

Electronic Supplementary Information

Materials

Ethanol (200 proof), glass slides (35 x 65 mm, no. 1), microcentrifuge tubes, Trypan Blue solution, and fetal bovine serum (FBS) were purchased from Fisher Scientific (Fair Lawn, NJ). 3-Mercaptopropyl trimethoxysilane was obtained from Gelest Inc. (Morrisville, PA) and the coupling agent GMBS (N-γ-maleimidobutyryloxy succinimide ester) was obtained from Pierce Biotechnology (Rockford, IL). SU-8-50 photoresist and developer were obtained from MicroChem (Newton, MA); silicone elastomer and curing agent were obtained from Dow Corning (Midland, MI). Phosphate buffered saline (PBS; 1x, without calcium or magnesium), and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Mediatech (Herndon, VA). Penicillin-streptomycin (PS) and 0.25% trypsin/ethylene diaminetetraacetic acid (EDTA) were obtained from Hyclone (Logan, UT). The peptide val-ala-pro-gly (VAPG) was purchased from Sigma-Aldrich (St. Louis, MO); arg-glu-asp-val (REDV) and arg-gly-asp-ser (RGDS) were obtained from American Peptide Company (Sunnyvale, CA). Blue [7-amino-4-chloromethylcoumarin, CMAC], green [5-chloromethylfluorescein diacetate, CMFDA], and orange [5-(and 6)-(((4-chloromethyl)-benzoyl)-amino)-tetramethyl-rhodamine, CMTMR] cell tracker dyes were also purchased from Molecular Probes. The adipose-derived stem cells (ADSCs) along with mesenchymal stem cell growth media (MSCBM) and supplements were purchased from Lonza (Basel, Switzerland). The A7r5 rat aortic SMC line was purchased from American Type Culture Collections (Manassas, VA). 3T3-J2 mouse embryonic FBs and H5V mouse cardiac ECs were kindly provided by Dr. Yaakov Nahmias at the Massachusetts General Hospital and Dr. George Coukos at the University of Pennsylvania, respectively.

Microfluidic device design and fabrication

The design and fabrication of the microfluidic devices followed previously described soft lithography techniques.^{1,2} The REDV- and VAPG- coated devices had a width of 1 mm each while the RGDS device was 1.7 mm in width. The height of all device types was $63 \pm 2 \mu\text{m}$. Each device had a single inlet and single outlet. The device layout was drawn using AutoCAD software and printed with high resolution on a transparency (FineLine Imaging, Colorado Springs, CO). The resulting photomask was used to generate a negative master for device fabrication at the George J. Kostas Nanoscale Technology and Manufacturing Research Center at Northeastern University. Silicon wafers were coated with SU-8-50 photoresist to a thickness of approximately $60 \mu\text{m}$ and then exposed to ultraviolet light (365 nm , 11 mW/cm^2) with the mask overlaid using a Quintel 2001 mask aligner. Unexposed photoresist was removed using SU-8 developer and feature height was measured using a Dektak surface profilometer (Veeco Instruments, Santa Barbara, CA).

To generate PDMS replicas, a mixture of silicone elastomer and curing agent (10:1 ratio) was poured over the master wafers, degassed, and allowed to cure overnight in an oven at 65°C . The cured PDMS was then cut using a scalpel and pulled off the wafers. Inlet and outlet holes were punched on each PDMS replica prior to bonding. The bonding process consisted of exposing the PDMS replicas and the glass slides to an oxygen plasma (100 mW with 8% oxygen for 30 sec.) in a PX-250 plasma chamber (March Instruments, Concord, MA), immediately bringing the PDMS replicas and the glass slides in contact, and then baking the device for 5 min at 65°C . This process creates an irreversible bond between the PDMS replicas and the glass slides and also prepares both surfaces for chemical surface modification.

Peptide attachment

Surface modification was performed on each device by using solutions of silane, GMBS, and peptides; the solutions were prepared as follows. A 4% (v/v) solution of 3-mercaptopropyl trimethoxysilane (a moisture sensitive compound) in ethanol was prepared under a nitrogen atmosphere. The coupling molecule, GMBS, was stored as a stock solution containing 50 mg GMBS in 0.5 mL DMSO; this stock solution was diluted with ethanol to create a 0.28% (v/v GMBS stock/ethanol) solution. Peptide solutions in PBS were prepared with concentrations of 0.1 mg/mL. Surface functionalization of the microfluidic devices was carried out in three steps. In the first step, the devices were flushed with silane solution and allowed to react at room temperature for 30 minutes. Unreacted silane was removed by flushing with ethanol. The GMBS solution in ethanol was then introduced into the devices and allowed to react for 15 min. The devices were flushed with ethanol to remove unreacted GMBS and then with PBS to remove the ethanol. The peptide solution was then flowed through the devices and allowed to react for 30 min before flushing with PBS. The devices were then directly used in experiments or stored at 4 °C. The same peptide attachment protocol was employed for all device types.

Cell culture

H5V ECs, A7r5 SMCs, and 3T3 FBs were cultured in DMEM supplemented with 4.5 g/L glucose and L-glutamine, 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. ADSCs were cultured in MSCBM (500mL bottle) supplemented with 50mL of MCGS (Mesenchymal stem cell supplement), 10mL of L-glutamine, and 0.5mL GA-1000. The cells were incubated in 75 cm² tissue culture flasks at 37 °C in a humidified atmosphere with 5% CO₂ and 95% air. Cells were grown to pre-confluence and isolated for experiments by

trypsinization using a 0.25% Trypsin-EDTA solution. For all experiments, cell suspensions were centrifuged at $190 \times g$ and then re suspended in PBS to the desired concentration (measured using a hemacytometer). For surface area optimization experiments and ADSC recovery flow experiments, 1×10^6 cells/mL solutions of ECs, SMCs and FBs were prepared separately and a 0.8×10^6 cells/mL solution of ADSCs was prepared. The solutions of ECs, FBs and ADSCs were incubated with cell tracker dyes. ECs, FBs and ADSCs were stained blue, green, and red, respectively. SMCs were not stained. For this step, the cell suspension drawn from the culture flask was centrifuged at $190 \times g$ for 5 min, resuspended in 15 mL of serum-free DMEM containing 5 μ M of cell tracker dye and incubated for 30 min at 37 °C. Centrifugation and resuspension in PBS were subsequently performed as described above. The suspensions of ECs, SMCs and FBs were then combined together such that the concentration of each of the three cell types was 0.33×10^6 cells/mL, giving a total cell concentration of 1×10^6 cells/mL. 10,000 ADSCs total were then spiked into the cell suspension of ECs, SMCs and FBs and this suspension was then loaded into a syringe.

Hele-Shaw flow experiments

Hele-Shaw experiments were run with a cell suspension of 1×10^5 ADSCs/mL flowed through Hele-Shaw devices at a flow rate of 118 μ L/min for a period of 5 min using a Harvard Apparatus PHD 2000 syringe pump (Holliston, MA). Cell adhesion within the devices was measured using a field finder (with 1 mm x 1 mm grids) placed under the microfluidic chamber. Adhered cells were manually counted at selected points along the device axis under a Nikon Eclipse TE2000 inverted microscope. Cell counts were taken between 3 and 35 mm from the device inlet, along the axis. All flow experiments were performed at room temperature.

Surface area optimization and ADSC recovery flow experiments

ADSC recovery experiments were performed with a total cell concentration of 1×10^6 cells/mL and flow rate of 7.54 $\mu\text{L}/\text{min}$. Three homogeneous suspensions of ECs, SMCs and FBs, each cell type labeled with a cell tracker dye of a particular color, were prepared in PBS to a cell concentration of 1×10^6 cells/mL. The three suspensions were then combined such that the concentration of each of the three cell types was 0.33×10^6 cells/mL. 0.6 mL of this suspension was collected in a microcentrifuge tube and a small volume ($\sim 20 \mu\text{L}$) containing approximately 10,000 ADSCs that were also labeled with a cell tracker dye was added. The resulting heterogeneous suspension was utilized for surface area optimization experiments as well as the three-stage experiments. The flow of cells into the devices was controlled by a syringe pump mounted such that the orientation of the syringe was vertical.³ To ensure 100% of the cell suspension flowed through the syringe and no cell loss occurred due to cell settling within the syringe, a steel ball bearing was placed inside the syringe and moved up and down by a motorized external magnet throughout the course of the flow.⁴ The depletion of each cell type in each device was determined by measuring cell concentrations from the device outputs using a hemacytometer in conjunction with fluorescence microscopy (to discern the different cell types labeled with cell tracker dyes) and comparing with the number of cells flowed into the device. (It should be noted that the inherent error in hemacytometer measurements is around 10%.⁵) The mixed cell suspension concentrations were measured using fluorescence microscopy (at 10x magnification) using an Nikon TE2000 inverted microscope using fluorescein ($480 \pm 30 \text{ nm}/535 \pm 40 \text{ nm}$), rhodamine ($540 \pm 25 \text{ nm}/605 \pm 50 \text{ nm}$), and DAPI ($360 \pm 40 \text{ nm}/460 \pm 50 \text{ nm}$) excitation/emission to count ECs, SMCs, FBs, and ADSCs. All fluorescence images were

collected using the Nikon microscope and all experiments were performed at room temperature and replicated five times.

Statistics and Data Analysis

The cell adhesion measurements reported represent average values over 5 repetitions, and the error represents the standard errors of the mean (standard deviation/ $\sqrt{n}$, where $n = 5$). The cell adhesion values and standard errors were rounded to the nearest integer in these tables to ensure that the data make physical sense.

References:

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3. B. D. Plouffe, M. Radisic and S. K. Murthy, *Lab Chip*, 2008, **8**, 462-472.
4. R. Cooper and L. Lee, *Lab Chip - Chips & Tips*, 2007, published online.
5. H. M. Shapiro, *Practical Flow Cytometry*, John Wiley & Sons, New York, 1995.

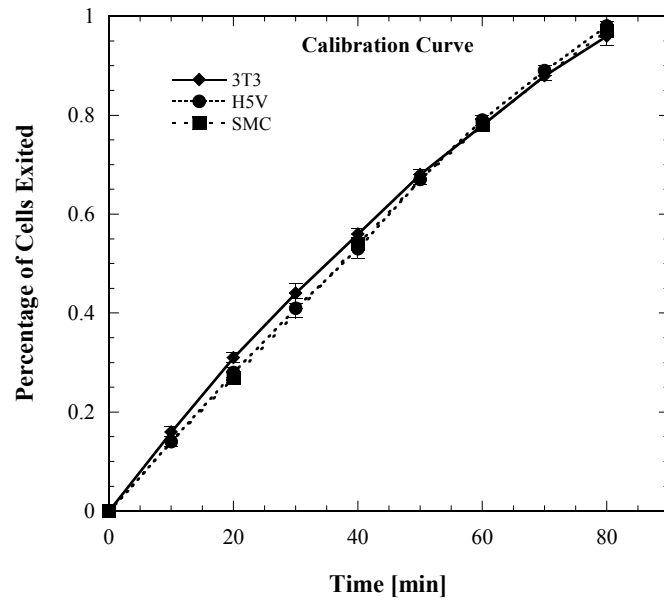


Fig S1: Calibration experiment comparing endothelial cell, smooth muscle cell, and fibroblast cells dispensed by the syringe as a function of time.