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Cassie-Baxter Surfaces for Reversible, Barrier-Free Integration of Microfluidics and 3D Cell Culture

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Abstract

3D cell culture and microfluidics both represent powerful tools for replicating critical components of the cell microenvironment; however, challenges involved in integration of the two and compatibility with standard tissue culture protocols still represent a steep barrier to widespread adoption. Here we demonstrate the use of engineered surface roughness in the form of microfluidic channels to integrate 3D cell-laden hydrogels and microfluidic fluid delivery. When a liquid hydrogel precursor solution is pipetted onto a surface containing open microfluidic channels, the solid/liquid/air interface becomes pinned at sharp edges such that the hydrogel forms the “fourth wall” of the channels upon solidification. We designed Cassie-Baxter microfluidic surfaces that leverage this phenomenon, making it possible to have barrier-free diffusion between the channels and hydrogel; in addition, sealing is robust enough to prevent leakage between the two components during fluid flow, but the sealing can also be reversed to facilitate recovery of the cell/hydrogel material after culture. This method was used to culture MDA-MB-231 cells in collagen, which remained viable and proliferated while receiving media exclusively through the microfluidic channels over the course of several days.

Graphical Abstract

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Conflicts of interest

There are no conflicts to declare.

Introduction

In the fields of cell biology and medicine, there is a pressing need for physiologically relevant *ex vivo* cell culture models. Traditional 2D cell culture fails to reproduce the biochemistry, mechanics and physiology of the *in vivo* cell microenvironment; as a result, critical cell-cell and cell-ECM interactions are missing, and complex behaviours such as cell migration cannot be reproduced.(1–5) On the other hand, animal models more accurately capture this complexity, but can be expensive, are difficult to observe with high resolution in real time, and present potential ethical issues. An ideal solution would have the observability and ease of use present in 2D cell culture coupled with the biological relevance of animal models, while avoiding the pitfalls of either method.

3D cell culture methods offer a partial solution to this problem; by replacing the stiff 2D tissue culture substrate with a compliant three-dimensional analogue of the extracellular matrix, cells are provided with a more physiologically relevant microenvironment.(6–9) When coupled with an integrated microfluidic network that mimics the dynamic nutrient delivery present in *in vivo* microcirculation, these 3D methods have the potential to become powerful research tools.(10, 11) Integrating microfluidics with 2D or 3D cell culture methods has made it possible to observe complex cell behaviours *ex vivo*, such as angiogenesis, chemotaxis and cell migration.(12–14) But for all the advantages of these methods, widespread adoption has been hindered by usability and repeatability issues. Integration of 3D culture with microfluidics typically involves injecting and curing a cell-hydrogel mixture inside an enclosed microfluidic device.(15–17) This process can be difficult to multiplex with high repeatability, makes the recovery of cells after culture very challenging, and necessitates the development of new, often complex, tissue culture protocols. In addition, injecting cells through a microfluidic channel generates shear stress that can adversely affect health and behaviour of cells(18), and the nature of these devices makes it difficult to retrieve cells after culture, precluding the use of post-culture biochemical or histological analyses.

In this work, we demonstrate a novel method of using topographically-induced entrapment of gas to integrate hydrogel into a microfluidic platform. This is accomplished by creating a microfluidic device that contains an array of open microchannels at the bottom of a macroscale well (Fig. 1 a, b); the topography of these microchannels serves as engineered surface roughness, making it energetically unfavourable for liquid to penetrate the microchannels from above. Therefore, a liquid hydrogel-cell suspension can be pipetted directly into the wells—in a manner identical to that used in standard cell culture—without flooding the microchannels below (Fig. 1c, d). The cured hydrogel serves as the fourth wall in the microfluidic channels and provides a fluid path for flowing media through the microchannels. This enables a dynamic nutrient delivery to the hydrogel site and diffusion-based solute exchange between the channels and cell-laden hydrogel. Combined with a removable lid that seals the top of the well, (Fig. 1e), this creates an effective, integrated platform that can be used for 3D cell culture with dynamic fluid flow to mimic solute delivery in microvasculature and interstitial fluid *in vivo*.

Materials and methods

Device fabrication

The microfluidic devices were made of three polydimethylsiloxane (PDMS) components: the reservoir layer, microfluidic layer, and removable lid (Fig. 1a). Each component was fabricated separately using soft lithography, and then the reservoir and microfluidic layers were plasma bonded together to create the complete device shown in Fig. 1b.

To generate the mold for the microfluidic layer, a 200 μm thick layer of photoresist (SU-8 3050, MicroChem) was spin-coated onto a flat silicon wafer at 1500 rpm for 30 seconds (Fig. 2a, b). After a prebaking sequence (1 min at 65°C, 60 min at 95°C, and 1 min at 65°C), the photoresist was exposed to UV light through a patterned photomask causing crosslinking of the exposed SU-8 (Fig. 2c). The wafer was post-baked (1 min at 65°C followed by 5 min at 95°C) and then agitated in SU-8 developer for 10–15 minutes in order to remove uncrosslinked SU-8 (Fig. 2d). The wafer was then rinsed in isopropyl alcohol (IPA 70% in water, Sigma-Aldrich) and dried with nitrogen gas. Then it was exposed to Trichloro(1H,1H,2H,2H-perfluoro-octyl)silane (Sigma-Aldrich) under vacuum for 40 minutes to produce a hydrophobic surface. Cast sheets of acrylic (McMaster-Carr) were laser-cut to form gaskets, which were clamped to the silicon wafers in order to complete the molds. Additional molds were made for the reservoir layer and removable lid components by laser-cutting features out of acrylic sheets.

PDMS components were cast by vigorously mixing PDMS base and curing agent (Sylgard 184, Dow Chemical) at a 10:1 ratio, followed by degassing under vacuum for 1 hour. The degassed liquid PDMS was poured into the completed molds and cured for 2 hours in a 65°C oven (Fig. 2e). After curing, the molds were removed from the oven and allowed to cool to room temperature; then, the final PDMS components were peeled from the molds (Fig. 2f).

The complete microfluidic devices were assembled by permanently bonding the reservoir and microfluidic layers together using oxygen plasma treatment⁽¹⁹⁾ (Fig. 2g). Note that exposure to oxygen plasma treatment makes PDMS more hydrophilic,^(20, 21) so the top

surfaces of the microfluidic channels were temporarily covered with tape during the plasma treatment steps to maintain hydrophobicity. After this step, the flat (feature-free) side of the microfluidic layer was plasma bonded to a glass cover slip to add mechanical rigidity.

Removable PDMS lids were made using the same PDMS casting process as the other components. The PDMS removable lids were thick blocks of material that contained a narrow rectangular groove on one side that was larger than the footprint of the reservoir. Pressing this side against the flat top surface of the microfluidic device and drawing a vacuum caused temporary sealing of the lid against the microfluidic device. The vacuum was generated by connecting tubing and a syringe to the vacuum gland of the lid, withdrawing the syringe plunger to generate negative pressure and mechanically restraining the syringe plunger in place. This method was sufficient to generate the necessary pressure in a portable manner to keep the lid in place for several days in culture. After experiments were complete, removal of the lid simply required releasing the vacuum by removing the mechanical restraint on the syringe plunger.

Wettability mode observation

Visual identification of surface wettability modes was performed using 1.5 wt% agarose on PDMS with periodic surface features. All PDMS surfaces were fabricated using standard soft lithography of SU-8/silicon molds, as described in the previous section. In some cases, a small amount of blue silicone pigment (Silc-Pig, Smooth-On) was added to the PDMS prior to the degassing step in order to aid in visualization. Low-melting point agarose powder (LMP, analytical grade, Promega) was dissolved in 77°C DI water at 1.5 wt%; 1.0 wt% of rhodamine B solution (Fluka Analytical) was added to the heated agarose solution in order to aid in visualization. 150 µL of this solution was pipetted onto PDMS micropatterned surfaces which had been preheated to 77°C in order to prevent immediate gelation of the agarose upon contact. The surfaces were then allowed to cool to room temperature to cause gelation of the agarose. The effect of temperature change on viscosity is negligible for 1.5 wt % agarose above 40°C (22). After the agarose solidified, a sharp razor blade was used to bisect the agarose and PDMS microchannels to obtain a cross section that could be observed under a microscope.

Contact angle measurement

PEGDA gel was made by diluting a mixture of 25 wt% of PEGDA 3500 powder (Sigma-Aldrich), 35mM of TEA(triethanolamine) (Acros), and 35 mM of VP(1-vinyl-2-pyrrolidinone) (Sigma-Aldrich) in PBS (Sigma-Aldrich). Gelatin Methacryloyl gel was made by mixing 3 wt% GelMA (BioBots), 1 wt% PEGDA powder, 35 mM TEA, and 35 mM VP in PBS. The high concentration collagen solution (70 vol%) was made by mixing F12 basic medium (Sigma-Aldrich), 10x DMEM (Sigma-Aldrich), 1 M NaOH (Sigma-Aldrich) and collagen (Bovine collagen type I, 3.18 mg/mL, Angio-Proteomie) in a 680:43:4:434 ratio. For low concentration collagen (37 vol%), the respective ratio was 340:43:4:434. Agarose gel was made by dissolving LMP agarose powder (Promega) in 77°C DI water at 1.5 wt%. Matrigel (Corning) was prepared on ice. The contact angle measurements were conducted by applying a 10 µL drop of each liquid solution on a flat PDMS surface and using an optical goniometer to read the contact angle.

Fluid flow and diffusion observation

To illustrate fluid flow in the microchannels, a liquid suspension of 1.5 wt% agarose in DI water was pipetted into the open reservoir of the microfluidic device and solidified. After solidification, a suspension of 20 μm diameter fluorescent microspheres (Phosphorex, Inc.) in DI water was introduced into the fluidic inlet at a constant volumetric flowrate of 25 $\mu\text{L}/\text{min}$. Time lapse images were taken every 400 milliseconds using a TI-E Nikon microscope to track the motion of the microspheres in the microfluidic channels. NIS-Elements software was used to calculate the average velocity of the microspheres in the microchannels.

In a separate set of experiments designed to demonstrate dynamic solute exchange between the hydrogel and fluid in the microchannels, a liquid suspension of 1.5 wt% agarose in DI water was pipetted into the open reservoir of the microfluidic device. After solidification of the agarose, water containing 1.0 wt% rhodamine was introduced into the inlet using a syringe pump at a constant volumetric flowrate of 25 $\mu\text{L}/\text{min}$. Time-lapse images were taken every 30 seconds; fluorescent intensity in the resulting images was measured using NIS-Elements software in order to track the rhodamine diffusion from the channels into the agarose hydrogel. The experiments were repeated with a solution of 2,000 kDa FITC-dextran (Sigma-Aldrich) at 0.6 mg/mL in DI water at the same flowrate.

3D cell culture in microfluidic device

The PDMS microfluidic devices were sterilized prior to use with cells using a two-step process. First, each device, including the fluidic pathways and cell reservoir, were rinsed with 70% ethanol (Sigma-Aldrich) and then placed in a 60°C oven for 20 minutes to dry. Then, immediately prior to introducing the cell/hydrogel suspension, each device was exposed to UV light (254 nm wavelength) in a class II biosafety cabinet for 30 minutes.

For all cell experiments, MDA-MB-231 Luc-GFP cells were suspended in a collagen solution or Matrigel at a concentration of 40,000 cells/mL. The collagen solution was made by mixing F12 basic medium (Sigma-Aldrich), 10x DMEM (Sigma-Aldrich), 0.2M NaOH (Sigma-Aldrich) and collagen (Bovine collagen type I, 3.18 mg/mL, Anglo-Proteomie) in a 680:43:43:434 ratio on ice. For cell culture in the microfluidic devices, 120 μL of the hydrogel-cell suspension was carefully pipetted into the cell culture reservoir and then cured in the incubator for 40 minutes at 37°C. The reservoir dimensions are 5mm $\times$ 5mm $\times$ 5 mm, and the device can be operated with the reservoir partially or fully filled with hydrogel-cell suspension. Once the collagen was cured, a removable lid was added to each device and sealed by using a syringe to draw a vacuum through the vacuum port. Media was continuously supplied through the microfluidic inlet ports at a rate of 2 $\mu\text{L}/\text{min}$ using a syringe pump. To obtain the proliferation rate, luciferase was measured using an IVIS imaging system (Xenogen IVIS 50) on days 0–4, and the relative population was calculated based on the number of cells on day 0.(6) Colony sizes were measured using dark field imaging (TI-E Nikon microscope).

Results and Discussion

The microfluidic device described here was designed to provide a simple, robust way to integrate 3D cell culture and microfluidic fluid flow without the need for a physical boundary separating the two. This allows exchange of solutes with the cells through both fluid flow and local diffusion—similar to solute exchange that occurs *in vivo* due to circulation and interstitial transport.

A number of methods to integrate 3D cell culture and microfluidics have been demonstrated previously, including the use of permeable membranes as boundaries.(23) The use of permeable membrane to separate cell culture and fluid flow is similar to the use of membranes in transwell plates; in this method, a thin (~10 μm) membrane with periodic perforations, typically 1–5 μm in diameter, is used to isolate cell culture on one side of the boundary until the cells have adhered and/or hydrogel has gelled. At this point, fluid can be introduced to the opposing side of the membrane, and solute exchange occurs through the membrane perforations. While much insight has been gained using this method, the membrane is both thicker—by an order of magnitude—and stiffer—by several orders of magnitude—than a typical capillary wall *in vivo*.(23, 24) Therefore, the presence of the membrane will alter both the diffusive exchange of solutes between the fluid and cells, and may alter physiological properties of cells cultured in contact with it.(25, 26)

Boundary-free integration of 3D cell culture and microfluidics has also been demonstrated previously, notably by *in situ* patterned gelation of hydrogel inside microchannels(15, 27, 28) or by molding the hydrogel itself to include microfluidic flow pathways.(29–31) The former method uses either defined flow pathways or UV radiation to trigger gelation only within specific regions inside a confined microchannel. This method offers excellent patterning resolution and has been particularly useful in elucidating mechanisms in cell migration.(11, 32–34) However, the complexity of this method, requirements for equipment often not available in most cell culture labs, and difficulty in retrieving cells after culture, make it challenging to implement in many applications. Molding microfluidic channels directly into hydrogels bypasses the integration issue entirely and can even be used to generate three-dimensional vascularized constructs when used in concert with a sacrificial material, but this method requires specialized equipment, and the low elastic modulus of the hydrogel material limits the size and resolution of the microchannels.(31, 35–37)

We have developed a microfluidic device that provides boundary-free integration of microfluidics and 3D cell culture by leveraging the entrapped-gas phenomenon that occurs in high-roughness surfaces. In this device, open microchannels serve as an engineered surface “roughness”, so cell-hydrogel suspensions can be directly pipetted onto the surface and the entrapped-gas phenomenon prevents the hydrogel from penetrating the microchannels. After gelation, the hydrogel surface forms the “fourth wall” of the channels in the microfluidic network, and fluid can be freely pumped into the channels.

Entrapped-gas effect in Cassie-Baxter surfaces

When a liquid droplet comes to contact with a flat, homogeneous, solid surface, there are three different interfacial energy values that determine the equilibrium shape of the droplet.

The surface tension between the solid and gas phases (γ_{SG}), the solid and liquid phases (γ_{SL}), and the liquid and gas phases (γ_{LG}) determine the angle of contact between the solid and liquid phases (θ_e), as shown in Fig. 3a. For smooth surfaces the wettability in terms of θ_e is governed by Young's equation(38):

$$\gamma_{SG} - \gamma_{SL} - \gamma_{LG} \cos(\theta_e) = 0 \quad (1)$$

This contact angle is defined for a set of solid, liquid and gas materials; and if it is greater than 90° for water, the surface is referred to as hydrophobic.

If the solid material has an inherent surface roughness, a liquid droplet introduced onto this surface will either completely wet the surface, displacing all gas and forming a continuous solid-liquid boundary (Wenzel mode), or it will only partially wet the surface, leaving pockets of gas trapped beneath the liquid and forming a complex series of solid-liquid, solid-gas, and gas-liquid boundaries (Cassie-Baxter mode), as shown in Fig. 3b. The favourability of either Cassie-Baxter or Wenzel mode for a given surface is governed by a combination of the microscale surface topography of the solid and the interfacial energy between the phases present.(39)

For materials with a high contact angle, it is energetically favourable to minimize the interfacial area between the liquid and solid phases. Surfaces with high roughness have a larger effective surface area than an equivalent flat surface. To minimize the solid-liquid interfacial area, the liquid often becomes pinned to the highest elevation features on the surface, trapping air pockets beneath the liquid in classic Cassie-Baxter (CB) mode. Cassie-Baxter mode surfaces exhibit much higher observed contact angles compared to Wenzel mode surfaces made of the same material. This effect can be observed in nature in the superhydrophobic lotus and water lily leaves(40); it has also been leveraged by numerous researchers to create super-hydrophobic self-cleaning surfaces.(41, 42) However, in this work it is the trapped gas phenomenon in CB surfaces that is of primary interest: by exploiting the stable entrapment of gas in open-top PDMS microfluidic channels to create boundary-free integration of hydrogels with microchannels.

In order to experimentally verify the formation of CB and Wenzel boundaries in PDMS surfaces, a series of SU-8/silicon surfaces with controlled, periodic topography were generated using photolithography and then replicated using soft-lithographic patterning of PDMS. Agarose was dissolved in water at 77°C at 1.5 wt%, pipetted onto the PDMS surfaces and then cooled to room temperature, causing gelation of the hydrogel. Cross-sections of PDMS and agarose surfaces prepared in this manner are shown in Fig. 3c. In high roughness surfaces, there is clear evidence of gas entrapment, indicative of the CB mode, while complete surface wetting occurs in the lower roughness surfaces.

The theoretical transition between CB and Wenzel modes occurs at the following boundary(43):

$$\cos \theta_e = \frac{-f_2}{r - f_1} \quad (2)$$

where f_1 is the percentage of the surface area under the liquid that is in contact with a solid surface and f_2 is the percentage of the surface area under the liquid that is in contact with gas. The roughness parameter, r is defined as the ratio of the total surface area of the solid to the projected surface area of the solid. For surfaces of the type shown in Fig. 3, where the liquid interface for CB mode is entirely in one plane, these parameters can be defined as:

$$f_1 = \frac{a}{a+b} \quad f_2 = \frac{b}{a+b} \quad r = \frac{a+b+2h}{a+b} \quad (3)$$

In equation (3), (h) is the feature height, (b) is the width of the cavities, and (a) is the width of solid wall separating the cavities. This makes it possible to express the transition between CB and Wenzel modes as a linear relationship between the minimum feature height that will sustain CB mode (h_{crit}) and the width of the cavities (b) for a given material:

$$h_{crit} = -\frac{b}{2} \left(\frac{\cos\theta_e + 1}{\cos\theta_e} \right) \quad (4)$$

The contact angle for water on PDMS is approximately $\theta_e = 104^\circ$.⁽⁴⁴⁾ Using the above relationship, this means that the feature height (h) needs to be at least ~1.6 times the gap dimension in order to induce CB mode on a PDMS surface.

This theoretical boundary is shown graphically in Fig. 4, along with a number of experimental data points. Each data point represents a PDMS surface that was tested using 1.5% agarose as in the manner shown in Fig. 3b; circles represent experiments where Cassie-Baxter mode was observed and an x represents experiments where Wenzel mode was observed. For all experimental cases in the region where theory predicts CB mode to occur ($h > h_{crit}$), CB mode was observed without exception. In the predicted Wenzel region, the vast majority of the experimental points agreed with theory, with only two outliers. Therefore, equation (4) appears to be a good model to utilize when trying to design for CB mode in a device – such as the work described here – but may incorporate some error when used to design for Wenzel mode.

The experimental outliers in Fig. 4 may be partially explained by the assumptions used in derivation of equation (4). An interesting observation about equation (4) is that wetting mode is dependent only on the contact angle, h , and b , while the a dimension does not appear. As shown in Fig. 4, in an area close to the transition line, there was one repeatable case in which the same pair of h and b , but different value of a , caused different wettability modes occur. For the same h and b , the higher a value corresponds to Cassie-Baxter mode, where the lower a value results in a Wenzel mode. Therefore, it can be a matter of interest to investigate the dependence of mode transition on a that has not been captured in the current model. In addition, other implicit assumptions in the derivation of equation (4), such as neglecting gravitational and pressure effects, could help explain the two outliers in Fig. 4.

The contact angle used in these calculations was for water on flat PDMS. Five hydrogel precursor solutions were tested on PDMS; of those five, three displayed contact angles similar to that of water, while two were slightly lower (Table 1). But all measured contact

angles were $>90^\circ$, indicating that each of these materials should be capable of forming CB interfaces with micropatterned PDMS surfaces. Lower values of θ_c lead to an increase in the slope of the transition line shown in Fig. 4, which means that it requires taller features to generate stable CB mode in periodic surfaces.

Microfluidic-hydrogel integration using CB surfaces

The knowledge gained from the previous analysis was used to design a series of open microchannels that result in Cassie-Baxter mode when hydrogel is pipetted onto the top surface. The combination of geometry and contact angle pins the interface, preventing the microchannels from flooding. When the hydrogel is solidified during incubation, it completes the circumferential area of the microchannel: the bottom, left and right side walls are made of PDMS, while the top wall is comprised of the hydrogel material. This creates an open fluid path for flow through the microchannels, combined with free exchange of solutes to and from the hydrogel via diffusion.

In order to demonstrate stable CB mode formation and unobstructed fluid flow in the microchannels of the microfluidic device, liquid agarose solution was pipetted into the reservoir and solidified; then, a dilute solution of 20 μm diameter fluorescent microspheres in water was introduced into the fluidic inlet at a flowrate of 25 $\mu\text{L}/\text{min}$. Time lapse images of the microchannels, shown in Fig. 5, demonstrate that while a few microspheres became attached to the surface of the agarose (yellow arrows), the majority of microspheres flowed freely through the channels (red arrows) from left to right at an average velocity of 64 ± 14 $\mu\text{m}/\text{s}$. Moving microspheres were observed in all channels in the device. Based on the known geometry of the microchannels below the agarose in the device (25 parallel channels, each $200 \mu\text{m} \times 100 \mu\text{m}$), the theoretical average velocity in the channels should be approximately 62 $\mu\text{m}/\text{s}$. The close agreement between the theoretical value and observed velocity of the microspheres implies that all of the microchannels were open to unrestricted fluid flow after the agarose solidified.

Another vital attribute of successful hydrogel/microfluidic integration is diffusion-based exchange of solutes between the hydrogel and the fluid in the microchannels. Figure 6a shows the numerical solution of a comparable scenario developed using COMSOL software: transient diffusion of a chemical species from a constant concentration source into a region with an initial species concentration of zero, representing diffusion from the microchannels into the hydrogel material. For this simulation, the diffusion coefficient of the species was modelled as $D = 3.5 \times 10^{-6} \text{ cm}^2/\text{sec}$, in order to simulate rhodamine in a 1.5% agarose hydrogel medium. The free diffusion coefficient of rhodamine in water is approximately $4 \times 10^{-6} \text{ cm}^2/\text{s}$; (45) by using this value in the Einstein-Stokes equation it is possible to estimate the hydrodynamic radius for rhodamine in water at room temperature (0.6 nm). A molecule of this size would diffuse through 2.0% agarose at approximately 80% of its free diffusion rate (46) ($3.2 \times 10^{-6} \text{ cm}^2/\text{s}$), while the diffusion rate through a less dense agarose gel, such as the 1.5% used in these experiments, would be similar or slightly higher.

The data from each timepoint in the simulation was integrated along the z-direction, normalized with respect to initial concentration in the hydrogel region and microchannel region. For example, summing species concentration along the z-direction at $y = 0$ correlates

to total amount of the species present in a column above the midpoint of one of the microfluidic channels. This data was used to generate the concentration versus time plot shown in Fig. 6a, where “above channel” corresponds to $y = 0$ while “above PDMS” corresponds to $y = 100 \mu\text{m}$.

The same scenario was recreated experimentally in our device: 1.5 wt% agarose in DI water was pipetted into the open reservoir of the microfluidic device and solidified. Water containing 1.0 wt% rhodamine was introduced into the microfluidic channels at a flowrate of $25 \mu\text{L}/\text{min}$. Time-lapse fluorescent images taken from beneath the microfluidic channels (Fig. 6b) show that a high fluorescent signal is initially localized within the microfluidic channels, but that an overall increase in fluorescent intensity and broadening of high-intensity regions occurs with time, signifying vertical and lateral diffusion of the rhodamine from the microchannels into the neighbouring hydrogel. Fluorescent intensity at several points—either centred directly beneath a PDMS wall or centred beneath a microfluidic channel—was tracked over time. This data, normalized with respect to the maximum and minimum fluorescent intensity at 1 second, is represented in Fig. 6b.

The concentration/time plot in Fig. 6a and the intensity/time plot in Fig. 6b and show very similar trends; additionally, incubating a PDMS surface in contact with a 1.5 wt% solution of rhodamine over 180 seconds showed no detectable diffusion of rhodamine into the PDMS. This information supports the conclusion that the fluorescent intensity increase seen in Fig. 6b is the result of unobstructed diffusion of rhodamine species from the microchannels into the adjacent hydrogel. The same experiment was repeated with a larger fluorescently-tagged species: a solution of FITC-dextran (2,000 kDa) in water was pumped through the microchannels of the device at $25 \mu\text{L}/\text{min}$. The much larger molecule flowed freely through the channels but did not penetrate the hydrogel (Fig. 6c), so there was no change in fluorescent intensity in the system over time. Because there was no detectable change in the fluorescent intensity of the hydrogel above the PDMS channels, this demonstrates sufficient sealing between the PDMS and agarose hydrogel to prevent seepage of fluid outside the defined microchannel geometry.

Because the microchannels are the only source of soluble factors in this device, the rate at which soluble factors diffuse to a specific location within the hydrogel will be dependent on the distance to the microchannels. The solution to Fick’s second law for diffusion in one dimension can be used as a rough estimate of diffusion time ($t \approx L_D^2/(4D)$) as a function of distance from the microchannels (L_D) and coefficient of diffusion (D). For example, oxygen ($D = 4.5 \times 10^{-10} \text{ m}^2/\text{sec}$ in collagen (47)) will take approximately 9 minutes to diffuse 1 mm, 37 minutes to diffuse a distance of 2 mm, and 3.8 hours for 5mm. But for a larger molecule like glucose ($D = 1.4 \times 10^{-10} \text{ m}^2/\text{sec}$ in collagen (48)), those numbers increase to 29 minutes, 2 hours, and 12.4 hours respectively. It should be noted that these diffusion coefficients are dependent on hydrogel type and crosslinking density (49, 50), so values will vary with different hydrogel formulations. But for all applications, diffusion time of important chemical species to the distal surface of the hydrogel should be taken into consideration when filling the well portion of the device, which can be used in a partially

filled (1–2 mm hydrogel thickness) or completely filled (5 mm hydrogel thickness) configuration.

Three-dimensional cell culture in CB microfluidic device

In order to demonstrate the utility of the CB microfluidic device in cell culture applications, MDA-MB-231 Luc-GFP cells were cultured in a collagen matrix within the device over a period of four days. First, a liquid collagen and cell mixture was prepared and pipetted into the reservoir of microfluidic devices and cured to solidify. The reservoirs of the microfluidic devices were then sealed using the removable lids and cell culture media was supplied through the microfluidic channels at a constant flowrate of 2 $\mu\text{L}/\text{min}$ for the duration of the experiments, to minimize media consumption. Microscopy images of cell colonies were captured after gelation of the hydrogel (day 0) and after 2, 4, and 6 days of continuous media flow. The diameter of 60 colonies in each device was measured using ImageJ; this data was used to calculate the approximate volume of cell colonies, shown in Fig. 7a. This demonstrates that cells cultured in the CB microfluidic device show an increase in colony size over the time period. Cell proliferation was also measured using IVIS imaging (Fig. 7b), and the results show a 10x increase in cell population over the time period, indicating a viable and proliferative cell microenvironment within the device.

The channels of the CB microfluidic device were created from optically transparent PDMS, which made it possible to observe cells inside the device during culture (Fig. 7a). In addition, using CB surfaces to integrate 3D cell culture with microfluidic channels also made it possible to easily retrieve cells after culture. This is in contrast to many microfluidic devices, which typically require cells to be introduced into the device via fluid ports; once cells are cultured in these systems it can be challenging to retrieve the cells without damage. To retrieve cells in the CB microfluidic device, the vacuum holding the removable lid to the device was released so that the lid could be removed. The device was inverted and a small amount of back pressure was applied simultaneously through the inlet and the outlet using air-filled syringes; this generated enough force to gently remove the cell/hydrogel plug from the reservoir, which made the cells available for further analysis.

The microchannels in this work were made from PDMS because this material is hydrophobic, biocompatible, optically transparent, and straightforward to fabricate with submicron fidelity. Because the device described here relies on gas entrapment associated with Cassie-Baxter surfaces, the microchannels could be fabricated from a number of alternate materials, as long as they can be used to generate a CB condition. This includes several polymers more commonly seen in tissue culture, such as polystyrene.^(51, 52) The geometry required for CB surface generation is also well within capabilities of high-throughput manufacturing processes such as hot embossing^(53, 54) and injection molding.⁽⁵⁵⁾ These features make this an attractive concept for future integration into commercially-available multi-well tissue culture plates.

Conclusions

We have shown that carefully designed micropatterned surfaces can be used to induce Cassie-Baxter mode in a hydrogel solution, and this method can be used to integrate 3D cell

culture with microfluidic fluid delivery. The use of CB mode eliminates the need for a barrier between the hydrogel and fluid path, creating a more direct pathway for diffusive transfer between the hydrogel and bulk fluid than is present in commercially available transwell plate systems or membrane-associated microfluidic devices. This reversible integration makes it easy to recover the cells after culture, enabling additional post-culture analyses. Moreover, because the integration step only requires simple pipetting of the 3D matrix onto the open channels without the need for complex new protocols, it provides an attractive option for adoption into many tissue culture labs.

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Fig. 1. Microfluidic tissue culture platform. (a) Devices are constructed from three polymer layers; the microfluidic and reservoir layer are plasma bonded together to form the base of the system, while the removable lid is used during cell culture to prevent media evaporation. (b) Completed microfluidic device, filled with food colouring to aid in visualization. Scale bar = 10 mm. (c-e) 3D tissue culture is integrated into the device by (c) pipetting a liquid/hydrogel cell suspension into the $5 \times 5 \times 5$ mm reservoir, where the Cassie-Baxter nature of the open microfluidic channels prevents the suspension from penetrating the channels. (d) Incubating the hydrogel (red) causes it to solidify, after which (e) the device can be sealed and tissue culture media (blue) flowed through the microfluidic channels. Inset images in (c-e) show cross section of channel and hydrogel geometry taken at each stage of assembly.

Fig. 2.

Fabrication of microfluidic tissue culture platform components using soft lithography. (a) Silicon wafer was (b) spin-coated with a layer of SU-8 photoresist, which was (c) selectively crosslinked using UV light through a photomask. (d) Removal of the uncrosslinked SU-8 left a positive set of features on the silicon. (e) Liquid PDMS was poured onto this positive mold and cured in an oven until (f) the final PDMS material could be removed from the mold. The reservoir layer and removable lid components were fabricated in a similar manner and (g) the microfluidic and reservoir layers were plasma-bonded together.

Fig. 3. Surface topography and surface energy determines Wenzel or Cassie-Baxter wetting modes. (a) Contact angles for hydrophilic (wetting) and hydrophobic (nonwetting) surfaces. (b) Schematic of Wenzel and Cassie-Baxter modes; in the former, the liquid wets the entire surface, while in the latter, gas becomes entrapped beneath the liquid layer, forming a complex three-phase system. (c) Micrographs showing Wenzel and Cassie-Baxter wetting modes in an agarose / PDMS system. A liquid suspension of agarose (dyed red) was introduced to the PDMS surface (dyed blue), and then cured and sectioned for visualization. Scale bar = 50 μm .

Fig. 4. Wettability modes for periodic microfabricated surface topographies. Solid line depicts theoretical boundary between Wenzel (white) and Cassie-Baxter (grey) modes for contact angle corresponding to water on PDMS ($\theta_e \approx 104^\circ$). Discrete points show experimental data for agarose hydrogel on PDMS.

Fig. 5. Fluid flow through microfluidic channels, moving from left to right. Time-lapse micrographs showing $20 \mu\text{m}$ diameter microspheres flowing through microchannels. Some microspheres became attached to the hydrogel surface (yellow arrows), but all channels contained freely-flowing microspheres (red arrows), demonstrating an open fluid path below the hydrogel. Dashed lines represent boundaries of PDMS microfluidic channels.

Fig. 6.

Solute transport for different molecular weight molecules. (a) Numerical simulation of diffusion of small molecules from microfluidic channels (μC) into hydrogel over time; dimensions given in μm . Dashed line represents boundary between μC and hydrogel. (b, c) Time-lapse micrographs showing fluorescent solutions being driven through channels at flowrate of $25 \mu\text{L}/\text{min}$, scale bar = $100 \mu\text{m}$; (b) Rhodamine, 0.48 kDa , diffuses readily into the neighbouring hydrogel, while very large molecules such as (c) FITC-Dextran, 2000 kDa , remain contained within the microfluidic channels.

Fig. 7.

Cell viability and sample removal in tissue culture platform. (a) Average colony radius for 3D cell culture of MDA-231 Luc-GFP cells in Matrigel in the microfluidic tissue culture platform; images of cell colonies in microfluidic device after 0 and 4 days, scale bar = 20 μ m, N = 3. (b) Cell proliferation data for MDA-231 Luc-GFP cells as measured by IVIS imaging; inset shows IVIS imaging of one cell/collagen suspension after being removed from the microfluidic device, scale bar = 5 mm, N = 3.

Table 1

Contact angle of water and liquid hydrogel precursor solutions on flat PDMS.

Liquid on PDMS	Contact angle	Temperature	
Water	$102.4^{\circ} \pm 3.3^{\circ}$	24°C	
Collagen (high)	$101.4^{\circ} \pm 3.8^{\circ}$	2–4°C	
Collagen (low)	$99.0^{\circ} \pm 4.6^{\circ}$	2–4°C	
PEGDA 3500	$93.4^{\circ} \pm 5.5^{\circ}$	**	24°C
GelMA	$97.4^{\circ} \pm 8.1^{\circ}$	2–4°C	
Agarose (1.5%)	$104.2^{\circ} \pm 5.0^{\circ}$	77°C	
Matrigel	$91.4^{\circ} \pm 1.5^{\circ}$	**	2–4°C

**

Statistically different from water value ($p < 0.05$)