One step antibody-mediated isolation and patterning of multiple cell types in microfluidic devices

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Cell-cell interactions play a key role in regeneration, differentiation, and basic tissue function taking place under physiological shear forces. However, current solutions to mimic such interactions by micro-patterning cells within microfluidic devices have low resolution, high fabrication complexity, and are limited to one or two cell types. Here, we present a microfluidic platform capable of laminar patterning of any biotin-labeled peptide using streptavidin-based surface chemistry. The design permits the generation of arbitrary cell patterns from heterogeneous mixtures in microfluidic devices. We demonstrate the robust co-patterning of α -CD24, α -ASGPR-1, and α -Tie2 antibodies for rapid isolation and co-patterning of mixtures of hepatocytes and endothelial cells. In addition to one-step isolation and patterning, our design permits step-wise patterning of multiple cell types and empty spaces to create complex cellular geometries *in vitro*. In conclusion, we developed a microfluidic device that permits the generation of perfusable tissue-like patterns in microfluidic devices by directly injecting complex cell mixtures such as differentiated stem cells or tissue digests with minimal sample preparation. © 2016 AIP Publishing LLC.

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INTRODUCTION

Tissue function critically depends on a range of homotypic and heterotypic cell-cell interactions that play a vital role in tissue maintenance and regeneration.¹ For example, primary hepatocytes lose their differentiated phenotype within days *in vitro* when cultured alone, but rapidly stabilize gene expression and function when co-cultured with endothelial cells or fibroblasts.^{2–5} The relatively recent development of micro-patterning techniques offers the ability to control the relative ratios between homotypic and heterotypic cell-cell interactions mimicking native tissue architecture.^{6,7} For example, stencil micro-patterning of primary hepatocytes demonstrated a critical island size of 500 μm below which homotypic interactions were insufficient to support liver-specific function.³

To date, a range of technologies has been developed to micro-patterning cells, including stencil-based patterning³ and micro-contact printing^{8–10} that are often limited to two cell types and static cultures. Alternative techniques include laser direct writing,⁴ dielectrophoresis,¹¹ photopatterning,^{12,13} topographic molding,¹⁴ and bioprinting^{15,16} that permit the patterning of multiple cell types in resolutions ranging from 5 to 200 μm , respectively. While several micro-patterning techniques can be used to pattern cells in microfluidic devices, they do so at the cost

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of a highly specialized equipment set-up (e.g., lasers, electrodes) or specialized biomaterials (e.g., UV responsive polymers).

Laminar flow patterning is an alternative methodology that can be used to precisely control fluid positioning in microfluidic devices without a physical barrier between neighboring streams due to low Reynolds (Re) numbers that govern flow on the microscale.¹⁷ Early work by Takayama and colleagues demonstrated the patterning of cells and their selective removal using trypsin in microfluidic channels.¹⁷ Subsequent work by Kenis and colleagues used the same approach to micro-pattern metals, organic polymers, crystals, and ceramics inside microfluidic channels with 10 μm resolution.¹⁸ In this context, the recent development microfluidic cell isolation using surface-chemistry bound antibodies^{19,20} offers a new opportunity to combine laminar flow patterning with cell isolation, creating arbitrary patterns from complex cell mixtures.

In this work, we present a microfluidic, antibody-based system capable of creating arbitrary patterns of multiple biotinylated antibodies. We demonstrate the calibration of antibody-based cell capture using a shear-generating microfluidic device, permitting a rapid single-step capture and patterning of multiple cell types from complex mixtures. We demonstrate the single-step co-patterning hepatocytes and endothelial cells in microfluidic devices as well as the patterning of empty space and isolated islands.

MATERIALS AND METHODS

Microdevice fabrication

Microfluidic devices were fabricated by photolithographically defining SU8 (SU-8 3025, MicroChem) on silicon wafers at the Harvey Krueger Center of Nanotechnology at the Hebrew University of Jerusalem as previously described.²¹ Microdevices contain a single inlet for surface chemistry and cell seeding and eight $180 \pm 2 \mu\text{m}$ wide inlets for antibody patterning (Fig. 1(a), supplementary Fig. 1³¹). Briefly, a negative relief of poly(dimethylsiloxane) (PDMS) was formed by curing the prepolymer (Sylgard 184, Dow-Corning) on the silicon mold. PDMS devices covalently bonded to a hydrofluoric acid-treated No. 1 glass coverslip using oxygen plasma activation. Device height was set at 25 μm to optimize cells-surface contact.

Numerical simulation

A computational fluid dynamic model was used to model the laminar flow within the device. A three-dimensional model of the geometry was designed and meshed using an extremely fine mesh of 5 μm tetrahedral elements. Finite element simulations were carried out using COMSOL Multiphysics 4.3b, coupling the Navier-Stokes equations with the convection and diffusion model for transport of diluted antibodies with a diffusion coefficient of $0.38 \times 10^{-10} \text{ m}^2/\text{s}$.²²

Streamline image velocimetry

Streamline image velocimetry (SIV) was carried out as previously described.²³ Briefly, microfluidic devices were incubated with 2% Pluronic F-68 solution for 1 h at 37 °C to prevent non-specific adhesion of fluorescent particles. Bangs Laboratories' 9.94 μm diameter green fluorescent microparticles (Fishers, IN) were suspended in 2% Pluronic F68 solution at a density of 2×10^6 particles/ml and introduced at flow rates of 4 and 6.25 $\mu\text{l}/\text{min}$. Images were taken using Zeiss LSM700 Microscope with 1.5 s exposure allowing particles to trace complete paths across the entire field of view. Multiple streakline images were converted to polynomial streamtube descriptors using MATLAB. Volumetric flow is constant in streamtubes for incompressible fluid allowing the derivation of relative fluid velocity profiles across the field of study. Using an analytically derived velocity field as a boundary condition, relative velocities were converted to absolute values.

FIG. 1. Microfluidic laminar co-patterning device. (a) The microfluidics design consists of 8 channel inlets $180\ \mu\text{m}$ in width and a single device outlet. The main chamber size is $1\ \text{mm} \times 1\ \text{mm}$ (supplementary CAD³¹). (b) Numerical simulation of the velocity field with superimposed streamlines demonstrates laminar flow behavior in the main chamber. (c) Numerical simulation of protein concentration field, when introduced in alternating channels. Protein lanes appear in the main chamber smudged by lateral diffusion along the perfusion path. (d) Cross-section of the flow field shows that $200\ \mu\text{m}$ from the entrance the distribution is undisturbed, over 90% of the pattern is retained even $800\ \mu\text{m}$ from the entrance (80% length). (e) Precision of laminar pattern $800\ \mu\text{m}$ from the entrance as a function of perfusion rate. Increased flow reduces residence time lowering the amount of time in which diffusion can occur. Error bars denote variances between streamlines in the device.

Streptavidin-based surface chemistry

Streptavidin-based chemistry was used to orient biotinylated antibodies on the device surface. Briefly, devices were sequentially perfused for 10 min in a flow rate of $50\ \mu\text{l/min}$ with 70% ethanol, deionized water, and phosphate buffered saline (PBS). Biotin-labeled albumin (A8549, Sigma-Aldrich) was perfused into the device at a concentration of $0.5\ \text{mg/ml}$ and incubated for 30 min at 37°C . The device was washed with PBS, perfused with $0.5\ \text{mg/ml}$ of streptavidin (85878, Sigma-Aldrich), and incubated for 30 min in 37°C . Excess streptavidin was removed by washing with PBS for 10 min at a flow rate of $50\ \mu\text{l/min}$. Surface passivation was carried out by a 1 h incubation with 10% Pluronic F-68 solution (P5556, Sigma-Aldrich), a non-ionic surfactant that prevent cells' attachment.²⁴ Homogenous distribution and stability of streptavidin on the surface was verified by perfusing $10\ \mu\text{g/ml}$ of Atto565-biotin (SC 319833, Santa Cruz Biotechnology) or fluorescein isothiocyanate (FITC)-biotin (B8889, Sigma-Aldrich) at a flow rate of $10\ \mu\text{l/min}$. Fluorescence was evaluated immediately following fluorophore binding and 72 h after storage at 4°C (Fig. 2(a)).

Micro-patterning of cell-capture antibodies under flow

Biotin-conjugated mouse anti-hCD24 (13-0247-80, eBioscience) and mouse anti-ASGPR1 (SC52623, Santa Cruz Biotechnology) were used to capture hepatocytes. Biotin-conjugated mouse anti-hCD202b/Tie2/Tek (334204, BioLegend) was used to capture endothelial cells. All three antibodies were used at a concentration of $10\ \mu\text{g/ml}$ and perfused into the microfluidic channels using a Fusion Touch 200 syringe pump (Chemyx) at a flow rate of $10\ \mu\text{l/min}$. The presence of the patterned $\alpha\text{-CD24}$ antibody on the surface was evaluated using PE-labeled goat anti-mouse IgG (R0480, DakoCytomation).

FIG. 2. Surface chemistry and antibody capture. (a) Rapid coating of albumin-biotin followed by streptavidin creates stable chemistry that persists for long periods of time. Homogenous distribution of streptavidin was evaluated using biotin-conjugated ATTO 565 fluorophores. Coating was stable even after 3 days of storage at 4 °C (b)–(d) Fluorescence images of biotin-conjugated FITC and ATTO 565 patterned using laminar flow on streptavidin coated device at 10 μ l/min flow rate. (e) and (f) Biotin-conjugated anti-CD24 antibody pattern on streptavidin-coated device at 10 μ l/min flow rate. Scale bars: a = 100 μ m; b–f = 330 μ m.

Flow cytometry

Flow cytometry was carried out on Becton Dickinson FACSAria III (Franklin Lakes, NJ). Cells were washed in PBS, dissociated using TrypLETM select (Invitrogen, Grand Island, NY), and centrifuged at 300 g for 5 min. Cell pellets were re-suspended in PBS containing 1% fetal bovine serum (FBS), filtered through a cell strainer to remove aggregates, and incubated with primary conjugated antibodies for 1 h on ice. Cells were then washed twice, suspended in PBS with 1% FBS, and analyzed. Live, single cells were gated by forward and side scatter emission. Unlabeled cells and isotype controls were used to set gates for negative populations. Data analysis was performed using TreeStar, Inc., FlowJo software (Ashland, OR).

Cell culture

All cells were cultured under standard conditions in a humidified incubator at 37 °C, under 5% CO₂. HepG2, Huh7, and 293T cells were obtained from the American Type Culture Collection (ATCC, USA). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS, 100 U/ml penicillin, and 100 μ g/ml streptomycin. Human neonatal microvascular endothelial cells (MVEC) were purchased from Vec Technologies (Rensselaer, NY) and grown on gelatin-coated tissue culture flasks in Endothelial Growth Medium EGM2-mv (Lonza, NJ). Cell diameter was evaluated microscopically in suspension to be 10, 13, and 18 μ m for MVEC, Huh7, and HepG2, respectively.

Calibration and optimization of cell capture from mixtures

Cell capture from mixtures was optimized in microfluidic linear stress devices (Fig. 4(a)) fabricated as previously described.²⁵ Non-specific cell binding was evaluated by perfusing mixtures of the target and non-related cells lines at equal concentrations and evaluating capture efficiency as a function of shear stress. Briefly, hepatocyte cell lines Huh7 and HepG2, primary MVEC, and off-target kidney cell line 293T were labeled using 5 μ M of either CellTrackerTM fluorescent probes Blue CMF₂HC (C12881) or Red CMPTX (C34552, Invitrogen Life Sciences) for 20 min at 37 °C. Cells were subsequently washed with PBS trypsinized, counted, mixed, and centrifuged at 300 g for 5 min at 4 °C. The dual-labeled mixed cell pellet was suspended with serum free DMEM to a combined suspension density of 1×10^6 cells/ml. Cell

mixtures were introduced to a linear stress device, which was precoated with specific antibodies for 30 min at a flow rate of 10 $\mu\text{l}/\text{min}$. The device was then washed with serum free DMEM for 10 min at a flow rate of 50 $\mu\text{l}/\text{min}$ to remove the unattached cells. Cell attachment was quantified by fluorescence using LSM700 confocal microscope (Zeiss, Germany). In each capture experiment, cell counts were obtained for five different shear stresses and represent cell counts averaged over 4 experimental repetitions. Cells were counted using the particle analysis module of Image J software 1.46r.

Co-patterning of hepatocytes and endothelial cells from mixtures

For co-patterning experiment, HepG2 and Huh7 cells were stably transfected with green fluorescent protein (GFP) under the control of the constitutive cytomegalovirus (CMV) promoter using the pGreenFireTM lentivirus system (System Bioscience, CA). The MVEC were similarly transfected with CMV-mCherry probe. Growing cells were trypsinized, counted, mixed, and centrifuged at 300 g for 5 min at 4 °C. The cell pellet was suspended with serum free DMEM to a suspension density of 1×10^6 cells/ml. MVEC were introduced to anti-Tie2 coated device at 4 $\mu\text{l}/\text{min}$, while Huh7 and HepG2 cells were introduced at 6.25 $\mu\text{l}/\text{min}$. Devices were subsequently washed with serum free DMEM for 10 min at a flow rate of 50 $\mu\text{l}/\text{min}$ to remove unattached cells.

Statistics

Unless otherwise noted, all experiments were carried out in experimental triplicates; data are shown as mean $\pm$ standard deviation; and statistical significance was determined by one-tailed Student's t-test with p-value of 0.05.

RESULTS

Microfluidic laminar co-patterning device

Laminar flow patterning was previously described in a simple capillary system with three inlets.¹⁸ As the technology is linearly extendible, we designed 1 mm² microfluidic chambers with 8 to 22 inlet channels (Fig. 1(a)). Device height was measured to be 26 ± 2 μm to maximize cell-surface interactions. For cell diameters ranging from 10 to 18 μm (*methods*), the sedimentation distance was about 500 μm , suggesting that cells are rolling on the glass surface well before shear-forces drop at the entrance to the main chamber, permitting cell capture.

Numerical simulations using COMSOL demonstrated linear streamline from 4 to 50 $\mu\text{l}/\text{min}$ (Fig. 1(b)). To evaluate the extent of lateral diffusion, we simulated the distribution of diluted antibodies with a diffusion coefficient of 5×10^{-7} cm²/s at 20 $\mu\text{l}/\text{min}$. Figures 1(c) and 1(d) show that concentrations retained 99% $\pm$ 1% stability of the initial concentration 200 μm , and 90% $\pm$ 4% stability 800 μm distance from the inlets, suggesting that the device is capable of patterning antibodies at high resolution as the short residence time limited cross-stream diffusion. Patterning precision increased with perfusion speeds ranging from 80% $\pm$ 5% at 4 $\mu\text{l}/\text{min}$ to 95% $\pm$ 2% at 50 $\mu\text{l}/\text{min}$ due to decreasing residence time limiting lateral diffusion (Fig. 1(e)).

Streptavidin-based surface chemistry permits antibody patterning in microfluidic devices

The non-covalent interaction of streptavidin with biotin is among the highest formation constants reported, about 10^3 – 10^6 times greater than for the interaction of ligands with their specific antibodies. This high affinity ensures that once formed, the complex is not disturbed by changes in pH or manipulations such as multiple washings.²⁶ Biotin-labeled albumin was homogeneously coated on the device surface (Fig. 2(a)), washed, and coated with 0.5 mg/ml of streptavidin that has four active binding sites. The streptavidin-coated devices could be dried and stored at 4 °C for extended periods of time. To evaluate the ability of the streptavidin-coated devices to pattern antibodies by laminar flow, we introduced biotin-conjugated FITC and

FIG. 3. Antibody specificity. Flow cytometry analysis showing the (a) selectivity of anti-CD24 antibody to Huh7; (b) selectivity of anti-ASGPR1 to HepG2; and (c) selectivity of anti-Tie2 antibody to MVEC.

FIG. 4. Shear effect on cell capture. (a) Geometry of linear shear stress microfluidic device that generates Hele-Shaw flow. (b) Linear shear stress profile developed in the device. (c) Capture of Huh7 and 293T cells on surfaces coated with anti-CD24 antibody as a function of shear stress. The dotted line shows that Huh7 comprise $97\% \pm 1\%$ of the captured cells at 0.8 Pa. (d) Capture of MVEC and HepG2 cells on device coated with anti-Tie2 antibody. MVEC comprise $86\% \pm 1\%$ of the captured cells at 0.5 Pa. (e) HepG2 and MVEC attachment on device coated with anti-ASGPR1 antibody. HepG2 comprise $80\% \pm 1\%$ of the captured cells at 0.8 Pa. Error bars denote standard errors for each point based on four repetitions.

ATTO 565 fluorophores in alternating inlets in flow rates ranging from 2 to 20 $\mu\text{l}/\text{min}$. While increasing flow rates enable higher precision due to low diffusion, decreasing residence time results in low fluorophores concentrations. In our hands, the optimal perfusion rate was 10 $\mu\text{l}/\text{min}$ and optimal antibody concentration was 10 $\mu\text{g}/\text{ml}$ (Figs. 2(b)–2(d), supplementary Fig. 3³¹). To validate our ability to pattern antibodies, we introduced biotin-conjugated anti-CD24 antibody and evaluated binding by probing the chamber with secondary anti-mouse antibody labeled with PE (Figs. 2(e) and 2(f)). The pattern was both selective and specific. The average width of antibody-patterned streamlines was $189 \pm 12 \mu\text{m}$. The variance between different devices was $\pm 3\%$.

Optimization of antibody-based cell capture in microfluidic devices

One of the basic requirements to capture specific cell types from complex mixtures is antibody selectivity. To characterize our cell population, we carried out flow cytometry analysis on the four cells types. Huh7 cells showed poor expression of hepatocyte specific marker ASGPR1 (data not shown) but robust expression of epithelial specific CD24²⁷ (Fig. 3(a)). HepG2 cells showed strong expression of ASGPR1 (Fig. 3(b)). Finally, MVECs showed strong expression of endothelial specific Tie2 (Fig. 3(c)). While the antibodies show little to no cross-reactivity with off-target populations, non-specific interactions with the device surface could lead to arbitrary cell adhesion. These weak non-specific interactions decrease with increasing fluid shear stress. However, specific binding can similarly decrease with increasing drag force, requiring optimization.

To optimize cell capture interactions, we introduced cell mixtures into a microfluidic linear stress device (Fig. 4(a)) fabricated as previously described.²⁵ The shear stress decreases linearly along the length of this device according to: $\tau = \frac{6\mu Q}{h^2 w} \left(1 - \frac{z}{L}\right)$, where τ is the shear stress, z is the axial position, Q is the volumetric flow rate, μ the fluid viscosity, h the chamber height, w the inlet width, and L the chamber length (Fig. 4(b)). This method permits the analysis of cell adhesion over a range of shear stress under identical experimental conditions. We show the isolation of Huh7 cells with a purity of $97\% \pm 1\%$ on anti-CD24 surface at a shear stress of 0.8 Pa, where non-specific binding of 293T cells becomes minimal (Fig. 4(c)). Isolation of MVECs from HepG2 mixtures using anti-Tie2 surface shows a purity of $86\% \pm 1\%$ at 0.5 Pa (Fig. 4(d)). Finally, isolation of HepG2 cells from MVECs using anti-ASGPR1 surface shows purity of $80\% \pm 1\%$ at 0.8 Pa (Fig. 4(e)). These results indicate the need for optimizing antibody selectivity as well as applied shear stress for a selective cell trapping under flow.

Co-patterning of hepatocytes and endothelial cells from mixtures

Following the antibody and shear optimization steps described above, we sought to co-pattern multiple cell types under flow using antibody-based cell capture. The shear stress in our co-patterning device (Fig. 1) was validated experimentally²³ (supplementary Fig. 2³¹). The microfluidic device was then perfused with biotin-conjugated antibodies against CD24, ASGPR1, or Tie2 in alternating inlets (Fig. 5(a)). mCherry-labeled MVECs perfused at 0.5 Pa were selectively captured in lanes coated with anti-Tie2 antibodies (Fig. 5(b), supplementary Fig. 4³¹). The average width of MVEC cell pattern on αTie2 -coated device was $167 \pm 18 \mu\text{m}$. The variance between different devices was $\pm 4\%$. GFP-labeled HepG2 and Huh7 cells were similarly captured in lanes coated with anti-ASGPR1 and anti-CD24 antibodies, respectively (Figs. 5(c) and 5(d), supplementary Fig. 4³¹), under a shear stress of 0.8 Pa. The average widths of HepG2 and Huh7 cell patterns were $174 \pm 14 \mu\text{m}$ and $186 \pm 32 \mu\text{m}$, respectively. Variance between different devices was $\pm 10\%$ and $\pm 6\%$ for HepG2 and Huh7 cells, respectively.

Finally, to demonstrate co-patterning of hepatocytes and endothelial cells, we perfused our microfluidic device with alternating pattern of biotin-conjugated antibodies against CD24 and Tie2 (Fig. 5(e)). As MVEC showed non-specific binding at 0.5 Pa (Fig. 4(e)), the mixture was introduced at a shear stress of 0.8 Pa, which is optimal for Huh7 cells (Fig. 4(c)), producing a co-pattern of both cell types under flow (Fig. 5(f)). Under these conditions, Huh7 cells show advantageous binding over MVEC producing wider patterns.

FIG. 5. Hepatocyte and endothelial cell patterning. (a) Schematic diagram of antibody pattern used to capture and pattern cells under flow. (b) Capture and patterning of MVEC on anti-Tie2 patterned device at a shear stress of 0.5 Pa. (c) Capture and patterning of HepG2 on anti-ASGPR1 coated device at a shear stress of 0.8 Pa. (d) Capture and patterning of Huh7 on anti-CD24 coated device at a shear stress of 0.8 Pa. (e) Schematic diagram of antibody pattern used to capture and co-pattern hepatocytes and endothelial cells under flow. (f) Capture and co-patterning of Huh7 and MVEC on an anti-CD24/Tie2 patterned device at a shear stress of 0.8 Pa. Under these conditions, Huh7 shows preferential binding. Scale bars = 100 μ m.

Patterning of cell islands in microfluidic devices

Previous work demonstrated the importance of controlling homotypic interactions, showing a critical island size for primary hepatocytes of about 500 μ m diameter.⁹ To explore such isolated islands under flow, we extended our design to 5×5 channels supporting cross exposure of patterned antibodies to cells in the orthogonal direction (Figs. 6(a)–6(d)). Fibronectin was patterned in alternating channels on one direction, and FITC-labeled mouse anti-fibronectin antibody was introduced in the orthogonal direction to mark precise islands (Fig. 6(e)). Similarly, Huh7 cells were introduced at 0.8 Pa in the orthogonal direction creating cellular island in a sealed microfluidic device under flow (Fig. 6(f)). Devices could be linearly extended (supplementary Fig. 5³¹).

DISCUSSION

In this work, we present an antibody-based, microfluidic system capable of patterning any biotin-conjugated set of antibodies using streptavidin-based surface chemistry. The design permits a one-step cell isolation and patterning in microfluidic devices permitting the generation of tissue micro-patterns from complex cell mixtures with minimal sample preparation. Our approach can be broadly employed to combine cell isolation with micro-patterning. The significance of such a combination is the ability to directly inject a complex starting sample such as differentiated stem cells or tissue digest into a patterning device with minimal sample preparation, resulting in a functional, perfusable tissue on a chip. One interesting application of such system lies in the differentiation of pluripotent stem cells. Human embryonic or induced pluripotent stem cells (hPSC) can be differentiated into mature cell types, but the process often results in complex cell mixtures in which target cell population is seldom more than 40% to 60%.^{28,29} For example, hepatocytes often co-differentiate with endothelial cells allowing similar systems to rapidly create tissue-like organization from a single experiment.^{5,14,30}

FIG. 6. Cross-patterning in microfluidic devices. (a) Image of microfluidic device consisting of a 5×5 array of inlets and outlets enabling the creation of a 2×2 matrix of patterned cells. (b) and (c) Numerical simulation of the velocity and concentration fields in the main chamber at $5 \mu\text{l}/\text{min}$ flow rate. Results show that perpendicular inlet and outlet ports do not disturb the laminar streams. (d) Flow inside the device was visualized using color dyes. Results show clear separation between each perfused color. (e) Fibronectin, an extracellular matrix protein, was lined-patterned in one direction and Huh7 were perfused orthogonally, creating a 2×2 matrix of cellular islands. Scale bars: (e) *left* = $500 \mu\text{m}$; *Right* = $100 \mu\text{m}$.

Our work explores some of the design principles governing laminar flow patterning. We note that the novelty of our work lie within the combination of cell capture and cell patterning in a single step. While microfluidic cell capture and patterning are established in their own rights, here, they are brought together in an enabling manner, as this is the first single-step micro-patterning of cells under flow.

We show that increasing flow rate decreases lateral diffusion due to lower residence time (Fig. 1). Increasing flow rate also decreases non-specific binding of off-target cells (Fig. 4). However, high shear rates also limit interactions between target cells and the surface, limiting antibody binding (Fig. 2), as well as cellular capture (Fig. 4). In our hands, shear rates of 0.5 to 0.8 Pa corresponding to 5 to $8 \mu\text{l}/\text{min}$ produced the best results. It is clear that similar optimization should be carried out for each application. It is worth noting that primary hepatocytes are sensitive to shear forces and lose function when exposed to shear stress above 1 Pa.¹ The cell lines used in our studies are not as sensitive, but similar design considerations hold.

CONCLUSIONS

In summary, our work demonstrates a microfluidic, streptavidin-based system capable of patterning any biotin-conjugated set of antibodies under perfusion. The system allows the capture and co-patterning of arbitrary cell types, exemplified by the one step patterns of hepatocytes and endothelial cells under flow.

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¹Y. Nahmias, F. Berthiaume, and M. L. Yarmush, *Adv. Biochem. Eng. Biotechnol.* **103**, 309–329 (2007).

²M. Shulman and Y. Nahmias, *Methods Mol. Biol.* **945**, 287–302 (2013).

- ³S. R. Khetani and S. N. Bhatia, *Nat. Biotechnol.* **26**(1), 120–126 (2008).
- ⁴Y. Nahmias and D. J. Odde, *Nat. Protoc.* **1**(5), 2288–2296 (2006).
- ⁵Y. Nahmias, R. E. Schwartz, W. S. Hu, C. M. Verfaillie, and D. J. Odde, *Tissue Eng.* **12**(6), 1627–1638 (2006).
- ⁶A. Folch and M. Toner, *Annu. Rev. Biomed. Eng.* **2**, 227–256 (2000).
- ⁷C. A. Goubko and X. Cao, *Mater. Sci. Eng. C* **29**(6), 1855–1868 (2009).
- ⁸J. C. Culver, J. C. Hoffmann, R. A. Poche, J. H. Slater, J. L. West, and M. E. Dickinson, *Adv. Mater.* **24**(17), 2344–2348 (2012).
- ⁹C. M. Nelson, M. M. Vanduijn, J. L. Inman, D. A. Fletcher, and M. J. Bissell, *Science* **314**(5797), 298–300 (2006).
- ¹⁰T. F. Didar, A. M. Foudeh, and M. Tabrizian, *Anal. Chem.* **84**(2), 1012–1018 (2012).
- ¹¹D. R. Albrecht, G. H. Underhill, T. B. Wassermann, R. L. Sah, and S. N. Bhatia, *Nat. Methods* **3**(5), 369–375 (2006).
- ¹²V. L. Tsang, A. A. Chen, L. M. Cho, K. D. Jadin, R. L. Sah, S. DeLong, J. L. West, and S. N. Bhatia, *FASEB J.* **21**(3), 790–801 (2007).
- ¹³A. M. Kloxin, A. M. Kasko, C. N. Salinas, and K. S. Anseth, *Science* **324**(5923), 59–63 (2009).
- ¹⁴K. R. Stevens, M. D. Ungrin, R. E. Schwartz, S. Ng, B. Carvalho, K. S. Christine, R. R. Chaturvedi, C. Y. Li, P. W. Zandstra, C. S. Chen, and S. N. Bhatia, *Nat. Commun.* **4**, 1847 (2013).
- ¹⁵K. Jakab, C. Norotte, F. Marga, K. Murphy, G. Vunjak-Novakovic, and G. Forgacs, *Biofabrication* **2**(2), 022001 (2010).
- ¹⁶V. Mironov, R. P. Visconti, V. Kasyanov, G. Forgacs, C. J. Drake, and R. R. Markwald, *Biomaterials* **30**(12), 2164–2174 (2009).
- ¹⁷S. Takayama, J. C. McDonald, E. Ostuni, M. N. Liang, P. J. Kenis, R. F. Ismagilov, and G. M. Whitesides, *Proc. Natl. Acad. Sci. U.S.A.* **96**(10), 5545–5548 (1999).
- ¹⁸P. J. Kenis, R. F. Ismagilov, S. Takayama, G. M. Whitesides, S. Li, and H. S. White, *Acc. Chem. Res.* **33**(12), 841–847 (2000).
- ¹⁹X. Luo, D. Mitra, R. J. Sullivan, B. S. Wittner, A. M. Kimura, S. Pan, M. P. Hoang, B. W. Brannigan, D. P. Lawrence, K. T. Flaherty, L. V. Sequist, M. McMahon, M. W. Bosenberg, S. L. Stott, D. T. Ting, S. Ramaswamy, M. Toner, D. E. Fisher, S. Maheswaran, and D. A. Haber, *Cell Rep.* **7**(3), 645–653 (2014).
- ²⁰B. Zhu, Y. Nahmias, M. L. Yarmush, and S. K. Murthy, *Stem Cells Transl. Med.* **3**(11), 1354–1362 (2014).
- ²¹T. Thorsen, S. J. Maerkl, and S. R. Quake, *Science* **298**(5593), 580–584 (2002).
- ²²S. Koutsopoulos, L. D. Unsworth, Y. Nagai, and S. Zhang, *Proc. Natl. Acad. Sci. U.S.A.* **106**(12), 4623–4628 (2009).
- ²³E. Keinan, E. Ezra, and Y. Nahmias, *Appl. Phys. Lett.* **103**(6), 63507 (2013).
- ²⁴Q. Cheng, K. Komvopoulos, and S. Li, *J. Biomed. Mater. Res. A* **96**(3), 507–512 (2011).
- ²⁵S. K. Murthy, A. Sin, R. G. Tompkins, and M. Toner, *Langmuir* **20**(26), 11649–11655 (2004).
- ²⁶E. P. Diamandis and T. K. Christopoulos, *Clin. Chem.* **37**(5), 625–636 (1991).
- ²⁷E. Coulstock, J. Sosabowski, M. Ovecka, R. Prince, L. Goodall, C. Mudd, A. Sepp, M. Davies, J. Foster, J. Burnet, G. Dunlevy, and A. Walker, *PLoS One* **8**(2), e57263 (2013).
- ²⁸R. E. Schwartz, K. Trehan, L. Andrus, T. P. Sheahan, A. Ploss, S. A. Duncan, C. M. Rice, and S. N. Bhatia, *Proc. Natl. Acad. Sci. U.S.A.* **109**(7), 2544–2548 (2012).
- ²⁹K. Si-Tayeb, F. K. Noto, M. Nagaoka, J. Li, M. A. Battle, C. Duris, P. E. North, S. Dalton, and S. A. Duncan, *Hepatology* **51**(1), 297–305 (2010).
- ³⁰R. Gaebel, N. Ma, J. Liu, J. Guan, L. Koch, C. Klopsch, M. Gruene, A. Toelk, W. Wang, P. Mark, F. Wang, B. Chichkov, W. Li, and G. Steinhoff, *Biomaterials* **32**(35), 9218–9230 (2011).
- ³¹See supplementary material at <http://dx.doi.org/10.1063/1.4944741> for CAD design, optical device characterization, experimental validation of numerical results, optimization of antibody concentration, whole device capture characterization, and cross-patterning of 11 × 11 microdevice.