma Scientific, Inc., Marietta, OH, USA) at 37 °C, 95% relative humidity, and 5% CO₂ before starting the perfusion cell culture. Perfusion cell culture was started by

adjusting the volumes in the source and waste wells such that 200 μl and 50 μl of cell culture medium were in each of the source and the waste wells, respectively (Fig. 4(c)).

D. Cell live/dead staining

We used the LIVE/DEAD[®] Viability/Cytotoxicity Assay Kit for mammalian cells (Molecular Probes, Inc., Eugene, OR, USA) to determine viability of the cultured cells following the standard protocol. Briefly, the fluorescent dye mixture was pipetted into the cell culture channel followed by a $1\times$ PBS buffer wash. Fluorescent live/dead cell staining images were captured using a Zeiss Axiovert 200 inverted fluorescence microscope equipped with an epifluorescence condenser and camera system.

E. End-point cell migration assay

The metastatic human breast cancer MDA-MB-231 cells (ATCC) were serum-starved overnight in Leibovitz' L-15 medium (Life Technologies, Carlsbad, CA, USA) containing 0.2% bovine serum albumin (BSA) (Sigma-Aldrich) in a sterile T75 cell culture flask (Corning Incorporated). MDA-MB-231 cells were then detached using 1 ml of cell dissociation buffer (Life Technologies), washed, and resuspended into single-cell suspension in 0.2% BSA/L-15 medium. After priming the device with cell culture medium, the middle cell culture channel was coated with an ECM before side-by-side seeding of MDA-MB-231 cells (6×10^5 cells/ml) into half of the cell culture channel as described above (Fig. 4). ECM coating was performed by pipetting 30 μl of human collagen IV solution (5 $\mu\text{g/ml}$ in $1\times$ PBS) (EMD Millipore, Billerica, MA, USA) into each of the waste wells (Fig. 4(a)) and then withdrawing 30 μl of liquid from the cell culture channel through the access port using a 100 μl pipette (Fig. 4(b)). Next, the human collagen IV solution was allowed to incubate inside the cell culture channel for 45 min at room temperature and additional 45 min at 37 °C. Compound concentration gradient was generated by pipetting 200 μl of 0.2% BSA/L-15 medium into one of the two source wells and 200 μl of human epidermal growth factor (EGF) solution (Sigma-Aldrich) (8×10^{-9} M) into the other source well, while 50 μl of 0.2% BSA/L-15 medium was pipetting into each of the waste wells (Fig. 4(c)). Bright field cell migration images were captured using a Zeiss Axiovert 200 inverted fluorescence microscope equipped with an epifluorescence condenser and camera system or using a continuous microscopy tracking method. Finally, cell number was counted manually from the bright field cell migration images.

III. RESULTS AND DISCUSSION

A. Microfluidic device assembly

The critical feature controlling the quality of this pump-free membrane-controlled perfusion microfluidic device assembly is to have a good seal along the two long edges of the porous membrane(s) without any gaps between them and the channel edges cut into the double-sided PSA sheet. The fluid perfusion flow in the pump-free membrane-controlled perfusion microfluidic device can be readily controlled by manipulating the liquid levels in the open wells along with using a porous membrane of appropriate dimensions and pore size. Since the cell culture well was enclosed and converted into a micron-sized deep chamber in the pump-free membrane-controlled perfusion microfluidic device, unlike the perfusion microplate design, a porous membrane is not required at every intersection between the open well and the enclosed micron-sized deep channels/chamber (see SM in Ref. 21 for details). As a result, we reduced the complexity of the device assembly and improved the scale-up and design flexibility of the pump-free membrane-controlled perfusion microfluidic devices.

In addition, we had incorporated an air vent hole (typically from 0.2 mm to 1 mm in diameter) located near the porous membrane into each micron-sized deep chamber. Although it is not absolutely necessary, this air vent hole significantly simplified priming, ECM coating, and/or cell seeding into the micron-sized deep chamber as trapped air could easily escape from the micron-sized deep chamber instead of going through the porous membrane. Also, liquid inside

the micron-sized deep chamber can be easily withdrawn through this air vent hole. Due to the small diameter of the air vent hole, liquid would not escape from the micron-sized deep chamber during the normal operation of the device as demonstrated in the fluid perfusion flow test experiments (Figs. 2 and 3).

B. Fluid perfusion flow test

As expected, fluid started to perfuse from the open well(s) with a taller liquid height through the micron-sized deep channel(s)/chamber(s) and porous membrane(s) towards the open well(s) with a lower liquid height until the liquid height in each open well reached the same level (Figs. 2 and 3). The fluid perfusion flow experiments demonstrated the versatility of the pump-free membrane-controlled perfusion flow method. We expect that both device configurations (Figs. 2 and 3) can be used for simple perfusion cell culture, conditioned medium dependent growth co-culture, and cell-based toxicity assays similar to the ones that we had previously reported¹⁶ as well as applications such as cell-cell communications, ADME/tox screening, and organs-on-a-chip but with lower sample consumption and more fast and efficient samples/drugs exchanges.

The fluid perfusion flow in the pump-free membrane-controlled perfusion microfluidic devices can be simulated based on Darcy's Law.^{16,19} For example, the mathematical simulation model for the two open well device with one porous membrane (Fig. 2) is given by (see SM in Ref. 21 for details)

$$\begin{aligned} V_1(t) &= \frac{1}{2}(V_1(0) - V_2(0))e^{\frac{-2t}{\tau}} + \frac{1}{2}(V_1(0) + V_2(0)) \\ V_2(t) &= -\frac{1}{2}(V_1(0) - V_2(0))e^{\frac{-2t}{\tau}} + \frac{1}{2}(V_1(0) + V_2(0)), \end{aligned} \quad (1)$$

where $V_1(t)$ and $V_2(t)$ are the fluid volumes in open wells 1 and 2, respectively, at time t , and $V_1(0)$ and $V_2(0)$ are the initial fluid volumes in open wells 1 and 2, respectively. τ is the characteristic fluid perfusion time of the porous membrane and is given by

$$\tau = \frac{\mu}{\kappa A_m} \frac{A_w L}{\rho g}, \quad (2)$$

where κ (m^2 or Darcy, which is equal to $1 \mu\text{m}^2$) is the permeability of the porous membrane and was fitted experimentally. From our previous study, the fitted permeabilities, κ , of the porous membrane with pore sizes of $1.2 \mu\text{m}$ (Whatman[®] ST 69, Catalog No. 10403012, Whatman), $5 \mu\text{m}$ (Order No. 12342-47-K, Sartorius Stedim Biotech GmbH), and $8 \mu\text{m}$ (Whatman[®] AE 99, Catalog No. 10400112, Whatman) were 0.15 Darcy, 0.8 Darcy and 4.0 Darcy, respectively.¹⁶ A_m (m^2) and A_w (m^2) are the cross-sectional areas of the porous membrane and the open wells, respectively, μ (Pa s) is the fluid viscosity, L (m) is the length of the porous membrane, ρ (kg m^{-3}) is the fluid density, and g (m s^{-2}) is the gravity constant. For the three open well device with two identical porous membranes (Fig. 3), the mathematical simulation model is given by¹⁶

$$\begin{pmatrix} V_1(t) \\ V_2(t) \\ V_3(t) \end{pmatrix} = \begin{pmatrix} \frac{1}{3} & \frac{2}{3} & \frac{1}{3} \\ \frac{1}{3} & -\frac{1}{3} & \frac{1}{3} \\ \frac{1}{3} & -\frac{1}{3} & -\frac{2}{3} \end{pmatrix} \begin{pmatrix} V_1(0) + V_2(0) + V_3(0) \\ \mathbf{a} e^{\frac{-3t}{\tau}} + \mathbf{b} e^{\frac{-t}{\tau}} \\ -\mathbf{a} e^{\frac{-3t}{\tau}} + \mathbf{b} e^{\frac{-t}{\tau}} \end{pmatrix}, \quad (3)$$

where $\mathbf{a} = \frac{1}{2}(V_1(0) - 2V_2(0) + V_3(0))$ and $\mathbf{b} = \frac{1}{2}(V_1(0) - V_3(0))$. $V_1(t)$, $V_2(t)$, and $V_3(t)$ are the fluid volumes in open wells 1, 2, and 3, respectively, at time t , and $V_1(0)$, $V_2(0)$, and $V_3(0)$ are the initial fluid volumes in open wells 1, 2, and 3, respectively. Both Eqs. (1) and (3) were validated with experimental fluid perfusion flow data using a pump-free membrane-controlled perfusion microfluidic device with two open wells and one porous membrane (Fig. 2) or with three open wells and two

FIG. 5. Experimental (circles) and simulated (lines) (a) fluid volumes and (b) flow rates in open well 1 (red) and 2 (black) of a pump-free membrane-controlled perfusion microfluidic device with two 6.5 mm diameter open wells and two 8 mm long $\times$ 5 mm wide $\times$ 137 μ m deep chambers as a function of time. A 2.45 mm long $\times$ 1.32 mm wide $\times$ 140 μ m deep 5 μ m pore size cellulose acetate membrane with $\kappa = 0.8$ Darcy was used to connect the two chambers together while each open well and each chamber were connected by a 2 mm long $\times$ 2 mm wide $\times$ 137 μ m deep channel. A 0.8 mm diameter air vent hole in each chamber was used to let air escape during device priming. Initial fluid volumes in open well 1 and 2 were 250 μ l and 50 μ l, respectively.

identical porous membranes (Fig. 3). The comparison between the experimental and the simulated fluid perfusion flow data shows excellent correspondence in both cases (Figs. 5 and 6).

By incorporating the micron-sized feature into the pump-free membrane-controlled perfusion microfluidic platform, fluid properties at the micro-scale could be fully taken advantage of with this microfluidic platform. For example, two separate fluid flow streams flowing side-by-side without mixing could be generated in the micron-sized deep channel due to the well-known properties of microfluidics²⁰ (Fig. 4(c) and see SM in Ref. 21 for details). This continuous laminar side-by-side fluid flow is not achievable with the previously reported perfusion microplate. The small length scale of the micron-sized deep channel precluded any possibility of eddy diffusivity due to turbulence and/or shear layer instability between the two fluids, and the only means of mixing was therefore molecular diffusion. The two fluid streams were separated by a very thin diffusion (mixing) layer, which grew relative to the amount of time the two fluids were in contact. The width of the diffusion interface is $\sqrt{2Dt}$, where D is the mass diffusion coefficient and t is the time ($t = L/U$, where L is the distance from the inlet, and U is the average fluid flow velocity). Thus, by exploring this thin diffusion layer, we had developed various pump-free membrane-controlled perfusion microfluidic devices that were capable of generating a spatially and temporally stable concentration gradient inside the micron-sized deep

FIG. 6. Experimental (circles) and simulated (lines) (a) fluid volumes and (b) flow rates in open well 1 (red), 2 (black), and 3 (green) of a pump-free membrane-controlled perfusion microfluidic device with three 6.5 mm diameter open wells and three 8 mm long $\times$ 5 mm wide $\times$ 137 μ m deep chambers as a function of time. Two identical 2.55 mm long $\times$ 1.32 mm wide $\times$ 140 μ m deep 5 μ m pore size cellulose acetate membrane with $\kappa = 0.8$ Darcy were used to connect the three chambers together, while each open well and each chamber were connected by a 2 mm long $\times$ 2 mm wide $\times$ 137 μ m deep channel. A 0.8 mm diameter air vent hole in each chamber was used to let air escape during device priming. Initial fluid volumes in open well 1, 2, and 3 were 50 μ l, 300 μ l, and 50 μ l, respectively. Note that green and red lines overlap on top of each other.

FIG. 7. Concentration gradient of FITC fluorescent dye solution across the channel width (black box area depicted in Fig. 4(c)) with either two identical 1.2 μm or 5 μm pore size cellulose acetate membranes under the continuous laminar side-by-side fluid perfusion flow. Each curve represents a measurement at a 15 min interval with a total time of 3 h.

channel for cell-based assay applications. This concentration gradient profile could be tuned by manipulating the fluid perfusion flow using different liquid heights in the open wells and/or different dimensions and pore sizes of the porous membranes (Fig. 7). Also, it is expected that the concentration gradient will lose its stability when the fluid perfusion flows from the two laminar fluid flow streams become insignificant, which can be estimated by Eq. (1). However, the stability of the concentration gradient profile can be maintained forever if the fluid volume in each open well is reset to its initial fluid volume every few hours.

We had also developed an alternative device design for this pump-free membrane-controlled perfusion microfluidic device by taping a circular porous membrane over the device opening before gluing the open wells (Fig. 8), instead of inserting the porous membrane into

FIG. 8. Schematic diagram depicting the assembly of an alternative design of the pump-free membrane-controlled perfusion microfluidic device with two open wells and one micron-sized deep chamber. A porous membrane is taped over the device opening for controlling the fluid perfusion flow. Two micron-sized deep channels are used to connect the chamber to the open wells. An air vent hole is used to let air escape during device preparation, such as priming, ECM coating, and/or cell seeding into the chamber.

FIG. 9. (a) A pump-free membrane-controlled perfusion microfluidic device, based on the device assembly depicted in Fig. 8, with two 6 mm diameter open wells and one 7 mm long $\times$ 2 mm wide $\times$ 137 μ m deep channel. Diameters of the device opening were (1) 4 mm, (2) 3 mm, and (3) 2 mm, respectively. Either a (1) 5 mm or (2) and (3) 4 mm diameter, 0.22 μ m pore size cellulose acetate membrane, which was taken from a Corning[®] 430517 1L Filter System (Corning Incorporated), was taped over each device opening. (b)–(f) Time lapse images during the fluid perfusion flow demonstration. The blue arrows indicate the flow direction. Initial fluid volumes in the top and bottom open wells were 200 μ l and 20 μ l, respectively. Image was taken every 4 min.

the cut double-sided PSA sheet (Fig. 1(b)). In this case, fluid perfusion flow was controlled by the diameter of the device opening, and the thickness and pore size of the porous membrane (Fig. 9). Also, the porous membrane assembly of this alternative device design is significantly easier as one only needs to cover the device opening with the porous membrane.

C. Perfusion cell culture and end-point cell migration assay

Other than generating spatially and temporally stable concentration gradient, the continuous laminar side-by-side fluid flow could be used to “pattern” cells in the micron-sized deep cell culture channel such that only half of the channel would be seeded with cells. This capability was demonstrated using C3A cells (Figs. 4 and 10). After being seeded into half of the cell culture channel, the bright field and the live/dead cell staining images before and after 42 h of perfusion cell culture showed C3A cells growing healthily and retained in that half of the cell culture channel (Fig. 10). Next, 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 then after cell attachment, we started the perfusion cell culture either using serum-free medium without EGF or generating an EGF concentration gradient across the cell

FIG. 10. Bright field images (black box area depicted in Fig. 4(c)) (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. 4(b) and (b) after 42 h of perfusion cell culture. (c) The corresponding live/dead cell staining image depicted in (b).

FIG. 11. Bright field images (black box area depicted in Fig. 4(c)) 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 laminar side-by-side fluid perfusion flow using the pump-free membrane-controlled perfusion microfluidic device depicted in Fig. 4. ((a) and (c)) 2 h and ((b) and (d)) 16 h after seeding MDA-MB-231 cells into half of the cell culture channel (red box area) using the side-by-side sample loading method depicted in Fig. 4(b). (e) Concentration gradient of FITC fluorescent dye solution across the cell culture channel width (black box area depicted in Fig. 4(c)). Each curve represents a measurement at a 30 min interval with a total time of 6 h.

culture channel width (Fig. 11). After 16 h of perfusion cell culture in serum-free medium without EGF, there were very few cells (approximately 25 cells) migrated towards the other half of the cell culture channel when only media without EGF were perfused into the cell culture channel. On the other hand, when EGF solution 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. Multiple repeated experiments showed that when compared with serum-free medium without EGF, approximately 2–3 fold more cells had consistently migrated towards the other half of the cell culture channel when EGF solution and medium were perfused side-by-side into the cell culture channel. These experiments demonstrated the feasibility of performing an end-point migration assay using the pump-free membrane-controlled perfusion microfluidic device.

IV. CONCLUSIONS

By adapting and incorporating the passive membrane-controlled pumping principle from the perfusion microplate into a microfluidic platform, we developed a pump-free membrane-controlled perfusion microfluidic platform. The benefits of this pump-free membrane-controlled perfusion microfluidic platform in microfluidic technologies were demonstrated with some exemplary pump-free membrane-controlled perfusion microfluidic devices. We also demonstrated applications for pump-free perfusion cell culture and an end-point cell migration assay with these pump-free membrane-controlled perfusion microfluidic devices. Other than cell culture and cell-based assay applications, we envision that the pump-free membrane-controlled perfusion microfluidic platform can also be used in chemical applications where perfusion flow is desired such as membraneless microfuel cell applications.

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