Published in final edited form as:

Lab Chip. 2013 October 7; 13(19): 3876–3885. doi:10.1039/c3lc50489a.

A contact line pinning based microfluidic platform for modelling physiological flows

Chih-kuan Tung^a, Oleh Krupa^b, Elif Apyadin^a, Jiun Liou Jr^b, Anthony Diaz-Santana^c, Beum Jun Kim^a, and Mingming Wu^a

Chih-kuan Tung: ct348@cornell.edu; Mingming Wu: mw272@cornell.edu

^aDepartment of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA.;
Tel: 1-607-255-9410

^bDepartment of Biomedical Engineering, Cornell University, Ithaca, NY, USA

^cSchool of Chemical and Biomolecular Engineering, Cornell University, Ithaca, NY, USA

Abstract

This work introduces a contact line pinning based microfluidic platform for the generation of interstitial and intramural flows within a three dimensional (3D) microenvironment for cellular behaviour studies. A contact line pinning method was used to confine natively derived biomatrix, collagen, in microfluidic channels without walls. By patterning collagen in designated wall-less channels, we demonstrated and validated the intramural flows through a microfluidic channel bounded by a monolayer of endothelial cells (mimic of a vascular vessel), as well as slow interstitial flows within a cell laden collagen matrix using the same microfluidic platform. The contact line pinning method ensured the generation of an engineered endothelial tube with straight walls, and spatially uniform interstitial fluid flows through the cell embedded 3D collagen matrix. Using this device, we demonstrated that the breast tumour cells' (MDA-MB-231 cell line) morphology and motility were modulated by the interstitial flows, and the motility of a sub-population of the cells was enhanced by the presence of the flow. The presented microfluidic platform provides a basic framework for studies of cellular behaviour including cell transmigration, growth, and adhesion under well controlled interstitial and intramural flows, and within a physiologically realistic 3D co-culture setting.

Introduction

Fluid flows in living systems can be broadly classified as intramural flows (flows within blood and lymphatic vessels) ¹ and interstitial flows (flows within tissues) ^{2, 3}. Fluid flows mediate the transport of macromolecules, and thus critically regulate a number of important homeostatic and pathologic processes in living systems, including morphogenesis during development ^{4, 5}, vascular tissue formation ^{6, 7}, immune cell trafficking ⁸ and tumour cell chemoinvasion ^{9–11}. Intramural flows cover a wide range of flow speed, from a few micrometres per second in lymphatic capillaries ¹ to tens of centimetres per second in

Correspondence to: Chih-kuan Tung, ct348@cornell.edu; Mingming Wu, mw272@cornell.edu.

[†]Electronic Supplementary Information (ESI) available. See DOI: 10.1039/b000000x/

arteries¹². Shear stresses induced by the intramural flows at the vessel wall have been found to directly regulate the physiology of the endothelial vessels^{13–16}, that the endothelial cells typically form tighter junctions in the presence of the flow¹⁷. Interstitial flows, driven by the hydrostatic and osmotic pressure differences between the arterial and venous, or arterial and lymphatic capillaries¹⁸, are typically in the order of a few micrometres per second² or Peclet number of about 1 at cell length scale. Recently, a number of works revealed that interstitial flows modulate the spatial distributions of the chemical secretions surrounding tumour cells via convective and diffusive transport, and as such guide breast tumour cell chemoinvasion^{9, 19}. In addition, interstitial flows influence tumour cells indirectly through matrix rearrangement by fibroblasts¹⁰.

Microfluidic models have emerged for modelling interstitial and intramural flows in the context of cell migration^{20, 21} and vascular tissue formation^{7, 16, 22–25}. The main advantages of microfluidic models are their ability to provide well-defined flow fields around cells at the micrometre scale, where the subsequent cellular dynamics can be followed using real time imaging at single cell resolution. This is particularly important for tumour cell studies as phenotypic and genotypic tumour cell heterogeneity and plasticity are hallmarks of cancer²⁶. In the current microfluidic interstitial flow models^{7, 20, 21}, two lines of micro-scale^{7, 20} or millimetre-scale²¹ PDMS posts were fabricated to confine the collagen gel within a channel and interstitial fluid flow were introduced perpendicular to the channel wall (e. g. lines of PDMS pillars)^{20, 21, 27}. These models have been used successfully in revealing roles of interstitial flows in angiogenesis^{7, 22}, and tumour cell chemoinvasion^{20, 21}. The limitations of current models come from the large cross sectional areas of PDMS posts (they block 44–63% of the cross section area) which lead to non-uniform interstitial flows within the collagen gel (see Fig. 2 of Haessler *et al.*²¹), and unwanted separations by the PDMS posts within the engineered endothelial wall. Furthermore, the PDMS post surfaces are also nucleation sites for air bubbles which eventually complicate the control of the flow. Although there have been methods for wall-less confinement of hydrogels^{28, 29}, these techniques have been limited to cellular co-culture studies and did not address the question of physiological flows.

In this paper, we present a contact line pinning method for generating interstitial flows within 3D biomatrices and intramural flows through an engineered micro-scale vascular vessel. Our concept is to use two parallel microfabricated ridges (cross section of $5\text{ }\mu\text{m} \times 10\text{ }\mu\text{m}$) for confining a hydrogel within a microfluidic channel (cross section of $400\text{ }\mu\text{m} \times 200\text{ }\mu\text{m}$). Interstitial flow is introduced to be perpendicular to the microfluidic channel, mimic of interstitial flows near the vascular vessels (see Fig. 1(c) of Kim *et al.*¹⁹). The advantage of this method is that the microfabricated ridges, in comparison to the micro-posts in the previous work^{7, 20, 21}, block significantly smaller cross section area (2.5% of the total channel cross section), enabling spatially uniform interstitial fluid flows and straight walls for the engineered endothelial tubes, and providing fewer nucleation sites for air bubbles. We validated the generation of both interstitial and intramural flows using experiments and COMSOL modelling. Furthermore, we used this device to study malignant breast cancer cells migrating in the presence of interstitial flows. By confining collagen in localized areas using microfabricated ridges, our method significantly improves the uniformity of interstitial

flows within an *in vitro* microfluidic model, and can be easily extended to construct tunable 3D co-culture models where cells of different types can be cultured on the same platform with designated spatial arrangements^{30–32} and in the presence of the flows.

Materials and Methods

Contact line pinning method and optimization of PDMS surface

The basic idea of the contact line pinning method is illustrated in Figure 1A. When a liquid drop is placed on a solid surface, the liquid wets the solid surface and a contact angle, θ , is formed at the air/liquid/solid contact point (See Figure 1A). In equilibrium, this angle is called static contact angle, θ_c . It quantifies the wettability of the fluid to the solid surface, and is governed by the Young – Laplace equation³³. In dynamic situation when fluid is added to (or extracted from the droplet), the contact line is pinned unless the contact angle exceeds the critical advancing angle θ_a (or smaller than the receding angle θ_r) (See Figure 1B). For a given surface and liquid, the larger the difference of the critical advancing and the critical receding angle, the larger the contact line pinning strength³⁴. The key feature of the contact line pinning method presented here is to use a micro-fabricated ridge (see the yellow square in Figure 1A) to increase the critical advancing and decrease the critical receding angle for a given surface, and thus to increase the strength of pinning a contact line in designated position. This method has been previously used to pin droplets on a substrate for protein crystallization work³⁵, high throughput spheroidal cell culture³⁶ as well as for priming microfluidic device³⁷. Here, we introduce this method to confine hydrogels in wall-less microfluidic channels. Figure 1A shows the concept of increasing the advancing angle by 90° using the microfabricated ridges.

The surface property (hydrophobicity) of the PDMS is optimized for obtaining maximum contact line pinning strength. PDMS is typically hydrophobic with a contact angle of 125° in room temperature and with DI water. However, PDMS can be rendered completely hydrophilic (contact angle of 0°) immediately after the oxygen plasma treatment through the creation of silanol groups (–SiOH) on the surface³⁸. With two weeks of time leaving the PDMS substrate in the room temperature, the PDMS surface eventually becomes hydrophobic. The main concept of surface optimization is to find a time point after plasma treatment at which the PDMS has the strongest pinning strength or the largest difference between the critical advancing and receding angles. Figure 1B shows the static contact angle, the critical advancing and the critical receding angle of a millimeter water droplet on PDMS surface at various time points (See Figure 1B), in which time 0 was defined as the time when the PDMS was treated with oxygen plasma (1 minute, high power intensity, Harrick Plasma Cleaner PDC-001, Harrick Plasma, Ithaca, NY, USA). To obtain the critical advancing/receding contact angles, we first took a time sequence of images [1 frame per second, with a goniometer (Ramé-hart 500 Advanced Goniometer/Tensiometer, Ramé-hart Instrument, Succasunna, NJ, USA)] of a water droplet, of which DI water was being injected/withdrew at a rate of 1 μ L/sec. The critical advancing and receding angles were then obtained by post processing the image series. The critical advancing/receding angles were defined when the contact line start to move upon injecting/withdrew liquid from the droplet. The diameters of the water drops were less than 3 mm in all measurements. The static

contact angles were measured with a 6 μL drop of water. All the measurements were carried out in room temperature. After every measurement, the water on the PDMS surface was removed using a nitrogen gun.

Figure 1B suggests that the pinning strength or the difference of the advancing and receding angles reaches a steady state at a time of 2–3 days after the oxygen plasma treatment of the PDMS surface. In our experiments, we typically wait for about one day after the PDMS was oxygen plasma treated before experiments. This decision is made after taking into consideration of three factors: (i) the ease with which to introduce collagen into the channel, (ii) the probability of introducing bubbles due to the hydrophobicity of the PDMS surfaces, and (iii) the pinning strength of the micro-fabricated ridges.

Microfabrication and assembly

The microfluidic device was fabricated using the standard soft lithography technique. The silicon master (See Figure 2A for the design) was first made with a two-step silicon etching methods, and the final microfluidic device was replicated from the silicon master using the PDMS stamping technique.

The fabrication of the negative silicon master was done at Cornell NanoScale Science & Technology Facility (CNF). We used a two-step etching process for (i) the trenches of 10 μm width and 5 μm depth, and (ii) the 200 μm depth channels. The detailed procedures are as follows. Photoresist (Microposit S1813, Shipley, Marlborough, MA, USA) was first spun on (3000 rpm for 45 sec) a silicon wafer (4" SEMI standard SSP, 0.5 mm thick), baked at 115 $^{\circ}\text{C}$ for one minute, and then exposed to (128.7 mJ/cm^2) on a contact aligner (Karl Suss MA/BA 6 aligner, Suss Microtech, Garching, Germany, soft contact mode). After developing the resist (60 sec MF-321 development, HamaTech-Steag Wafer Processor, Santa Clara, CA, USA), the wafer was then etched by a Botsch deep silicon etching method (Unaxis 770 Deep Silicon Etcher, Oerlikon, Pfäffikon, Switzerland) to obtain the 5 μm trenches. The second layer that defines the microfluidic channels were made onto the silicon wafer using the same instruments and methods, except with a different photoresist (Megaposit SPR220-7.0, Shipley, 2500 rpm, 40sec), exposure dosage (1.05 J/cm^2), an additional post-exposure wait time (90 minutes), developer (AZ 726 MIF), and etching depth (200 μm). After stripping the photoresist (AURA 1000 Resist Strip, GaSonics, San Jose, CA, USA), the wafer was treated with (1H,1H,2H,2H-Perfluorooctyl) Trichlorosilane or FOTS using a vapour deposition method (Molecular Vapor Deposition, Applied Microstructures, San Jose, CA, US) to ensure the easy release of PDMS from the silicon master.

Standard 10:1 PDMS (SYLGARD 184 Silicone Elastomer Kit, Dow Corning, Midland, MI, US) procedures were used for making an inversed PDMS replica from the silicon master. After curing, 2 mm holes were punched using biopsy punches (Miltex, Inc, York, PA, USA) for the access of the channels. The PDMS pieces were then autoclaved, and oxygen plasma treated for a minute on high power setting using the same plasma cleaner as in previous section. Our PDMS pieces were kept in room temperature for a day before usage. For the experiment, the PDMS piece was sandwiched between a Plexiglas manifold and a glass

slide. To avoid drying of the PDMS device, 1% agarose gel was added to surround the PDMS device, in the space between the Plexiglas manifold and glass slide.

Flow control and measurements

Both intramural and interstitial flows were generated by a syringe pump (KDS-230, KD Scientific, Holliston, MA, USA), and syringes (1 mL or 3mL, BD, Franklin Lakes, NJ, USA). Intramural flows were measured using a particle tracking method with 0.51 μm diameter fluorescent beads (Dragon Green, Bangs Laboratories, Fishers, IN, USA). Images of the fluorescent beads with long exposure time (e. g. 0.5s) were acquired to obtain the flow speed across the channel. A flow rate range of 0 – 0.15 $\mu\text{L}/\text{min}$ were used, but 0.12 $\mu\text{L}/\text{min}$ is found to be optimal for our setup. Interstitial fluid flows were measured using a fluorescence recovery after photo bleaching (FRAP) method with an epi-fluorescence microscope (IX81, Olympus America, Center Valley, PA, USA). 10^{-4} M fluorescein sodium (Fisher Scientific, Pittsburgh, PA 15275) solution was introduced to the flow channel at the designated flow rate for at least 30 minutes before measurement. A focused light spot was brought to the center portion of the imaging area for 6 seconds by a high-magnification objective (40X/0.60, LUCPlanFL, Olympus) and a xenon lamp (LB-LS/30, Sutter Instrument Company, Novato, CA, USA). A sequence of the images of the bleached area was then taken immediately by a low-magnification objective (4X/0.13, UPlanFL, Olympus). This process was repeated at three different positions along the middle channel at each of the flow rates. These images were used later for obtaining the interstitial flow rates.

Imaging and data analysis

All the images were taken by a CCD camera (Orca-ER, Hamamatsu Photonics, Japan) in conjunction with the epi-fluorescence microscope (IX81, Olympus). For FRAP data analysis, we used ImageJ (freely available at <http://rsb.info.nih.gov/ij/>), as well as a MATLAB program developed by Jönsson in 2010³⁹. For cell morphology analysis, a “Fit Ellipse” function within ImageJ was used to compute the long and short axis of the cell. For motility analysis, the cells were tracked using ImageJ, and the trajectories were analysed using GraphPad Prism and an in house MATLAB program.

Cells and 3D cell culture

MDA-MB-231 breast cancer cell line was provided by the Center for the Microenvironment and Metastasis at Cornell. The growth media consists of DMEM (Gibco, Grand Island, NY, USA), 10% Fetal Bovine Serum (FBS, Atlanta Biologicals, Lawrenceville, GA, USA), 100 units/mL of penicillin, and 100 $\mu\text{g}/\text{mL}$ of streptomycin. Human Umbilical Vein Endothelial Cells (HUVECs, Lonza, Walkersville, MD, USA) carry green fluorescence genes through a near permanent Lenti-viral transfection. The growth media consist of Medium 199 (Gibco), 150 units/mL of penicillin, 150 $\mu\text{g}/\text{mL}$ of streptomycin (Gibco), 2 mM of L-glutamine (Gibco), 5 units/mL of heparin (Fisher Biotech, Fair Lawn, NJ, USA), 18% of FBS, and 20 $\mu\text{g}/\text{mL}$ of Endothelial Cells Growth Supplement (CGS, Millipore, Temecula, CA, USA).

Type I collagen was extracted from rat tails (Pel-Freez, Rogers, AR, USA) using a modified protocol⁴⁰ and stored at 5 mg/mL in 0.1% acetic acid at 4 °C. MDA-MB-231 cells from 50–75% confluence of a T-75 tissue culture dish were used for experiments. Cell numbers were

counted using a hemocytometer (Bright-Line Hemocytometer, Hausser Sci., Horsham, PA). For a typical preparation of a 200 μL 3D cell culture, 60 μL 5 mg/mL collagen, 20 μL 10X M199, 1.3 μL 1N NaOH, and 34.7 μL cell culture were mixed with DMEM on ice to achieve a PH ~ 7 and cell density $10^6/\text{mL}$. The cell embedded collagen was then introduced to the cell channel, and then incubated at 37°C upside down for 15 minutes and then right side up for 15 minutes, to allow for even cell distribution along the z-axis during collagen polymerization. The empty channels were then filled with proper media.

For fixing and straining, HUVECs were first fixed with 4% formaldehyde (Polysciences, Warrington, PA, USA) in Phosphate Buffer Solution (PBS, Fisher), followed by a PBS wash. 1% TitronX-100 (Sigma, St. Louis, MO, USA) was used to permeate the cell membrane, and 3% Bovine Serum Albumin (BSA, Sigma) in PBS was used to prevent nonspecific protein binding. DAPI (Invitrogen, Eugene, OR, USA) was used to stain the nuclei, and AlexaFluor Phalloidin (Invitrogen) was used to stain actins.

Experimental setup

For the generation of intramural flow experiment, both PDMS piece and the glass slide were treated with poly(ethyleneimine) (Fluka, St. Louis, MO, USA) and glutaraldehyde (Fluka) before the experiments to promote adhesion of both cells and collagen to the surfaces⁴⁰. Type I collagen was first introduced into the two side cell channels (as shown in Figure 2B), and then placed in an incubator (100% humidity, 37°C , and 5% carbon dioxide) for 30 minutes until the collagen was polymerized. HUVECs were harvested close to confluent, and were introduced to the central channel at a concentration of 6×10^6 cells/mL. After confirming there was no convective flow under an optical microscope, the device was incubated for two hours, to allow cells to be attached to the surfaces. To facilitate the cells to adhere to the top PDMS surface, the device was first incubated upside down for an hour, and then was flipped back, and incubated for another hour. The excess cells were then removed by a slow flow of HUVEC growth media at a flow rate (0.12 $\mu\text{L}/\text{min}$). Initially this flow was used to remove the excess cells. Once the excess cells were fully removed, an identical flow was continually used to provide nutrients to the attached cells during the entire culture process, which typically took two to three days. As a result, this flow rate was chosen such that it was fast enough to bring nutrients into the channel, yet still gentle enough not to displace the collagen in the two side channels over several days. The devices were then kept in the incubator with the above mentioned flow to allow HUVECs to proliferate and cover the area.

For the generation of interstitial flow, MDA-MB-231 cell embedded type I collagen was introduced to the central cell channels (either one, or two, or three channels). After the collagen polymerization, the empty channels were filled with media. The device was then connected with syringes, and placed in a 5% CO_2 , 37°C and humidified (humidity 90%) microscope environment chamber for imaging.

Results and discussion

Confine collagen in wall-less microfluidic channels using micro-fabricated ridges

The key feature of the microfluidic platform is to use micro-fabricated ridges to confine collagen in wall-less channels. A schematic drawing of the device is shown in Figure 2A. In the actual experimental setting, three such devices were fit onto a 1" by 3" glass slide for multiple parallel experiments. Within each device, there are three parallel cell channels, each is 400 μm wide and 200 μm deep, which are designed to contain collagen or cell laden collagen. The 200 μm depth is an important aspect of our device, since it provides the cells with a 3D ECM environment. In our previous work, we have found that cells mostly migrate along the surfaces of the channels when the channel depth is less than 100 μm ^{41, 42}. We choose 400 μm for channel width since cancer cells migrate about 20 μm per hour, 400 μm allows us for carrying out cell migration studies for about 10 hours⁴³. The central portion of the cell channels (where the interstitial flows pass through) are bounded by the microfabricated ridges with 5 μm $\times$ 10 μm cross section area (See the close up image in Figure 1A). The dimensions of the ridges are optimized to provide the strongest pinning strength, as demonstrated by Kalinin *et al.*³⁴. The interstitial flow is introduced by the flow channel to be perpendicular to the cell channels, which is a mimic of *in vivo* interstitial flow configuration near vascular vessels¹⁹.

The microfluidic platform can be easily re-configured for introducing either intramural flow alone, or interstitial flow alone, or both. We provide two examples here. (i) For studies of intramural flow, type I collagen was introduced into the two side cell channels (see Figure 2B). In this design, endothelial cells will be introduced into the central channel at a later stage to form an engineered vascular vessel. (ii) For studies of the interstitial flow alone, collagen was introduced into the middle cell channel (see Figure 2C), and interstitial flow will be introduced from the flow channel. The top view (bright field images in the top row of Figure 2B,C) and the side view (confocal images in the lower row of Figure 2B,C) of the three cell channels confirmed that the micro-fabricated ridges successfully confined the collagen within the wall-less channels. A confocal image of the collagen matrix (Figure 2D) shows the fibrous structure of the collagen gel, which demonstrates the successful polymerization of the collagen within the device.

Intramural flow characterization in an engineered endothelial tube

Figure 3A demonstrates the establishment of an engineered endothelial tube using the collagen channel configuration shown in Figure 2B. Type I collagen matrix was first introduced into the two side cell channels. After the collagen was polymerized, HUVECs (6×10^6 cells/mL) expressing green fluorescent proteins were introduced into the middle cell channel. The device was left in the incubator with no flow for 2 hours to allow HUVECs to attach to all surfaces. A flow of 0.12 $\mu\text{L}/\text{min}$ (or 50 $\mu\text{m}/\text{sec}$) was then introduced into the middle cell channel (or the endothelial tube) to provide nutrient, and the device was incubated for 2–3 days with the flow to form HUVEC monolayer. Figure 3A are confocal images of the top and side view of the endothelial tube. Here, HUVECs were fixed and stained for the nuclei (blue) and the actin filaments (red) (Figure 3A). The side view of the

vessel demonstrates that a single layer of HUVECs covered on all four surfaces, attaching to both the PDMS and glass surfaces, as well as the two collagen side walls.

Figure 3B shows the intramural flow field revealed by a particle tracking method. One the left is a micrograph of 0.5 μm diameter fluorescent beads in the channel with exposure time of 0.5 sec. The lengths of the streaks of fluorescent particles were used to compute the velocity profile across the channel. The quadratic Poiseuille's flow profile indicates that the endothelial cell covered collagen walls provide a non-slip boundary condition to the centre channel, which agrees with the numerically calculated values (See Figure S1 in supplementary material).

Figure 3 demonstrates the feasibility of creating an engineered blood vessel using the contact line pinning based microfluidic device. The advantage of the platform is the continuous and straight wall of the blood vessel, and the compatibility with optical imaging. This platform is particularly attractive for studies of animal cell transmigration in the presence of well controlled intramural flows.

Interstitial flow characterization through a 3D collagen matrix

Interstitial flows within the microfluidic device were characterized using a modified Fluorescence Recovery After Photo Bleaching (FRAP) method (See Figure 4). Briefly, to measure a slow flow (at Peclet number close to 1) within a biomatrix, a localized spot within a fluorescent collagen matrix was bleached using an intensified fluorescent light beam, and the movement of the bleached spot was imaged and post processed for obtaining the flow rates. Here, we used an experimental setting that is similar to the one shown in Figure 2C, with an exception that the collagen were introduced into all three cell channels instead of one. Typical images of the bleached spot are shown in Figure 4A, where both convective and diffusive transport of the fluorescent dye are seen. We used two different methods to calculate the flow speed, an ImageJ based (Figure 4B), and a MATLAB based (Figure 4C) method. In the ImageJ based method, we used the minimum of the light intensity of the area of interest (marked in Figure 4A) as the position of the bleached spot. In the MATLAB method, we used a program developed by Jönsson et al.³⁹ to compute the center of mass of the bleached spot. The results from both analysis were consistent.

Within the time duration of the flow measurements, the bleached spot moved at a uniform speed, as shown in Figure 4D. To verify the spatial uniformity of the flow rate, we measured flow speed at three different locations that are 1 mm apart and along the middle cell channel (locations are marked in Figure 4G), and demonstrated that the flows are constant at these three locations at various pumping rates (Figure 4E). In addition, we validated the measured flow rate against the calculated flow rate using the known pumping volume flow rate (Figure 4F). For physiological representations of interstitial flow in the tumour microenvironment (2–10 $\mu\text{m}/\text{sec}$), low pumping rates (0.05–0.15 $\mu\text{L}/\text{min}$) are required. The spatial uniformity of the interstitial flows were further verified by computing the flow speed profiles within the three dimensional space of the collagen filled channels using a COMSOL Multiphysics software. The top and side view of the flow speed profiles are shown in Figure 4G–H. In Figure 4G, the variation of the flow speed along the y-axis is 8% within 80% of

the middle cell channels. In Figure 4H, the variation of the flow speed along the vertical direction is 2.3% within the 85% of the middle cell channel.

Figure 4 demonstrates the capability of contact line pinning device for generating spatially uniform interstitial flows within collagen matrices. This spatial uniformity is facilitated by the small cross sections of the microfabricated ridges that were used to confine the collagen within the cell channels. This platform can be easily reconfigured to study cell-cell interactions in a co-culture platform in the presence of interstitial flows. It should be noted that the modified FRAP utilizes a wide field fluorescent microscope, instead of a specially designed laser beam system or confocal microscope^{21, 44, 45}. This method is straightforward to implement, and can be made readily available to many biology laboratories who do not own a confocal microscope.

Interstitial flow modulates tumour cell morphology and motility

Using the experimental setting presented in Figure 4, we studied breast tumour cell (MDA-MB-231 cell line) morphology and motility in the presence/absence of interstitial flows. Figure 5 shows that tumour cell morphology and motility were both modulated by the presence of the flow (See also Movie S1).

In the absence of the flow (Figure 5A, top row), cells became more elongated. This is reasonable, because at $t=0$, the cells were a few minutes after they were seeded into the cell channel, and they were mostly rounded because they did not have time to adhere to the matrices. However, with time, cells started to attach to the collagen matrix via adhesion sites and as a result stretched out into a more elongated morphology. This observation is consistent with previous report that cells are more elongated when fibronectin is added to the culture media⁴⁶. The aspect ratios of the cells covered a wide range, and varied from 1.0 (round) to 10 (highly elongated), as shown in Figure 5B.

In the presence of the flow (Figure 5A, bottom row), cells remained to be mostly rounded during the course of 16 hours. This is likely to be caused by the absence of the cell secreted adhesion molecules, such as fibronectin, which were carried away by the flows before being polymerized. It has been reported that mammary epithelial cells express fibronectin, which is essential for cell adhesion to the collagen fibers⁴⁷. The cells were motile in the presence/absence of the flow as shown in Movie S1. Furthermore, we found that the average cell aspect ratio increases with time (Figure 5C) in the absence of the flow, and this trend of increase is less pronounced in the presence of the flow (Figure 5D). The coexistence of mesenchymal (elongated) and amoeboid (round) motilities have been known in oncology research⁴⁸, and a number of studies have been carried out to understand the molecular signatures of mesenchymal-amoeboid transition⁴⁹. It is likely that the elongated and rounded cells observed here correspond to mesenchymal and amoeboid cells⁵⁰, however, the detailed molecular mechanism for the impact of fluid flows on cell morphology has yet to be explored.

Cell migration velocities along the flow direction, V_x , are plotted against their aspect ratio in the presence/absence of the flow in Figure 5E–F. Although no directed cell migration along the flow direction was observed in our experiments^{20, 21}, it is clear that the maximum

velocity of the tumour cells is correlated with its cell aspect ratio, it decreases as the cell aspect ratio increases. Within the round cell sub-population (aspect ratio between 1–2), the variance of the velocity is 9.12 $\mu\text{m/hr}$ in the presence of the flow, which is significantly larger than 5.50 $\mu\text{m/hr}$, that in the absence of the flow. This observation is consistent with previous work from the Swartz lab in which cells are more motile in the presence of the flow ²¹.

Figure 5 demonstrates that interstitial flows modulates tumour cell morphology in a significant way, cells stay more rounded in the presence of the flow than those in the absence of the flow. Cell motility is consistently enhanced in the presence of the flow for the rounded cell sub-population (aspect ratio between 1–2 and 2–3). However, the cell motility (or variance of V_x) of elongated cells (aspect ratio larger than 3) did not show an apparent trend in relation to the flow (See table S1 in supplementary materials).

Conclusion and future perspective

We introduced a contact line pinning based method for patterning collagen gels in wall-less channels. Using this method, we successfully introduced and characterized the intramural flows through an engineered vascular tube, and interstitial flows through collagen matrices. Using this microfluidic platform, we revealed that interstitial flows modulate tumour cell (MDA-MB-231 cell line) morphology in a significant way, and the overall cell aspect ratio decreases in the presence of the flow. Furthermore, motility of rounded cell sub-population (aspect ratio less than 3) is enhanced in the presence of the flow.

The main advantage of the presented contact line based microfluidic platform versus the micro-post based platform in the existing literature ^{20, 21, 24} is the ability to create spatially uniform interstitial flows within collagen matrices, and to provide straight and continuous endothelial vessel walls. These features are particularly important for cell migration and transmigration studies. It should be noted that this platform can be easily re-configured for 3D co-culture studies in the presence of the flows.

Given the increasingly accepted importance of physiological flows in immune and tumour cell migration and invasion ^{51, 52}, the presented platform overcomes the limitation of the traditional migration assay, the Boyden chamber, in that the flows are difficult to control and results are population and end-points. This is particularly important for cancer research as cancer cell phenotypic and genotypic plasticity and heterogeneity are hall marks of cancer ²⁶. It has been hypothesized that the outliers of the tumour cells (the fast movers) may play important roles in tumour cells ability to metastasize to a distant site. The presented platform enables us to interrogate a sub-population of the cells (e. g. grouped according to their aspect ratio) and open door for studying rare cell events.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

MW would like to thank Melody Swartz for many insightful discussions, MW and CKT would like to thank Abraham Stroock's group for their help with the endothelial cell culture, and Dr. Rajesh Bhaskaran for the help with COMSOL Multiphysics. This work was primarily supported by the National Cancer Institute (Award No R21CA138366), partially supported by the Cornell Center on the Microenvironment & Metastasis (Award No U54CA143876 from the National Cancer Institute), the Cornell NanoScale Science & Technology Facility and the Cornell Nanobiotechnology Center.

Notes and References

1. Berk DA, Swartz MA, Leu AJ, Jain RK. Am J Physiol Heart Circ Physiol. 1996; 270:H330–H337.
2. Chary SR, Jain RK. Proc Nat Acad Sci USA. 1989; 86:5385–5389. [PubMed: 2748592]
3. Starling EH. The Journal of physiology. 1896; 19:312–326.
4. Culver JC, Dickinson ME. Microcirculation. 2010; 17:164–178. [PubMed: 20374481]
5. Le Noble F, Moyon D, Pardanaud L, Yuan L, Djonov V, Matthijsen R, Breant C, Fleury V, Eichmann A. Development. 2004; 131:361–375. [PubMed: 14681188]
6. Helm CLE, Fleury ME, Zisch AH, Boschetti F, Swartz MA. Proc Nat Acad Sci USA. 2005; 102:15779–15784. [PubMed: 16249343]
7. Song JW, Munn LL. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:15342–15347. [PubMed: 21876168]
8. Fleury ME, Boardman KC, Swartz MA. Biophys J. 2006; 91:113–121. [PubMed: 16603487]
9. Shields JD, Fleury ME, Yong C, Tomei AA, Randolph GJ, Swartz MA. Cancer Cell. 2007; 11:526–538. [PubMed: 17560334]
10. Shieh AC, Rozansky HA, Hinz B, Swartz MA. Cancer Res. 2011; 71:790–800. [PubMed: 21245098]
11. Jain RK. Cancer and Metastasis Reviews. 1990; 9:253–266. [PubMed: 2292138]
12. Nagatomo I, Nomaguchi M, Matsomoto K. Clin Auton Res. 1992; 2:197–200. [PubMed: 1498566]
13. Ng CP, Helm CLE, Swartz MA. Microvascular Res. 2004; 68:258–264.
14. Price GM, Wong KHK, Truslow JG, Leung AD, Acharya C, Tien J. Biomaterials. 2010; 31:6182–6189. [PubMed: 20537705]
15. Li YSJ, Haga JH, Chien S. Journal of Biomechanics. 2005; 38:1949–1971. [PubMed: 16084198]
16. Hsu YH, Moya ML, Abiri P, Hughes CCW, George SC, Lee AP. Lab on a Chip. 2013; 13:81–89. [PubMed: 23090158]
17. Hur SS, del Alamo JC, Park JS, Li YS, Nguyen HA, Teng D, Wang KC, Flores L, Alonso-Latorre B, Lasheras JC, Chien S. Proc Nat Acad Sci USA. 2012; 109:11110–11115. [PubMed: 22665785]
18. Heldin CH, Rubin K, Pietras K, Ostman A. Nature Reviews Cancer. 2004; 4:806–813. [PubMed: 15510161]
19. Kim BJ, Wu M. Annals of Biomedical Engineering. 2012; 40:1316–1327. [PubMed: 22189490]
20. Polacheck WJ, Charest JL, Kamm RD. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108:11115–11120. [PubMed: 21690404]
21. Haessler U, Teo JCM, Foretay D, Renaud P, Swartz MA. Integrative Biology. 2012; 4:401–409. [PubMed: 22143066]
22. Vickerman V, Kamm RD. Integrative Biology. 2012; 4:863–874. [PubMed: 22673733]
23. Kim S, Lee H, Chung M, Jeon NL. Lab on a Chip. 2013; 13:1489–1500. [PubMed: 23440068]
24. Yeon JH, Ryu HR, Chung M, Hu QP, Jeon NL. Lab on a Chip. 2012; 12:2815–2822. [PubMed: 22767334]
25. Zheng Y, Chen J, Craven M, Choi NW, Totorica S, Diaz-Santana A, Kermani P, Hempstead B, Fischbach-Teschl C, Lopez JA, Stroock AD. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109:9342–9347. [PubMed: 22645376]
26. Hanahan D, Weinberg RA. Cell. 2011; 144:646–674. [PubMed: 21376230]
27. Vickerman V, Blundo J, Chung S, Kamm R. Lab Chip. 2008; 8:1468–1477. [PubMed: 18818801]

28. Lee SH, Heinz AJ, Shin S, Jung YG, Choi SE, Park W, Roe JH, Kwon S. *Anal Chem.* 2010; 82:2900–2906. [PubMed: 20210331]
29. Hwang H, Park J, Shin C, Do Y, Cho Y-K. *Biomedical Microdevices.* 2012;10:1007/s10544-10012-19733-10549
30. Wong AP, Perez-Castillejos R, Love JC, Whitesides GM. *Biomaterials.* 2008; 29:1853–1861. [PubMed: 18243301]
31. Kunze A, Giugliano M, Valero A, Renaud P. *Biomaterials.* 2011; 32:2088–2098. [PubMed: 21159379]
32. Zervantonakis IK, Hughes-Alford SK, Charest JL, Condeelis JS, Gertler FB, Kamm RD. *Proceedings of the National Academy of Sciences of the United States of America.* 2012; 109:13515–13520. [PubMed: 22869695]
33. De Coninck J, Dunlop F. *J Stat Phys.* 1987; 47:827–849.
34. Kalinin YV, Berejnov V, Thorne RE. *Langmuir.* 2009; 25:5391–5397. [PubMed: 19317420]
35. Kalinin Y, Berejnov V, Thorne RE. *Microfluid Nanofluid.* 2008; 5:449–454. [PubMed: 21423834]
36. Hsiao AY, Tung YC, Qu XG, Patel LR, Pienta KJ, Takayama S. *Biotechnology and bioengineering.* 2012; 109:1293–1304. [PubMed: 22161651]
37. Vulto P, Podszun S, Meyer P, Hermann C, Manz A, Urban GA. *Lab Chip.* 2011; 11:1596–1602. [PubMed: 21394334]
38. Chen IJ, Lindner E. *Langmuir.* 2007; 23:3118–3122. [PubMed: 17279784]
39. Jönsson P, Jonsson MP, Tegenfeldt JO, Höök F. *Biophysical Journal.* 2008; 95:5334–5348. [PubMed: 18567628]
40. Cross VL, Zheng Y, Choi NW, Verbridge SS, Sutermaster BA, Bonassar LJ, Fischbach C, Stroock AD. *Biomaterials.* 2010; 31:8596–8607. [PubMed: 20727585]
41. Haessler U, Kalinin Y, Swartz MA, Wu M. *Biomed Microdevices.* 2009; 11:827–835. [PubMed: 19343497]
42. Haessler U, Pisano M, Wu M, Swartz MA. *Proc Natl Acad Sci U S A.* 2011; 108:5614–5619. [PubMed: 21422278]
43. Kim BJ, Hannanta-anan P, Chau M, Kim YS, Swartz MA, Wu M. *PLoS ONE.* 2013 accepted. 10.1371/journal.pone.0068422
44. Berk DA, Yuan F, Leunig M, Jain RK. *Biophysical Journal.* 1993; 65:2428–2436. [PubMed: 8312481]
45. Chary SR, Jain RK. *Proceedings of the National Academy of Sciences of the United States of America.* 1989; 86:5385–5389. [PubMed: 2748592]
46. Montanez-Sauri SI, Sung KE, Berthier E, Beebe DJ. *Integrative Biology.* 2013; 5:631–640. [PubMed: 23340769]
47. Williams CM, Engler AJ, Slone RD, Galante LL, Schwarzbauer JE. *Cancer Res.* 2008; 68:3185–3192. [PubMed: 18451144]
48. Friedl P. *Curr Opin Cell Biol.* 2004; 16:14–23. [PubMed: 15037300]
49. Pačková K, Rösel D, Novotný M, Brábek J. *Cell Mol Life Sci.* 2010; 67:63–71. [PubMed: 19707854]
50. Condeelis J, Segall JE. *Nature Reviews Cancer.* 2003; 3:921–930. [PubMed: 14737122]
51. Shieh AC, Swartz MA. *Physical Biology.* 2011;8.
52. Swartz MA, Lund AW. *Nature Reviews Cancer.* 2012; 12:210–219. [PubMed: 22362216]

Fig. 1. Illustration of a contact line pinning method

A. The contact angle of a water drop on a flat substrate is enhanced by the presence of the micro-fabricated ridges (from θ to θ^*) as seen in the illustrations (left column) and micrographs (right column). The micrograph shows a millimetre size water drop placed on a PDMS surface, with a measured contact angle 51° (top), and 139° (bottom). Yellow squares denote the ridge locations, not to scale. **B.** Advancing (θ_a), static (θ_c), and receding (θ_r) contact angles of a millimetre size water droplet placed on a PDMS surface as a function of the time. $t=0$ is defined as the time when the PDMS is rendered hydrophilic via oxygen plasma treatment. The advancing and receding of the contact line are achieved by injection or withdrawal of water at $1 \mu\text{L}/\text{sec}$ rate with a needle using a goniometer. The two insets show images of an advancing (top) and a receding (lower) droplet. The hysteresis between the advancing and receding angles determines the contact line pinning strength. The static measurements were done using a $6 \mu\text{L}$ water drop. The dashed lines are the general trend lines. The untreated PDMS surface has a contact angle close to 125° .

Fig. 2. Design principle of a contact line pinning based microfluidic device for modelling physiological flows

A. Layout of the microfluidic device. The three parallel channels are bounded by the microfabricated ridges for housing collagen or cell laden collagen. The horizontal channel is used to introduce interstitial flow into the cell-laden collagen. **B–C.** Bright field (top view) and confocal (side view) images of the three cell channels. In B, collagen matrix is introduced into two side cell channels. This configuration is designed for generating an engineered endothelial tube for intramural flow studies. The intramural flow is along the channel direction. In C, collagen is introduced in the middle cell channel. This configuration is designed for interstitial flow studies, in which the flow is in the horizontal direction. Scale bar is 100 μm . **D.** A reflective confocal micrograph of the collagen fibre matrix. Scale bar is 20 μm .

Fig. 3. Intramural flow measurements in an endothelial tube

A. Top and side view of an endothelial cell covered microfluidic channel. GFP-expressing HUVECs were used in this experiment, the red is the actin stain and blue is the nuclei stain. The scale bar is 50 μm . **B.** Characterization of intramural flow in the centre channel. Left: 0.2 μm green fluorescent beads are used as tracing particles, with 0.5 sec exposure time. It can be seen that the flow is faster at the centre of the channel, and nearly no flow close to the side wall. The scale bar is 50 μm . Right: flow speed analysis shows a Poiseuille flow profile across the channel.

Fig. 4. Interstitial flow measurements within collagen gel using FRAP

A. Images of the photo bleached spot within a collagen gel taken at two consecutive time points and in the presence of a flow. The scale bar is 200 μm . **B–C.** Illustration of flow speed analysis using ImageJ software (B) and a MATLAB program (C). In B, light intensity profiles averaged along the y- axis of the boxed regions in A are plotted, and the displacement of the profile minimum along the x-axis is used to calculate the speed. In C, the centre of mass of the bleached area is located using an iterative computation algorithm developed by Jönsson et al. (2010), and its displacement is used for flow speed calculation. Cross is where the center of mass is. The polygon is drawn around the bleached spot to facilitate the center of mass calculation. The scale bar is 200 μm . **D.** Flow speed is shown to be constant and at a rate of 13 $\mu\text{m}/\text{sec}$. **E.** Flow speed is shown to be constant at three spatial locations in the middle cell channel that are 1mm apart. The locations A, B, and C are indicated in (G). **F.** The flow speed from FRAP measurements (y-axis) is validated against the calculated speed using the known pumping volume flow rate (x-axis). **G–H.** The spatial distribution of the flow speed within the cell channels from a COMSOL Multiphysics computation. In (G), the variation of the flow speed along the y-axis is 8% within 80% of the middle cell channels. In (H), the variation of the flow speed along the vertical direction is 2.3% within the 85% of the middle cell channel. The two dashed lines mark the boundary between the flow channel and the collagen filled space.

Fig. 5. Interstitial flow modulates breast tumour cell morphology and motility

A. Images of breast tumour cells (MDA-MB 231 cell line) embedded in a type I collagen matrix at two time points, $t=0$, and $t=16$ hrs., in the absence (top row) and presence (low row) of a flow (flow rate is $11.5 \mu\text{m}/\text{sec}$). $t=0$ is defined as the time when the flow started. Rounded cells (defined as aspect ratio less than 3) are marked in blue and elongated cells (aspect ratio larger than 3) in red. The scale bar is $100 \mu\text{m}$. **B.** Close up image of the tumour cells for cell morphology studies, the ellipses are obtained using Fit Ellipse function in Image J. The aspect ratio is defined as the length of the major axis divided by that of the minor axis of the ellipse. The lower panel is the aspect ratio distribution of cells at time point 16 hrs. in the absence of a flow. **C.** Scatter plot of cell aspect ratios at three time points in the absence of the flow. **D.** Cell aspect ratio versus time at three different flow rates. **E–F.** Scattered plot of the cell velocity V_x versus aspect ratio, in the absence (E) and presence (F) of a flow of $3.5 \mu\text{m}/\text{sec}$. The red dots denote the variances of V_x with an aspect ratio bin size of 1.