

Suspended microfluidics

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Although the field of microfluidics has made significant progress in bringing new tools to address biological questions, the accessibility and adoption of microfluidics within the life sciences are still limited. Open microfluidic systems have the potential to lower the barriers to adoption, but the absence of robust design rules has hindered their use. Here, we present an open microfluidic platform, suspended microfluidics, that uses surface tension to fill and maintain a fluid in microscale structures devoid of a ceiling and floor. We developed a simple and ubiquitous model predicting fluid flow in suspended microfluidic systems and show that it encompasses many known capillary phenomena. Suspended microfluidics was used to create arrays of collagen membranes, micro Dots (μ Dots), in a horizontal plane separating two fluidic chambers, demonstrating a transwell platform able to discern collective or individual cellular invasion. Further, we demonstrated that μ Dots can also be used as a simple multiplexed 3D cellular growth platform. Using the μ Dot array, we probed the combined effects of soluble factors and matrix components, finding that laminin mitigates the growth suppression properties of the matrix metalloproteinase inhibitor GM6001. Based on the same fluidic principles, we created a suspended microfluidic metabolite extraction platform using a multi-layer biphasic system that leverages the accessibility of open microchannels to retrieve steroids and other metabolites readily from cell culture. Suspended microfluidics brings the high degree of fluidic control and unique functionality of closed microfluidics into the highly accessible and robust platform of open microfluidics.

high throughput metabolomics | multiplexed cell culture | spontaneous capillary flow | passive biphasic systems | arrayed migration platform

Open microfluidic systems offer unique advantages, creating platforms that provide increased accessibility, robustness, functionality, and simplicity of fabrication. An inherent feature of these systems is the introduction of an air–liquid interface (1), a component that makes understanding and characterizing fluid flows in these systems complex. However, these phase interfaces can be leveraged to provide unique functionality, integrating the enabling features of phase interfaces demonstrated in closed systems while maintaining the benefits of the open systems. Air–liquid and liquid–liquid phase interfaces in closed systems [reviewed by Atencia and Beebe (2)] have been demonstrated for ultrahigh-throughput screening (3), protein purification (4), organ models (5), cell invasion assays (6), and small-molecule extraction (7). These closed microfluidic approaches often use pinning to create a vertical interface between a series of pillars or ridges (8–11), or require the use of tubes and actuation devices (12–14). Although open systems provide a significantly different approach to interfacing with microscale devices (15–18), the lack of control over the fluid flow prevents the development of platforms with the complexity and functionality necessary for many applications (19). Recently demonstrated open microfluidic platforms have yielded systems with unique functionality through connected microfluidic cell cultures to protein detection platforms (20), neutrophil migration platforms (21), or single-cell actuation devices (15). The need for control within these platforms is paramount, with studies evaluating the robustness of fluid handling

within open devices (22, 23), but these open devices have still been limited to methods that rely on active pumping or patterning methods and are difficult to fabricate and operate. Interestingly, Melin et al. (23) demonstrated a closed microfluidic system that was directly interfaced with an open system to leverage benefits of both to build complexity into accessible and reliable open microfluidic systems.

Here, we develop an encompassing concept for microscale capillary flow, called suspended microfluidics, that enables a high degree of control for channels operating with multiple air or immiscible fluidic interfaces. In these systems, fluid flow occurs in an open microchannel, such that the fluid is “suspended” between two or more air environments or between air and a second immiscible liquid. The phenomenon relies on the generation of spontaneous capillary flow (SCF) in a channel that has neither a ceiling nor a floor (Fig. 1 A–D). In the channel, the fluid front advances (defined as SCF occurring) when the energy reduction associated with the larger liquid–solid surface area outweighs the energy increase associated with the larger liquid–air interface area. Suspended microfluidics is a robust and accessible class of microscale capillary flow that allows the creation of horizontal phase interfaces in open microfluidic systems. The platform technology developed here enables accessible, multiplexed systems for biochemical and cell-based assays and can impart unique functionality to microfluidic assays. Importantly, SCF can be used for many fluids with properties including aqueous solutions, hydrogels, oils, and organic immiscible solvents, and in a wide range of open geometries. This makes suspended microfluidics well suited for a large scope of applications, and to highlight this aspect, we demonstrate here the ability to create (i) a highly multiplexed 3D biochemical assay for tumor cell response, (ii) horizontal microtranswells for cellular invasion assays, and (iii) a microscale culture and solvent extraction platform for metabolomics assays.

Results and Discussion

Modeling SCF. To determine geometrical considerations that allow the robust design of channels enabling SCF, we developed an analytical model in which a fluid flows in a channel containing open air–liquid interfaces without external pressure applied to initiate flow. At each point down the length of the channel, the cross-section can be defined with sections of solid wall, which

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treatments were highly sensitive to ECM composition, a factor that should be strongly considered when testing potential anti-cancer drugs (25) (Fig. 3G). To achieve comparable levels of multiplexing using traditional closed microfluidic systems would require complex valving, fabrication, and control systems for high-throughput screening arrays. The benefits of SCF yield open systems that simultaneously provide control over the fluid flow of traditional closed systems and the experimental simplicity and density of open microwell approaches. This unique combination resulted in a platform that is capable of exploring a wide range of factors found in the cellular microenvironment critical to understanding the complexities of cancer regulation and progression.

A Cellular Invasion Platform. Virtual membranes created by SCF in the μ Dot array also enable a unique membrane-free cell invasion assay inspired by the macroscopic gold-standard transwell platform. We demonstrate this application with an assay designed for studying the collective invasion of prostate cancer (PC3-MM2) cells through a 3D ECM (Matrigel; BD Biosciences). We introduced a range of chemoattractants in the medium below the matrix by adding varying amounts of fetal bovine serum (FBS) and epidermal growth factor (EGF) into the bottom channels (Fig. 3H). Confocal imaging was used to track the extent of cellular invasion after 24 h in culture (Fig. 3I). We observed that cells placed on the opposite side of a high chemoattractant concentration migrated the farthest into the gel, whereas cells seeded on gel above control medium without chemoattractant did not display noticeable invasion (Fig. 3J). Importantly, the horizontal configuration of the μ Dots enabled us to discern the collective invasion of the group of cells, because each cell is placed at the same “starting line” and exposed to the same gradient of chemoattractant. This phenomenon displays interesting readout ability because all the cells can invade together toward a chemoattractant source in a parallel plane to the starting position of the cells, as opposed to being disseminated migrating toward an in-plane chemoattractant source as most in vitro invasion assays necessitate. The μ Dot embodiment of the transwell enables high-density invasion assays using a passive multiplexing

method, allowing a simple approach to explore large-parameter spaces (e.g., screening assays) for a functional cellular end point.

Biphasic Metabolomic Platform. SCF is a ubiquitous method for generating flow in both open systems containing air–liquid interfaces, as described previously, and immiscible liquid interfaces. We demonstrated the ability to flow an immiscible organic solvent in a suspended microfluidic system over an aqueous solution, allowing the creation of open, passive, and static biphasic platforms. This enabling property was used to develop a microsystem for the extraction of small molecules secreted by cells in culture into organic solvents to perform metabolomic studies. We confirmed that the same equation describing the flow of aqueous fluid in an open microchannel could be applied to organic solvent flowing in an open U-shaped channel with apertures in the floor (Fig. 4A). Specifically, we flowed pentanol in a U-shaped channel containing an array of apertures in the floor, creating contact between the pentanol and aqueous media from mammalian cell culture in a channel below (Fig. 4B and C). This fluidic connection allows the diffusion of specific small-molecule metabolites from the aqueous phase to the organic phase. Here, we highlight the utility of this platform in a proof-of-concept experiment in which we studied adrenal cell steroidogenesis and chose pentanol to extract steroids with ranging polarities.

The study of steroidogenesis has become an important area of research in human development and molecular toxicology. It is a critical driver of normal development, and aberrant steroidogenesis can occur in response to in utero toxicant exposure, resulting in birth defects (26). Likewise, steroidogenic pathways are hijacked in late-stage prostate cancer, significantly contributing to mortality (27). To understand and develop treatments for aberrant steroidogenesis better, as well as to identify environmental toxicants that may disrupt normal steroidogenesis, more advanced in vitro techniques are required. The reduced culture volumes inherent to microfluidic cell culture models enable the ability to work with limited primary cell samples, such as cells from biopsies in patients with prostate cancer and in fetal rodent models, and the technique presented herein streamlines analysis by incorporating on-chip isolation of steroids for downstream

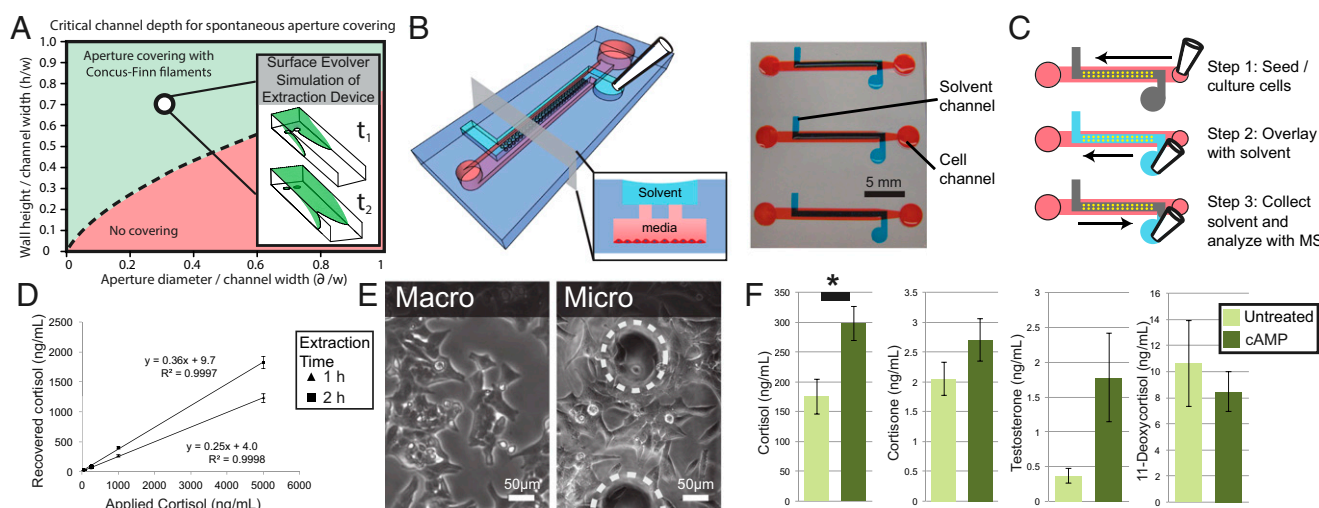


Fig. 4. Suspended microfluidics for solvent extraction of small molecules. (A) Extraction device geometrical parameters are indicated with an open circle on the plot of parameter space. (Inset) Surface Evolver simulation at two stages of the simulation. Schematic of the device illustrates the solvent channel overlaid on a cell culture channel with apertures connecting the two (B) with the device operation shown (C). (D) Standard curve shows robust steroid retrieval over a range of cortisol levels from 50 to 5,000 ng/mL with $24 \pm 1\%$ recovery for a 1-h extraction and $38 \pm 1\%$ recovery for a 2-h extraction ($n = 5$). Error bars represent SE. (E and F) Solvent extraction of steroids from cell culture medium. (E) Adrenal (NCI-H295A) cells within the device (micro) display a similar morphology to those cultured in a six-well plate (macro). Dotted circles outline extraction apertures. (F) Steroids extracted from microfluidic cell culture. Cortisol production increased significantly ($*P < 0.05$) with 8Br-cAMP (0.5 mM) treatment ($n = 3$). Error bars represent SE.

Supporting Information

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SI Materials and Methods

Numerical Simulations. We used Surface Evolver software to perform numerical simulations on the advancement or stalling of the liquid front in suspended microfluidic configurations. In the case of the rail-shaped channels, geometries were defined by creating two parallel rectangular beams. We filled the space between the beams toward one end of the setup with fluid, and the pressure of the outer face was set to zero to simulate an infinite source of fluid. We determined the conditions for which spontaneous capillary flow (SCF) was impossible (i.e., when the liquid front stalled, when an energetic-equilibrium point could be found). When the software was not able to determine an equilibrium point, we considered that SCF was possible (i.e., that the energy profile did not contain a minimum and was constantly decreasing). In the case of U-shaped channels, we added a floor with circular apertures joining the bottom of the two beams in the previously defined geometries. Two unique Surface Evolver software scripts were developed to solve the problems of mesh sizes increasing too fast at the triple line in the corners of the U-groove and numerical errors at the advancing triple line over an aperture (*SI Materials and Methods, Numerical Simulation* below).

Mold and Microdevice Fabrication. We fabricated the microchannels in polydimethylsiloxane (PDMS; Sylgard 184; Dow Corning). We created the silicon-SU8 molds for the microchannels by spinning SU-8 50 and 100 (Microchem) on 75- and 150-mm-diameter wafers (WRS) according to the manufacturer's specifications (1). Lithography masks were printed by ImageSetter, LLC, and we performed the UV exposure with an OMNISCure light source (EXFO). After development, we placed the wafer on a hot plate, mixed PDMS curing agent and base silicone elastomer in a 1:10 ratio, and poured degassed PDMS over the mold. We pressed a cellulose acetate sheet on the uncured PDMS, after which we placed a layer of thin silicone foam, an acrylic rectangle, and a 15-kg weight on top. We cured the PDMS at 85 °C for 3 h and peeled the layer off the wafer. PDMS channels used for solvent extraction experiments were coated with 15 μm of parylene C (PD2010; SCS). Channels were plasma-treated after parylene coating at 50 W for 50 s, with an oxygen flow rate of 5 standard cubic centimeters (sccm) (FEMTO; Diener Electronic).

SCF. PDMS microdevices were designed for two sets of validation experiments. Parallel rail suspended microfluidic channels were designed with constant widths ranging from 500 to 1,000 μm in 100- μm intervals with a height of 700 μm . Channels containing apertures in the floor of the channel were designed with a constant height of 700 μm and width of 800 μm with apertures increasing in size from 200 to 800 μm over the course of the channel. To render the surfaces hydrophilic, the devices were subjected to plasma treatment (FEMTO) at 100 W for 50 s, with an oxygen flow rate of 5 sccm. A red aqueous dye (Allura Red) mixed 1:1 with deionized water was added to the device to characterize the SCF.

Suspended μDot Array. We designed a network of channels leveraging the U-shaped channels containing apertures in the floor, such that the apertures connected with a channel in a layer of PDMS below it. Using a series of U-shaped channels in a top layer of PDMS and a series of channels in a bottom layer of PDMS perpendicular to the top channels, we created an array of these apertures, each accessible from the top by one channel and

from the bottom by another channel. We created the μDots in the apertures by flowing and polymerizing a plug of gel in them. In this study, we created two μDot arrays, one with 3 channels on top and 3 channels on the bottom, representing a 9-hole μDot array, and the other containing 4 channels on the top and 10 channels on the bottom, representing a 40-hole μDot array. The top U-shaped channels were 20 mm long and 1 mm wide. The bottom channels were 20 mm long and 1.5 mm wide. The input port for both devices had a diameter of 1.5 mm, the outputs for both were 3 mm, and all channels were 500 μm tall. Two μDot designs were demonstrated. The first design was a circle with a 500- μm diameter and a depth of 250 μm . The second design was an ellipse with a short axis of 500 μm , a long axis of 750 μm , and a depth of 250 μm . To make all surfaces hydrophilic, the devices were plasma-treated channel-up at 100 W for 50 s, with an oxygen flow rate of 5 sccm, and placed on top of each other so that the through-holes connected the two channels. The 40-hole array was designed with similar hole considerations as the 9-hole arrays, with longer U-shaped channels to span the extra channels, and they were 50 mm long and 1 mm wide.

μDot Array for Parametric Multiplexing. To expand on the combinatorial assay using the μDot array described in the main text, we prepared three solutions of 2 μm FITC surface-labeled polystyrene particles (Spherotech) (0.1% wt/vol, 0.01% wt/vol, and 0.001% wt/vol), and then mixed the solutions in a 1:1 ratio with 8.8 mg/mL Matrigel (BD Biosciences). These Matrigel solutions were added to the U-shaped top channel of the μDot array and were able to flow over and into the aperture by means of SCF. We subsequently removed the gel from the channels by aspirating the excess, leaving suspended gels in each of the through-holes. We incubated the devices for 20 min at 37 °C. Three solutions of Texas Red dye and 10 kD of dextran (Invitrogen) were added to the bottom channels at concentrations of 10 μM , 2 μM , and 0.4 μM , and were incubated at 37 °C for 30 min. The bottom channels were then washed with PBS before imaging on an Olympus IX-70 microscope with a 10 $\times$ fluorescent objective (Fig. S4).

Cell Culture. The prostate cancer (PC3-MM2) cell line was maintained in RPMI 1640 medium supplemented with 10% FBS, 100 U/mL penicillin (Gibco), and 100 $\mu\text{L/mL}$ of streptomycin (Gibco), and was incubated at 37 °C in 5% CO_2 . Human T47D breast carcinoma cells (generously donated by M. Gould, University of Wisconsin) were cultured in flasks with low-glucose DMEM medium (1.0 mg/mL; Gibco), supplemented with 10% (vol/vol) FBS, 100 U/mL penicillin, and 100 $\mu\text{L/mL}$ of streptomycin, and were incubated at 37 °C and 5% CO_2 . NCI-H295A cells (generously donated by the laboratory of G. D. Hammer (University of Michigan, Ann Arbor, MI) were maintained in phenol red-free DMEM/F-12 medium (Sigma-Aldrich) containing 5% (vol/vol) Nu-Serum I (BD Biosciences), 1% ITS+ Premix (BD Biosciences), 100 U/mL penicillin, and 100 $\mu\text{L/mL}$ streptomycin, and were incubated at 37 °C with 5% CO_2 . Culture methods were adapted from Samandari et al. (2). All cells were maintained in standard culture flasks before seeding into microchannels.

Microculture for Solvent Extraction. The microchannel array for solvent extraction was created from two layers of PDMS. The bottom layer, forming a channel used for cell culture, was 300 μm deep, 18 mm long, and 1.5 mm wide. The top channel, a U-shaped channel used for pentanol extraction, was 650 μm deep, 12 mm long, and 0.8 mm wide. The apertures connecting the top

and bottom channels were 150 μm in diameter and 170 μm deep, and they were patterned in an array of 2×33 . NCI-H295A cells were suspended in cell culture medium with or without 0.5 mM 8-bromoadenosine 3',5'-cyclic monophosphate (Sigma-Aldrich) at a concentration of 2,600 cells/ μL , and 13.0 μL of cell suspension was added to the bottom channel of each microfluidic device. To prevent evaporation, the microfluidic device was placed in a Nunc Omnitray (Thermo Fisher Scientific) containing 250 μL of distilled water (50×5 - μL drops), which was then placed in a larger tray with an additional 90 mL of distilled water and kept in a humidified incubator.

Steroid Extraction from Microculture on Chip. After 48 h in microculture, steroids were extracted from cell culture medium by adding 9 μL of 1-pentanol (Sigma-Aldrich) to the top channel of the microfluidic device. The device was placed on a rocker (Vari mix platform rocker; Thermo Scientific) during extraction. After 30 min, the pentanol was removed from the channel and an additional 9 μL of pentanol was added to the top channel for 30 min, at which point the pentanol was removed and the channel was washed with 9 μL of pentanol. Combined pentanol extracts were stored at -20°C before HPLC-MS/MS analysis. This experiment was performed three times with two to four replicates within each experiment.

Extraction of Cortisol Standard Solutions on Chip. Cortisol standard solutions were prepared in media used for the NCI-H295A cell culture with 0.1% methanol at the following concentrations: 50 ng/mL, 250 ng/mL, 1,000 ng/mL, and 5,000 ng/mL. Cortisol solution (13 μL) was added to the bottom channel of the microfluidic device, and the device was placed in a humidified Nunc Omnitray within a humidified incubator as described above for 1 h before extraction. Samples were extracted for a total of 1 h following the protocol outlined above. Samples were extracted for an additional 1 h (2 h total) by adding 9 μL of pentanol to the top channel, waiting 1 h, removing the pentanol, and washing the channel with 9 μL of pentanol. Pentanol extracts were stored at -20°C before HPLC-MS/MS analysis. This experiment was performed using five microfluidic channels for each cortisol concentration.

HPLC-MS/MS Analysis of Pentanol Microfluidic Channel Extracts for Hormones. Microfluidic channel pentanol extracts in polypropylene autosampler vial inserts (National Scientific) were spiked with 5.0- μL aliquots of a mass-labeled internal standard stock solution containing 1,000 ng/mL d_4 -cortisol and d_5 -testosterone (C/D/N Isotopes) using a glass syringe (Hamilton). Calibration standards were prepared by aliquoting 1- μL , 2- μL , 5- μL , and 10- μL aliquots of standard stock solution containing 1,000 ng/mL cortisol, cortisone, testosterone, and 11-deoxycortisol (Sigma-Aldrich) to vial inserts that had been spiked with mass-labeled internal standard as described above. The vial inserts were then placed into open 1.5-mL microcentrifuge tubes (FisherBrand), and the solvents were reduced to dryness using a TurboVap Solvent Concentrator (Biotage) operating at 30°C and 5–10 psi nitrogen gas flow. The vial inserts containing the sample residue were then placed into 2-mL amber glass screw-capped autosampler vials (National Scientific) and reconstituted in 30 μL of 18 M Ω /cm water/methanol (80:20 vol/vol; Honeywell B&JI). The reconstituted extracts were then vortexed vigorously for 30 s and stored refrigerated until analysis.

Hormone analysis was performed by binary gradient reversed phase separation of 10- μL injections of extracts through a Kinetex 2.6- μm , 100-Å 150 $\times$ 4.6-mm C_{18} column at 40°C (Phenomenex) using an integrated HPLC system (Prominence UFLC XR; Shimadzu) coupled to a quadrupole-linear ion trap mass spectrometer (QTRAP 5500 MS/MS; AB/SCIEX) operating with atmospheric pressure chemical ionization in positive ionization

mode. Pertinent mass spectrometer parameter settings are as follows: source temperature = 500°C , nebulizer gas = 40 psi, collisionally activated dissociation gas = medium setting, corona discharge needle current = 3.0 V, and entrance potential = 10. Additional details for the HPLC-MS/MS analysis, including the HPLC binary gradient pump program and multiple reaction monitoring MS/MS parameters, are listed in Tables S1 and S2.

Imaging. Phase contrast images were taken after 48 h in culture using an Olympus IX70 microscope equipped with an ORCA-AG CCD camera (Hamamatsu) with a 10 $\times$ phase objective.

Confocal Microscopy. We prepared a red cell-tracker dye solution (Invitrogen) in PBS, flowed it in the top and bottom channels of the μDot array, and incubated the device for 30 min. After washing with PBS, we fixed, permeabilized, and stained the cells by flowing 4% (vol/vol) paraformaldehyde, 0.1% Triton, and DAPI stain, respectively. We imaged the device on a Digital Eclipse C1 Plus confocal microscope (Nikon) with a slice height of 5 μm and a 5- μs pixel dwell time. We read the image stacks using the ImageJ-based software Fiji (Freeware, NIH) and created final rendered images using the freeware Osirix (Freeware, www.osirix-viewer.com/).

Multiplexing the Effect of ECM Components, Soluble Factors, and Matrix Metalloproteinase Inhibitors on T47D Cell Growth. A stock collagen solution was prepared by mixing collagen type I (10.59 mg/mL, rat tail; BD Biosciences) with 100 mM Hepes buffer in $2\times$ PBS in a 1:1 dilution ratio, and it was incubated inside a bucket with ice for 10 min. Cells were trypsinized and resuspended in serum-free (SF) DMEM medium. FBS and T47D cells were added to the collagen gels, and SF DMEM was used to adjust the final concentrations to 5×10^5 cells/mL, 4% FBS, and 3.0 mg/mL collagen. For experiments that included fibronectin or laminin, either fibronectin (1 mg/mL, human; BD Biosciences) or laminin (1.88 mg/mL, mouse; BD Biosciences) was mixed with the collagen gels, such that the final collagen concentration was 3.0 mg/mL and the final fibronectin or laminin concentration was 100 $\mu\text{g/mL}$. Gels containing T47D cells were loaded on the bottom channels of the μDot array. After the loading was done, arrays were placed inside an incubator at 37°C for 10 min to polymerize the collagen gels.

Human mammary fibroblast (HMF) conditioned medium (CM) was collected from a 2D culture of HMFs that were grown in a flask with high-glucose DMEM medium (4.5 mg/mL; Gibco), supplemented with 10% (vol/vol) calf serum (CS) and 1% penicillin/streptomycin. The collected HMF CM was mixed with 25% (vol/vol) fresh medium (4.5 mg/mL glucose, 10% CS, 1% penicillin/streptomycin). A broad-spectrum matrix metalloproteinase (MMP) inhibitor (5.0 mM GM6001; Millipore) was diluted to 5 μM using SF DMEM, mixed with HMF CM to a get final GM6001 concentration of 500 nM, and then mixed with 25% (vol/vol) fresh medium. As a control, fresh DMEM medium was used to treat T47D cells without the influence of MMP inhibitors or growth factors secreted by HMF cells. All soluble formulations (HMF CM, MMP inhibitor medium, and DMEM) were added through the top channels of the μDot arrays, and changed every other day for 7 d.

Analytical Model of SCF in an Open Microfluidic Geometry. We analyze the case of two simple channel geometries for suspended microflows: the rail-shaped channel (a channel devoid of ceiling and floor) and the U-shaped channel with circular apertures in the floor. However, the analysis performed here can be equally performed on any geometry, and thus will be generalized. It is supposed that the liquid originates from a sufficiently large reservoir, such that the pressure at the inlet port is zero, and we

investigate the conditions for SCF. The model consists of calculating the energy variation with an infinitesimal progression of the liquid in the channel inducing the inflow of a volume dV . The internal pressure P at the liquid front is given by Eq. S1, where dE/dV is the variation of free energy per unit volume, γ is the surface, and dA is the variation in areas, with the indices L , S , and G representing liquid, solid, and gas phases, respectively:

$$P = \frac{dE}{dV} = \left(\gamma_{SG} \frac{dA_{SG}}{dV} + \gamma_{SL} \frac{dA_{SL}}{dV} + \gamma_{LG} \frac{dA_{LG}}{dV} \right). \quad [\text{S1}]$$

Considering that any increase of the solid–liquid surface A_{SL} is made at the expense of the solid–gas surface A_{SG} , we write $dA_{SG} = -dA_{SL}$, and using Young's law with θ being the contact angle of the fluid on the solid surface, we obtain Eq. S2:

$$P = \gamma_{LG} \left(\frac{dA_{LG}}{dV} - \cos(\theta) \frac{dA_{SL}}{dV} \right). \quad [\text{S2}]$$

With the reservoir pressure being zero, and given that the condition for flow is $P < 0$, the condition for SCF can be written in Eq. S3:

$$\frac{dA_{LG}}{dV} < \cos(\theta) \frac{dA_{SL}}{dV}, \quad [\text{S3}]$$

Eq. S3 can be reduced to Eq. S4:

$$\frac{dA_{LG}}{dA_{SL}} < \cos(\theta). \quad [\text{S4}]$$

For simplicity purposes, we consider here all liquid interfaces to be straight lines without curvature (Fig. S1). The expressions for the surface area variations in the two cases considered here are written in Eq. S5, where dx is the length of advancement of the fluid front, p_f is the perimeter of free (unbounded) surfaces, and p_w is the perimeter of wetted surfaces at the cross-sectional plane of the channel at the fluid front:

$$\begin{aligned} dA_{SL} &= p_w dx, \\ dA_{LG} &= p_f dx. \end{aligned} \quad [\text{S5}]$$

On elimination of dx in Eq. S5 and using Eq. S5, a global condition for SCF in a suspended microfluidic channel is written in Eq. S6:

$$\frac{p_f}{p_w} < \cos(\theta). \quad [\text{S6}]$$

SCF Condition in Different Geometries. The condition for SCF derived in Eq. S6 has two main assumptions: (i) The source pressure of the fluid is negligible, and (ii) the interfaces are modeled as straight lines. The cross-sectional shape of the channel modeled is divided into two, possibly discontinuous, sections, with one representing the solid walls and the other representing the unbound air–liquid interface. The model developed does not present any restrictions on the number or shape of these sections, provided they do not make the second assumption invalid. We thus find that the equation describing the condition for SCF flow can be written for suspended and open microfluidic systems but can also predict phenomena, such as the Concus–Finn limit and capillary flow in a tube. Several geometries illustrated in Fig. 2 are detailed here.

We can write more specific SCF conditions for different channel geometries. A simple suspended microfluidic system consists of a channel with a rectangular cross-section of width w

and height h , devoid of a ceiling and floor. For this geometry, Eq. S7 provides a condition on w , h , and θ for the occurrence of SCF:

$$\frac{p_f}{p_w} = \frac{w}{h} < \cos(\theta). \quad [\text{S7}]$$

For a channel with a rectangular cross-section, devoid of a ceiling (i.e., a U-shaped cross-section), of width w and height h , Eq. S8 provides a condition for the occurrence of SCF:

$$\frac{p_f}{p_w} = \frac{w}{2h + w} < \cos(\theta). \quad [\text{S8}]$$

For a channel with a rectangular cross-section, devoid of a ceiling (i.e., a U-shaped cross-section), of width w and height h , containing a circular aperture of diameter δ in the floor, Eq. S9 provides a condition predicting the advancement of the fluid front over the aperture at the largest point of the aperture. One complexity in this case, which will be studied in more detail in the following, is the possibility for the liquid to separate into two Concus–Finn filaments for certain values of θ , and thus not validate the second hypothesis developed earlier:

$$\frac{p_f}{p_w} = \frac{w + \delta}{2h + w - \delta} < \cos(\theta). \quad [\text{S9}]$$

In a wedge forming an angle of 90° between a floor and a wall, fluid flow can occur (e.g., a Concus–Finn filament). In this case, the width and height of the fluid filament are equal and the condition for flow in a wedge can be written in Eq. S10. Interestingly, Eq. S10 yields the same condition for the extension of filaments in a wedge as the Concus–Finn limit:

$$\frac{p_f}{p_w} = \frac{\sqrt{(2)}}{2} < \cos(\theta) \text{ (i.e., } \theta < 45^\circ \text{)}. \quad [\text{S10}]$$

In a V-shaped groove with a bottom angle Φ , Concus–Finn fluid filaments can extend. We note w as the half-width of the groove in the top and h as the depth of the groove. The condition for flow in the groove can be written in Eq. S11. Interestingly, Eq. S11 yields the same condition for the extension of filaments in a wedge as the Concus–Finn limit for any angle:

$$\frac{p_f}{p_w} = \frac{w}{\sqrt{(h^2 + w^2)}} < \cos(\theta) \text{ (i.e., } \theta < \frac{\Phi}{2} \text{)}. \quad [\text{S11}]$$

Although capillary flow in closed capillary tubes is not an open microfluidic system, it is interesting to find that Eq. S6 still holds. We write the trivial condition for flow in a closed tube in Eq. S12 and show that it matches the well-known 90° limit for observing capillary rise in a tube/channel of small dimensions:

$$\frac{p_f}{p_w} = 0 < \cos(\theta) \text{ (i.e., } \theta < 90^\circ \text{)}. \quad [\text{S12}]$$

Numerical Simulation. From a numerical standpoint, two-phase flows are difficult to model: Finite element methods require a bulky 3D meshing of the computational domain; a remeshing that follows the interface motion; and a complicated time-consuming model for the tracking of interface location, such as the level-set model. On the other hand, surface integral methods only require a 2D meshing of the interfaces but cannot deal with viscous and inertial forces. However, these latter models are well adapted for the prediction of equilibrium states of multiphase components occupying a defined domain. From an initial non-equilibrium topological state, an equilibrium shape is searched. In the present study, we use Surface Evolver numerical software,

which is widely used and documented in the literature, and we make use of its potentialities by noting that if an interface is not at equilibrium, it will evolve to minimize its surface energy. In our case, if the computation does not find an equilibrium state, it means that the SCF continues. More specifically, we created Surface Evolver data files describing the geometries studied (i.e., the parallel rail channel and the U-groove with holes) and set up an initial interface at the inlet (the source codes are available at <http://mmb.bme.wisc.edu/links/links.htm?id=links>). When SCF occurs, the interface moves at each iteration of the numerical simulation, mimicking the progression of the flow, although not respecting the kinetics of the flow. However, for systems in which the time scale of surface tension equilibration is far less than other characteristic time scales (e.g., velocity, inertia), the progression of the simulation resembles the actual progression of the flow. When the interface stops, an equilibrium point has been determined.

Two types of Surface Evolver data files were set up for this study. The first type corresponds to the geometry of parallel rails (i.e., a rectangular cross-section devoid of a floor and ceiling; Fig. S24) with three options: constant gap distance, increasing gap distance (i.e., diverging rails), and decreasing gap distance (i.e., converging rails). The second type corresponds to a groove with a U-shaped cross-section (Fig. S24) pierced with holes in the bottom plane. In both cases, the pressure is set to zero for simulating an infinite reservoir at the inlet and the initial “artificial” interface is flat and located at the channel inlet. The surface tension is applied on the free surfaces (i.e., the “facets” in Surface Evolver terminology). The capillary force at the wall is taken into account by Green’s theorem on the edges comprising the liquid–solid–air contact line; thus, the liquid–solid interface facets do not have to be explicitly represented. It can be shown that the free energy to be minimized is

$$E = \gamma_{LG} A_{LG} - \gamma_{LG} \int_{A_{SL}} \cos(\theta) dA, \quad [\text{S13}]$$

where A_{LG} is the free liquid surface area, A_{SL} is the surface in contact with the walls, and θ is the Young contact angle (we use the static contact angle because we are looking for the departure from equilibrium). In Eq. S13, the first term on the right-hand side corresponds to the surface tension force exerted on the free surface, and the second term corresponds to the capillary constraints. The surface tension and contact angle are parameters that can be easily changed. As a matter of fact, in the SCF problem, the value of the surface tension does not affect the threshold for SCF but only conditions the dynamics of the flow; hence, the surface tension that is taken into account is that of water $\gamma_{LG} = 72$ mN/m.

For the parallel rail geometry and a simple U-shaped groove, with a given contact angle ($\theta = 35^\circ$), a comparison between the numerical and theoretical results is shown in Fig. S24. For small dimensions (i.e., <10 μm), the assumption of planar interfaces produces results close to that of Surface Evolver. A discrepancy starts for large dimensions (i.e., more than several hundred micrometers), where the influence of curved interfaces is larger. Nevertheless, the discrepancy remains acceptable to use Eq. S6 as a design guideline for open microfluidic systems.

Surface Evolver Command Scripts. To solve the flow of fluid over an aperture in the floor of a U-shaped channel, we developed two unique commands to adapt to the particular geometry considered. First, because the “numerically” fast motion of the triple line in the corners of the U-groove produced increasingly large meshes, we implemented a regularization command for the size of the meshes by finding the average length of the edges along the triple line and then refining the longer edges and deleting the

shorter edges. Second, because of errors occurring with the advancing triple line at the contact of a hole, we added a command to fix in place the vertices that hit the edge of the hole. These commands are regularly repeated during the iterations of the numerical energy minimization. We performed the rest of the simulation in a similar manner to the rail-shaped simulation. The source codes developed for performing a simulation of a fluid flowing over an aperture are available at <http://mmb.bme.wisc.edu/links/links.htm?id=links>.

For the flow over an aperture in a U-shaped groove, with a contact angle of $\theta = 35^\circ$, an initial comparison between the theory and the simulation is shown in Fig. S2B. A U-shaped groove, 150 μm wide and 250 μm tall, was designed in Surface Evolver, and apertures of increasing diameter were placed on the floor of the channel. The condition for SCF given by Eq. S9 was plotted in Fig. S2B, and Surface Evolver simulations for different ratios of δ/w were performed. Results show that for this geometry, the theory and the simulations are coherent.

Filling an Aperture in a U-Shaped Channel Above Concus–Finn Limit.

We have shown in the preceding section that SCF depends on the geometry and on the contact angle. In this section, we investigate SCF in a U-groove with a hole in the bottom plate. This geometry is slightly more complex, given that two modes of flow can occur: one in which the fluid flows over the aperture and the other in which it flows around it without covering it. Thus, we investigate under which conditions the flow is blocked by the aperture, flows over the aperture, separates into two filaments on each side of the aperture, or flows around the aperture and subsequently fills the aperture. The considered geometry is that shown in Fig. 1C.

For SCF to occur over an aperture in the floor of a U-shaped channel, SCF must occur first in the “plain” U-groove, which is determined by Eq. S8. However, this condition is not sufficient when the triple line reaches the hole. A more restrictive condition is imposed by the presence of the hole. Two situations can occur: The contact angle of the fluid can be lower than the Concus–Finn limit, in which case the fluid front will split into two filaments, or the contact angle of the fluid can be higher than the Concus–Finn limit, in which case the front moves over the hole in a unified way. These two cases are explored here.

Condition for SCF over an aperture for a fluid with a contact angle over the Concus–Finn limit. Let us assume first that the contact angle is above the Concus–Finn limit (i.e., for a 90° corner, $\theta > 45^\circ$). The condition for SCF given by Eq. S9 can be written:

$$\frac{\delta}{w} = \frac{\cos(\theta) \left(2 \frac{h}{w} + 1 \right) - 1}{\cos(\theta) + 1}. \quad [\text{S14}]$$

This shows that the SCF condition depends only on three parameters h/w , δ/w , and θ . We can determine a parameter space identifying all the possible geometries given by h/w and δ/w , and plot the line for a specific θ separating the region that supports filling and the region that does not (Fig. S34).

However, the situation is much more complex when the contact angle is below the Concus–Finn limit. In such a case, two filaments stretch in the two corners. These filaments can eventually merge together downstream from the hole and may fill the hole depending on the ratio h/w and the value of the contact angle.

Condition for SCF over an aperture for a fluid with a contact angle below the Concus–Finn limit. In the case in which the contact angle is below the Concus–Finn limit, the fluid front splits in two filaments around the aperture. At this point, the fluid acts as two separate flows in a wedge and does not validate the straight interface assumption used to develop the equation for SCF (Eq. S6). Downstream of the aperture, the filaments can continue as filaments without merging, and therefore without filling the U-shaped



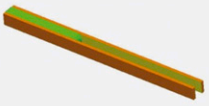
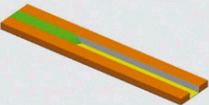
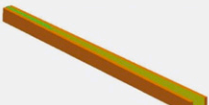
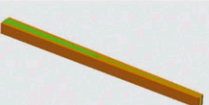
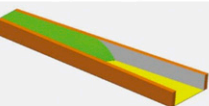
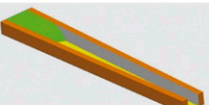
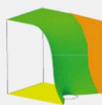
Geometry	Conditions for SCF	View
Parallel rail	$\frac{w}{h} < \cos(\theta)$	
Parallel U-shaped groove	$\frac{w}{2h + w} < \cos(\theta)$	
Diverging rail	$\frac{w_{\text{end}}}{h} < \cos(\theta)$	
Converging rail	$\frac{w_{\text{start}}}{h} < \cos(\theta)$	
Diverging U-shaped groove	$\frac{w_{\text{end}}}{2h + w} < \cos(\theta)$	
Converging U-shaped groove	$\frac{w_{\text{start}}}{2h + w} < \cos(\theta)$	
U-shaped groove with pinholes	$\begin{cases} \frac{w}{2h + w} < \cos(\theta) \\ \frac{h}{\delta} > 2.5 \end{cases}$	

Fig. S4. Different spontaneous flows for different geometry channels, with (1) type of channel, (2) an equation describing the conditions for flow, and (3) a Surface Evolver schematic.

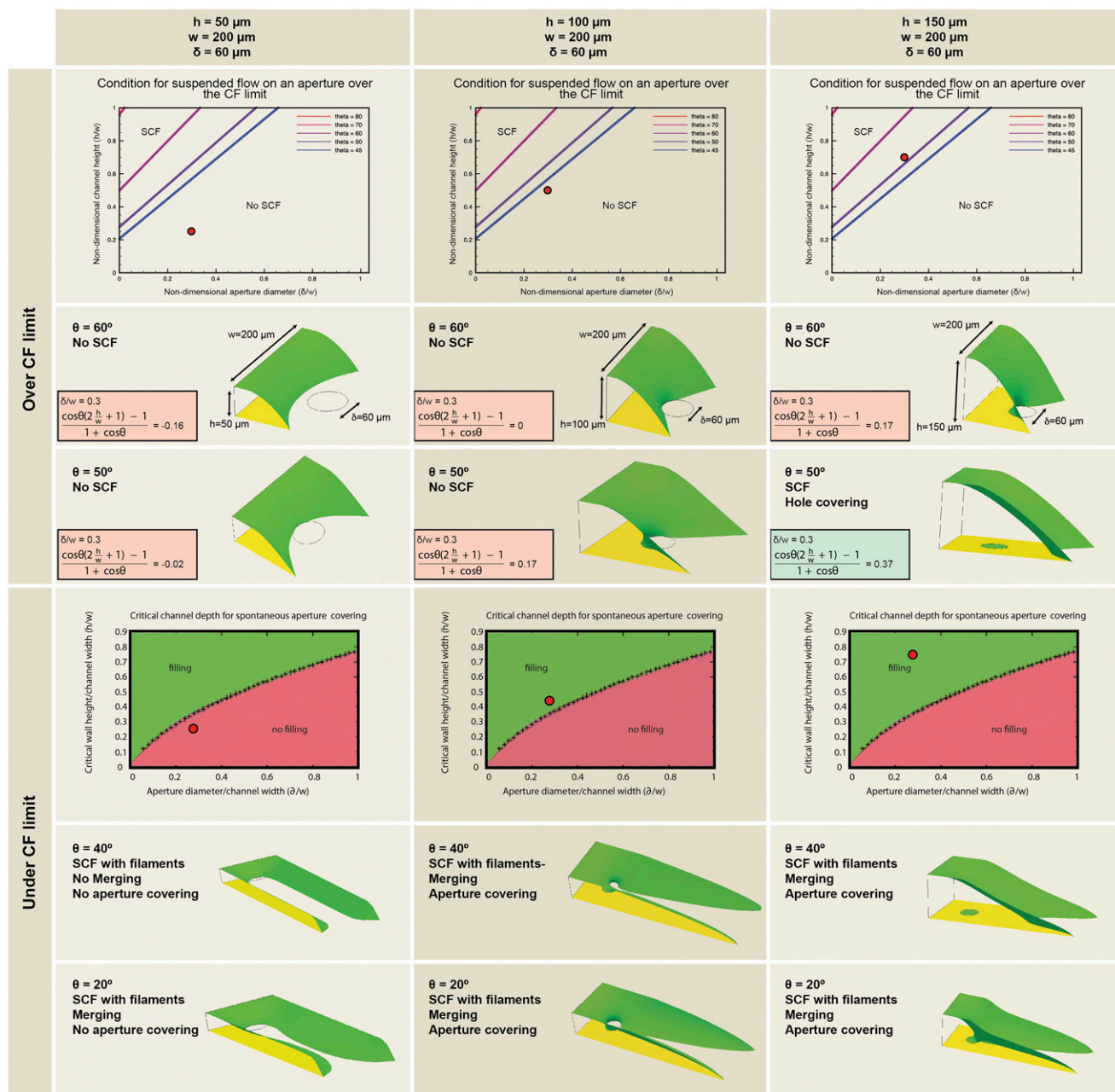


Fig. S5. Summary of aspect ratios for filling an aperture in a U-shaped channel.

