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Steady flow of pressure-driven water-in-oil droplets in closed-open-closed microchannels

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ABSTRACT

Open microfluidics is an emerging field of bio/medical applications that need direct energy/matter exchange between microfluids and environment. This paper presents the design, simulation, fabrication, and test of a microfluidic chip for a water-in-oil (WiO) two-phase flow in closed-open-closed microchannels. The chip, fabricated from PDMS using soft lithography, consists of a flow-focusing structure for WiO droplet generation and a long closed-open-closed channel for droplet flow. A negative pressure applied to the end of the channel is used as the driving force for WiO droplets to flow through the open channel. It is found that the negative pressure that is capable of driving a steady flow for a given flow rate, without overflow and air suction, falls into a pressure range instead of being an exact value. The mechanism for the pressure range is investigated theoretically and experimentally and is attributed to the surface tension. Yeast cells have been incorporated in the droplets, and the successful flow through the open channels verifies the function of the chips.

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I. INTRODUCTION

Open microfluidics, in which fluids flow in a microchannel with at least a part of the sealing walls being absent, is an emerging area in biological and medical applications for direct exchange of energy or matter between fluids and environment.1,2 As open microfluidics provides the accessibility to fluids during flowing, a device-to-world interface can be achieved in dynamic modes, and direct fluid treatment or manipulation that is impossible in conventional sealed microfluidics can be performed. For example, in atmospheric room temperature plasma (ARTP) mutation, a new technology that uses RF plasma to perform whole-cell mutagenesis for high mutation rates at low temperatures,3,4 the fluids that contain cells should be exposed directly to plasma during flowing to perform fast plasma treatment. Besides direct energy/matter exchange, the open area makes it easy to remove detrimental air bubbles that are inevitable in sealed microfluidics, which are difficult to predict and hard to remove. Such unique features make open microfluidics an enabling technology in a wide range of applications such as cell culture, rare cell isolation, cell signal studying, metabolomics, etc.

Most open microfluidic studies focus on a single-phase flow in open channels.2,3,12 In single-phase open microfluidics, the most critical challenge is to prevent overflow of the fluid from the channels at places where physical walls are absent. So far, surface tension confinement and wettability-contrast confinement have been developed for open microfluidics.6,13,14 Surface tension confinement exploits the surface tension of the liquid-air interface at the open areas where the top and/or the bottom walls are removed to confine the flow of liquid in open channels.15–18 Wettability-contrast confinement employs the surface tension difference between hydrophilic and hydrophobic patterns, like virtual walls, to confine the flow in specific areas.19–21

The second challenge in open microfluidics is the method to drive the flow. So far, the fluid transport and manipulation in an open channel normally depend on external energy such as pumps, valves, or voltage.2,22,23 As the surface tension for confining flows is quite small, the pumping pressure should be low enough to avoid overflow at the open areas. Recently, flow-driven techniques using capillary forces, such as the spontaneous capillary fluid (SCF) technique, have attracted extensive attention because they are easy to implement by using capillary forces instead of external facilities and
energies,\textsuperscript{16,26,27} However, capillary action is inherently one-off and somewhat inflexible in controlling flow rates. In addition, because the directions and the flow rates highly depend on the fluid properties and the geometries of channels, surface pretreatments are inevitable, and the storage time is short.

The third challenge in the implementation of open channels is the fast evaporation of liquids at the open areas because of the large surface-to-volume ratio at the air-liquid interface, in particular, for aqueous solutions that are indispensable for cell culture.\textsuperscript{2,8,9} One possible solution is to use a water-in-oil (WiO) two-phase liquid in open channels. However, manipulating two-phase flows in open microfluidics requires consideration of numerous factors,\textsuperscript{28} and the investigation on the two-phase flow is relatively nonthorough.\textsuperscript{29} So far, two-phase droplets have been realized in paper-based open channels by producing a nanofibrillar texture on top of the paper microfibers with oxygen plasma etching prior to fluoro-silanization.\textsuperscript{29} More recently, a spontaneous capillary flow of two-phase systems has been demonstrated in open channels fabricated on PMMA.\textsuperscript{29}

Aiming at cell treatment applications like ARTP, the two-phase flow in open channels with a narrow width, large flow rate range, and stable flow speed are highly required. Here, we present an open microfluidic device that is able to generate and transport WiO two-phase droplets in a long, close-open-close channel by pressure. This device demonstrates the following features for open microfluidics. (1) A negative pressure is applied to the outlet of the channel such that a high flow speed, steady and continuous flow, and good tolerance to liquid properties (such as wettability) can be achieved. (2) By using flow focusing, WiO two-phase flows have been implemented, which use nonvolatile oil that has low evaporation at the open area to protect the volatile aqueous liquid.

II. MATERIALS AND METHODS

A. Chip design

Figure 1 shows the schematic diagram of the closed-open-closed microfluidic chip. The chip has three layers. The middle polydimethylsiloxane (PDMS) layer is fabricated with channels and sandwiched in-between a bottom glass layer used for mechanical support and a top sealing PDMS layer. The top PDMS layer is opened with small holes to provide inlets, outlets, and the open area for the channel. The channels consist of a droplet generation part and a transport channel part. The first part consists of an oil channel and a straight water channel that passes through the oil channel. The transport channel part starts from the intersection point and consists of two straight sealed channels connected by an open channel.

Continuous oil and water flows are generated from their own inlets and then driven into their own channels by syringe pumps. At the intersection, two-phase WiO droplets are created using the flow-focusing structure.\textsuperscript{30,31} Being dispersed in the oil, the two-phase WiO droplets can be driven by syringe pumps to flow through the first closed channel 1 to the open channel and then are driven to flow through the open channel to enter the second closed channel by the negative pressure that is applied to the end of channel 2.

Without physical walls at the open area, there is no confinement to prevent overflow at the open area. Although the surface tension and the associated pinning effect can act as a confinement to the fluid that is not wetting the walls, the surface tension confinement is unreliable if the flow rates are high. To drive the droplets to flow into the closed channel 2, a negative pressure is applied to the outlet, which causes a pressure difference to suck the droplet into channel 2 reliably. In the open area, the oil sealed droplets are stable and are not easy to evaporate, so each droplet can be regarded as an individual liquid unit.\textsuperscript{31} Compared to the capillary flow, a negative pressure can drive continuous flows with flow rates in a large range by tailoring the negative pressure.

B. Fabrication process

The fabrication processes are shown in Fig. 2. First, an SU-8 master for molding microchannels is fabricated using photolithography. The SU-8 master is fluoro-silanized with perfluorooctyl-trichlorosilane to facilitate demolding. Then, the PDMS prepolymer mixed with a curing agent (10:1) is poured onto the master and cured on a hot plate at 120 °C for 10 min. The PDMS is then peeled off to obtain a thick PDMS layer with channels. Then, a thick, flat PDMS layer is fabricated on a supporting wafer, followed by drilling through-holes in the PDMS layer to form the inlets/outlets and the open area. Immediately after oxygen plasma treatment (70 W, 80 sccm, 30 Pa, 10 s), the two PDMS layers are bonded and baked in an oven at 120 °C for 10 min. Following cutting the inlets and outlets in the sealing PDMS layer, the two PDMS layers are bonded to a glass slide after the bottom PDMS surface is treated with oxygen plasma.

C. Flow generation

Syringe pumps are used to drive the continuous flow of fluorocarbon oil (dynamic viscosity of 1.35 mPa s and density of 1600 kg/m$^3$) and deionized water. The surface tension of oil is 15.58 mN/m, and the interfacial tension between oil and water is estimated to be 8.52 mN/m using the pendant drop method. When the two flows intersect at the focusing point, WiO droplets are generated and flow into the straight channel. The flow speeds and the sizes of the water and oil droplets can be adjusted by changing the flow rates of syringe pumps.

To generate a proper negative pressure to drive the droplets flowing through the open channel, a simple setup based on
FIG. 2. Schematic of fabrication process: (a) SU-8 coating and UV exposure, (b) SU-8 developing and hard bake, (c) PDMS casting and curing, (d) PDMS demolding from the SU-8, (e) drilling holes in the top PDMS layer, (f) bonding two PDMS layers, and (g) bonding the two PDMS layers to a glass.

With the pressure generator, the negative pressure can be simply controlled by the water level difference through lifting or dropping the two bottles. A 10 Pa pressure resolution can be obtained at 1 mm difference in the water levels. It should be noted that the negative pressure varies theoretically during the experiment as a result of the rise in the liquid level in the collector. However, as the flow rate to the collector is in the order of $\mu$L/min, whereas the bottle capacity is 100 ml, the changes in the liquid level during experiments are extremely slow, and thus the resulting pressure changes are neglected.

III. RESULTS AND DISCUSSIONS

A. Chip fabrication

Figure 4 shows the optical photograph of the open microfluidic chip. The middle PDMS layer is 1 mm thick, and the sealing PDMS layer is 5 mm thick. A through window with a diameter of 4 mm is opened in the sealing PDMS layer such that the channel beneath the window is opened. All the channels have a rectangular cross section with a 50 $\mu$m width and an 80 $\mu$m depth. The orifice for flow-focusing is 20 $\mu$m wide.

It should be noted that hydrophobicity is preferred at the open area so that the WiO droplets can be formed easily and transported.
with the outer phase oil. However, oxygen plasma treatment for PDMS bonding changes the PDMS surfaces from hydrophobicity to hydrophilicity temporarily. This temporary change recovers with time, and after 48 h placement at room temperature, the PDMS surfaces recover to hydrophobicity.

**B. Operation principle**

Due to the low flow speed, the flow in the microchannels can be approximated as a steady-state to illustrate the operation principle. As shown in Fig. 5, when there is no negative pressure applied to channel 2, the pressure inside the droplet differs from the environment air pressure because of the Laplace pressure, a pressure difference across the curved surface of a liquid caused by the surface tension of the interface between liquid and gas. In a closed channel, the pressure gradient for driving the flow can be obtained by the Poiseuille equation,

\[ \Delta P_{\text{closed}} = \frac{128\mu Q L}{\pi D^4}, \]  

where \( \mu \) is the dynamic viscosity, \( L \) is the length of the channel, \( Q \) is the flow rate, and \( D \) is the hydraulic diameter of the channel that is given by \( D = 2WH/(W + H) \) for rectangular channels, with \( W \) and \( H \) being the width and the depth, respectively.

This equation indicates that it is a linear relationship between the pressure difference and the flow rate. However, this equation is not applicable to open channels. As the pressure is used to overcome the flow resistance caused by the surface tension from the channel walls, it is reasonable to use the ratio of the wall area between the open channel and the closed channel, \( (2H + W)/(2H + 2W) = 0.8 \), to obtain the rough pressure of an open channel. Using this relationship, the following equation is used to estimate the pressure gradient in an open channel:

\[ \Delta P_{\text{open}} = 0.8\Delta P_{\text{closed}}. \]  

To initiate the flow into channel 2, as shown in Fig. 5, the pressure gradient across the whole length of channel 1 and the open channel is

\[ \Delta P_{12} = P_{in} - P_k = \Delta P_1 + \Delta P_{open} = \frac{128\mu Q}{\pi D^4} (L_1 + 0.8L_{open}). \]  

The air in channel 2 can be treated as an air bubble, across which the pressure difference that is needed to drive the air to flow is given by\(^{32,33}\)

\[ \Delta P = P'_{air} - P_{out} = \frac{C_l}{A} \gamma \frac{y}{H^2}. \]  

where \( A \) is the cross-sectional area of channel 2, \( C_l \) is the capillary number, and the factor \( C_{1D} \) determined by the geometry and the length of channel 2.\(^{31}\)

The relationship between \( P_k \) and \( P'_k \) can be obtained from the Laplace equation,

\[ P_k = P'_k + 2\gamma/r_k, \]

where \( \gamma \) is the surface tension of the liquid, and \( r_k \) is the curvature radius of the droplet surface and is positive for convex surfaces and negative for concave surfaces.

Using Eqs. (4)–(6), the \( P_{out} \) that is needed to initiate a flow into channel 2 can be expressed as

\[ P_{out} = P_{in} - \frac{128\mu Q}{\pi D^4} (L_1 + 0.8L_{open}) - \frac{C_l}{A} \frac{\gamma}{H^2} - \frac{2\gamma}{r_k}. \]  

It indicates that to initiate a flow into channel 2, the pressure of the outlet of the chip should be lower than the \( P_{out} \) determined by Eq. (7).

It should be noted that once the fluid enters channel 2, the length of the air in channel 2 decreases, while the fluid in channel 2 proceeds, so the pressure should change dynamically if a stable flow rate is preferred. However, as it is complex or even impossible to know and control the exact pressure that changes dynamically, the pressure gradient should be larger than the maximum pressure at which the fluid fully fills channel 2, i.e.,

\[ P_{in} - P_{out} = \frac{128\mu Q}{\pi D^4} (L_1 + 0.8L_{open} + L_2). \]  

Once the flow begins to enter the tube that connects the outlet of the chip and the collector, an extra pressure is also required to drive the flow through the tube. Therefore, the maximum pressure gradient is given by

\[ P_{in} - P_{\text{collector}} = \frac{128\mu Q}{\pi D^4} (L_1 + 0.8L_{open} + L_2) + \frac{128\mu Q}{\pi D_{\text{tube}}^4} L_{\text{tube}}. \]  

where \( D_{\text{tube}} \) and \( L_{\text{tube}} \) are, respectively, the hydraulic diameter and length of the tube. In the experiment, the tube hydraulic diameter is 0.3 mm; one has \( \frac{L_{\text{tube}}}{D_{\text{tube}}} \approx 0.01 \frac{L_{\text{tube}}}{D_{\text{tube}}} \), so the last term in Eq. (9) is much smaller than the first term and is neglected.

With Eq. (9), the pressure difference needed to establish a stable flow, i.e., the difference in the pressure between the collector and the air is

\[ \Delta P = P_{air} - P_{\text{collector}} = P_{air} - P_{in} + \frac{128\mu Q}{\pi D^4} (L_1 + 0.8L_{open} + L_2). \]  

Therefore, the pressure difference should be larger than \( \Delta P \) such that the fluid can be driven to flow through the open channel and into the second closed channel.

To verify the function of the chip, numerical simulation is performed using COMSOL, and the results are shown in Fig. 6. The model is a laminar flow governed by the Navier-Stokes equation. The surface condition is a steady free surface with zero pressure.
FIG. 6. Negative-pressure-driven open channel flow (cross section): (a) the fluid overflows from the open channel when $\Delta P = 0$, (b) the fluid partly overflows from the open channel for a low negative pressure, (c) the fluid flows along the open channel for a proper negative pressure, and (d) air is sucked and drawn into the channel with a large negative pressure. $U_{\text{max}}$ is $1.5 \times 10^{-3}$ m/s for (a)–(c) and $2.8 \times 10^{-3}$ m/s for (d). The input average velocity is $1 \times 10^{-3}$ m/s, and the output pressure is $0$ Pa, $-1$ Pa, $-3$ Pa, and $-6$ Pa for (a)–(d), respectively.

Figure 6(a) shows if there is no pressure difference between the open area and the outlet, the fluid will overflow from the open area instead of flowing into channel 2 because of the flow resistance in channel 2. When applied with a negative pressure, as shown in Fig. 6(b), the fluid is constrained or sucked into channel 2.

For a given flow rate, the negative pressure is determined for driving the fluids into channel 2 in a steady mode without overflow or air suction from the open area. If the negative pressure is insufficient, the droplets cannot be completely sucked into channel 2, and overflow occurs at the open area, as shown in Fig. 6(c). On the contrary, if the negative pressure is too large, air will be also sucked into the channel from the open area, as shown in Fig. 6(d).

C. Single phase flow

The behaviors of a single phase flow in the open channels highly depend on the wettability of the fluids. By applying only water into the channels, water cannot flow into channel 2 but overflow at the open area, even when a negative pressure is applied to the outlet, as shown in Fig. 7(a). When applying only oil into the channels, it flows through the open area and flows into channel 2 when a negative pressure is applied to the outlet, as shown in Fig. 7(b).

This distinct difference can be attributed to the opposite features in wettability and surface tension of oil and water. Because the PDMS channel walls are hydrophobic and oleophilic, the oil flow driven by the syringe pumps and the capillary force at the tip of the flow are unlikely to overflow at the open area and likely to flow into channel 2 when a negative pressure is applied. For water, the surface tension of the hydrophobic PDMS causes water to overflow from the open area and form a large water droplet, instead of wetting channel 2. This results in air instead of water being sucked into channel 2 until the water droplet expands largely enough to cover the entrance of channel 2.

D. Formation of water-in-oil droplets

The WiO droplets are generated by the flow focusing structure. With continuous syringe pumping, the continuous outer phase (oil) squeezes the dispersed phase (water) at the channel intersection to form droplets, as shown in Fig. 8(a). The WiO droplets larger than the channel size are of a column shape with two spherical crowns on the two ends, but the surface tension changes the droplets collected in a large area to a perfect circular shape, as shown in Fig. 8(b). This allows the WiO droplets to be sucked into a closed channel with a negative pressure they accumulate even before suction.

The WiO droplets in the channel have a length of 100–250 $\mu$m in the major axis and a width of 50 $\mu$m in the minor axis, depending on the flow rate ratios of the water phase to the oil phase. Figure 9 shows the measured droplet size ratios (length to width, $L/W$) vs the flow rate ratios (water flow rate to oil flow rate, $Q_w/Q_o$). It can be seen that the $L/W$ of the droplets increase linearly with the flow rate ratios, while the width of the droplets is constant (the width of the channel). The slopes of the fitting lines are almost identical, indicating that the length increment changes linearly with the flow rate ratios.

The frequencies of droplet formation at the intersection, indicating the intervals between two droplets, are also proportional to the flow rate ratios, as shown in Fig. 10. The frequencies of droplet formation is in the range of 1–50 Hz for 0.05–2 $\mu$l/min flow rates. As the flow rate is linear with the frequency, the droplet formation can be controlled by adjusting the flow rates. It also shows that the slope
of the fitting lines increases with the oil flow rates. The dependence of the droplet sizes and the frequency on the flow rate ratios are in agreement with literature reports.

E. Water-in-oil droplets in open channels

After generation, the WiO droplets are driven to pass the first closed channel 1 under the syringe pumping pressure and then reach the open channel where the pneumatic pressure is released. At the open channel, the WiO droplets continue to flow along the open channel instead of spilling due to the inertial velocity of the droplets and the surface tension of the channel walls. The wettability of the fluid to the channel surface, i.e., the oleophilic property of PDMS, is critical for the droplets to continue flowing until they reach the starting point of the second closed channel. Once a WiO droplet covers the opening of channel 2, the negative pressure is applied to the droplet and it is sucked into channel 2, as shown in Fig. 11(a).

Figure 11(b) shows the optical photos of the WiO droplets flowing along the closed-open-closed channels. The droplets flow steadily upon the action of a negative pressure. The magnitude of the negative pressure is determined by the flow rates in channel 1 to ensure an identical flow rate in channel 2. Figures 11(c) and 11(d) show the details of the droplets that are passing through the boundary between a closed and an open area defined by the window drilled in the sealing PDMS layer. As the channels in the two areas are identical, the droplets have no distinct change before and after crossing the boundary.

The flow of WiO droplets in more complex U-shaped channels is also tested. Under proper negative pressure driving, the droplets can also flow along the open U-shaped channel, as shown in Fig. 12. It should be pointed out that in long U-shaped open channels, the oil between two water droplets are more likely to spill at the open area because of the relatively larger flow resistance.
F. Negative pressure

Experiments show that the negative pressure applied to the end of the second closed channel to drive the droplets to flow along the open channels without overflow is not a fixed value but a pressure range. By defining the maximum and the minimum values of the pressure range as $\Delta P_{\text{MAX}}$ and $\Delta P_{\text{MIN}}$, respectively, the changes in the maximum and the minimum negative pressures vs the flow rates are obtained, as shown in Fig. 13. It clearly shows that the negative pressure is not a one-to-one correspondence to the flow rate of the droplets, and the maximum and the minimum negative pressures are linear with the flow rates in the range of 0.25 μl/min to 2.5 μl/min.

The fact that a negative pressure range can maintain a steady flow can be attributed to the changes in the surface tension induced by the changes in droplet shapes at the open area. For a steady flow in the open channels, the fluid is in a steady-state and thus the negative pressure applied to the fluid equals the sum of the pressure difference for overcoming the flow resistance to drive the fluid and the pressure difference across the fluid surface at the open area. According to Laplace theory, a pressure difference, so-called the Laplace pressure, exists across the surface of a curved liquid as a result of the surface tension of the liquid. For a given flow rate in the first and the second closed channels, the pressure differences to drive the flow in these two channels are unaltered, and the variation in the negative pressure is balanced by the pressure difference in the liquid at the open channel by changing the shape of the liquid.

By defining the largest negative pressure (corresponding to the lowest $P_{\text{collector}}$) when the air is sucked into channel 2 and the lowest negative pressure (corresponding to the largest $P_{\text{collector}}$) when the fluid overflowed at the open channel, the range of the negative pressure $\Delta P$ can be obtained using Eqs. (2), (6), and (10) through the following relation:

$$\frac{128\mu(0.8L_{\text{open}} + L_z)Q}{\pi D^4} \leq \Delta P \leq \frac{128\mu L_2 Q}{\pi D^4} + \gamma \left( \frac{1}{R_1} + \frac{1}{R_2} \right).$$ \hspace{1cm} (11)

For high $P_{\text{collector}}$ (low $\Delta P$), the fluid at the open channel is squeezed to a convex shape, and the pressure inside the fluid is higher than $P_{\text{air}}$ according to Laplace theory. Further increasing the $P_{\text{collector}}$ increases the pressure inside the fluid at the open channel. Once the surface tension cannot withstand the inside pressure, the fluid overflows. This threshold inside pressure is used to define the highest $P_{\text{collector}}$ (the lowest $\Delta P$). If the $P_{\text{collector}}$ is low, the fluid at the open channel is a concave because of the low pressure in the fluid according to Laplace theory. With the increase in the negative pressure, the fluid shape at the open area becomes more concave such that the pressure difference induced by the shape deformation of the fluid also increases to balance the increase in the negative pressure, as shown in Fig. 14. Once $P_{\text{collector}}$ is lower than a specific value, which is used to define the lowest $P_{\text{collector}}$ (the highest $\Delta P$), air is sucked into channel 2 from its opening.

Figure 14 also shows that the negative pressure required to drive the flow of WiO droplets is larger than that required to drive the pure oil flow with the same flow rate, meaning that the flow resistance of the two-phase flow is greater than that of single-phase oil. In addition, the fitted lines for the maximum and the minimum pressures of the single phase oil flow are parallel, and the interval of about 250 Pa is the maximum pressure that can be offered by the changes in the fluid shape at the open area. For the two-phase WiO flow, while the maximum pressure is also parallel, the minimum pressure has a higher slope and intersects the maximum pressure at 2.5 μl/min.
FIG. 14. Changes in the negative pressure is balanced by the changes in the surface tension caused by the shape changes of the fluid in the open area.

G. Droplets with yeast

By introducing yeast cells in the water phase, droplets enclosing the yeast cells can be generated. The droplets of the yeast cells can also flow through the open channel. Figure 15 shows the yeast enclosed in droplets, and after flowing, the droplets are collected and viewed under a microscope. It can be seen that the yeast cells distribute evenly in each droplet, and there is no cell loss due to adhesion to the channel surfaces and overflow at the open area. Each droplet, as an independent cell culture unit, can be treated by plasma when passing through the open channel, and the duration for plasma treatment can be adjusted by tailoring the rates of the oil and the water.

IV. CONCLUSION

An open microfluidic chip consisting of a droplet generator and closed-open-closed channels has been designed, fabricated, and characterized for a water-in-oil two-phase flow. By applying a negative pressure to the outlet of the channel end, a steady two-phase flow has been successfully obtained without an overflow at the open channel. The influences of the negative pressure and the flow rates on the flow states have been investigated, and it is found that a pressure range instead of an exact value can operate the chip well. The mechanism of the pressure range is interpreted by using the surface tension induced Laplace pressure. Cell transportation tests have been implemented using wrapped yeast cells, and the results show that the chip works well for cell transportation using a water-in-oil two phase flow.

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