Live-cell imaging of invasion and intravasation in an artificial microvessel platform

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SUPPLEMENTARY VIDEO LEGENDS

S1: Supplementary Video 1.mp4

Title: Phase-contrast movie of a HUVEC microvessel under shear stress (12-15 dyne cm^{-2}).

Legend: A confluent HUVEC endothelium in focus on the bottom of the vessel. The time series comprises 83 frames captured at 10 min intervals resulting in an approximately 14 h time-lapse video.

S2: Supplementary Video 2.mp4

Title: MDA-MB-231 breast cancer cells moving within a proteolytically degraded ECM track next to an HMVEC vessel under shear stress (12-15 dyne cm^{-2}).

Legend: The breast cancer cells (BCCs) express RFP (red) in their cytoplasm. Due to population heterogeneity, only one of the BCCs expresses GFP (green) in its nucleus. A proteolytically degraded ECM track is visible under phase-contrast. A BCC protrusion is temporarily inserted into the ECM/vessel interface. The time series comprises 77 frames captured at 12 min intervals resulting in a 15 h time-lapse video.

S3: Supplementary Video 3.mp4

Title: Multiple MDA-MB-231 breast cancer cells moving within a predefined matrix tunnel next to an HMVEC vessel under shear stress (12-15 dyne cm^{-2}).

Legend: The breast cancer cells (BCCs) express RFP (red) in their cytoplasm and GFP (green) in their nuclei. A predefined ECM track, about 300 μm in length and visible under phase-contrast, directs the cells towards the vessel wall. Approximately 7 cells are migrating at high speeds and directedness due to confinement within the predefined ECM track. The cells change polarity after encountering the terminal ends of the ECM track, including the vessel wall, and often crawl over one another to persist in their direction of migration. A single BCC is visibly intravasating into the interior of the vessel. The time series comprises 77 frames captured at 12 min intervals resulting in a 15 h time-lapse video.

S4: Supplementary Video 4.mp4

Title: A single MDA-MB-231 breast cancer cell invading through the ECM towards an HMVEC vessel under shear stress (12-15 dyne cm^{-2}).

Legend: The single breast cancer cell (BCC) expresses RFP (red) in its cytoplasm and GFP (green) in its nucleus. The BCC invades the ECM at an angle towards the vessel. After it is bifurcated by an obstruction in the ECM, it visibly extends protrusions into the ECM/vessel interface. The time series comprises 77 frames captured at 12 min intervals resulting in a 15 h time-lapse video.

S5: Supplementary Video 5.mp4

Title: A single MDA-MB-231 breast cancer cell invading and intravasating into an HMVEC vessel under shear stress (12-15 dyne cm^{-2}).

Legend: The HMVEC vessel is labeled green through uptake of BSA conjugated to Alexa Fluor 488 (green) from permeability measurements. The single breast cancer cell (BCC), located about 50 μm away from the vessel, expresses RFP (red) in its cytoplasm and GFP (green) in its nucleus. On day 3, the BCC retracts one of two protrusions from the ECM/vessel interface. On day 4, the BCC is able to invade into the ECM/vessel interface; the BCC exhibits 2 nuclei within its cell body. On day 5, the BCC contracts, rounds up, and is carried away by flow. The event is magnified and displayed at a slower frame rate. The time series comprises 333 frames captured at 10 min intervals resulting in a 55 h time-lapse video.

S6: Supplementary Video 6.mp4

Title: A single MDA-MB-231 breast cancer cell intravasating into an HMVEC vessel under shear stress (12-15 dyne cm^{-2}).

Legend: The single breast cancer cell migrates along the side of the HMVEC vessel visible under phase-contrast. The HMVEC endothelium is visibly disrupted after intravasation. The time series comprises 123 frames captured at 10 min intervals resulting in a 20 h time-lapse video.

S7: Supplementary Video 7.mp4

Title: An HMVEC vessel under shear stress (12-15 dyne cm^{-2}) extending an angiogenic sprout towards a cluster of MDA-MB-231 breast cancer cells.

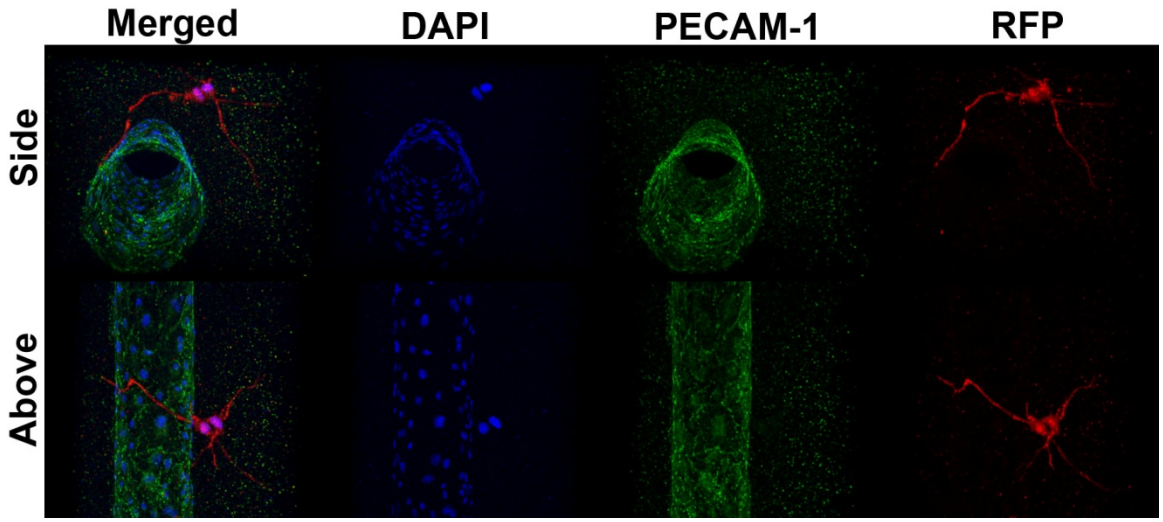
Legend: The breast cancer cells express GFP (green) in their nuclei. The time series comprises 83 frames captured at 10 min intervals resulting in an approximately 14 h time-lapse video.

S8: Supplementary Video 8.mp4

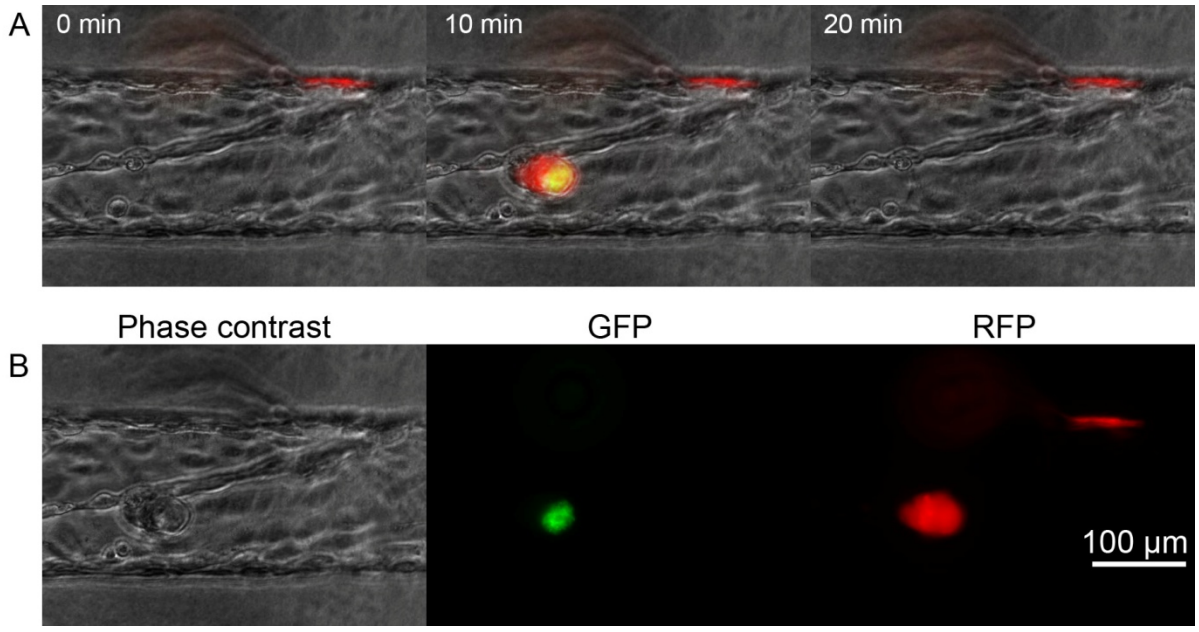
Title: Angiogenic sprouts and neighboring HMVECs oscillating in position relative to a cluster of MDA-MB-231 breast cancer cells.

Legend: The MDA-MB-231 breast cancer cells (BCCs) express GFP (green) in their nuclei. The activated cell's left (blue arrow) and right (green arrow) tips fluctuate in length in the direction of the cluster of BCCs. The left and right neighbors of the activated endothelial cell are traced to obtain their centroid positions. The time series comprises 83 frames captured at 10 min intervals resulting in an approximately 14 h time-lapse video.

SUPPLEMENTARY FIGURES



Supplementary Figure S1. 3D projection from above and side of a confocal z-stack showing a PECAM-1 (green) stained HUVEC channel with 2 separate RFP expressing HT-1080 fibrosarcoma cells (red) extending protrusions near the vessel. Nuclei are stained with DAPI (blue). Fluorescence channels are merged and displayed separately.



Supplementary Figure S2. A, Time lapse images of an intravasating tumor cell (same cell depicted in Figure 3D) temporarily adhering to a downstream portion of the HMVEC vessel before complete removal by shear stress. Flow within the vessel is from left to right. B, Separate phase and fluorescence images reveal a single nucleus within the intravasating tumor. The nucleus exhibits discrete GFP focal points that suggest the cell is condensing its chromatin during mitosis.

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Fabricating a perfusable cylindrical ECM scaffold

A custom nozzle machined out of PEEK tubing of 1.6 mm in diameter and 1 cm in length was inserted in series within the PDMS channels (Fig. 1C). The taper of the nozzle was made through uniform sanding with an electric drill on a belt sander and the concaved receiving end was bored with a centering tool on a lathe. The PDMS housing was additionally modified to include inlet and outlet reservoirs by hole-punching and press fitting Teflon tubing preceding the nozzle and at the end of the to-be-formed collagen channel 1.5 cm downstream of the nozzle. Bubbles in the gel were removed by placing the device on an ice block typically for 2-4 h. After bubble removal, template rods were slowly pulled out, leaving behind a cylindrical channel. Media placed in the Teflon outlet reservoir would ensured that the channel would fill with liquid as the rod was removed. Once flow was confirmed, the device was connected to a recirculating perfusion setup to condition the gel (Fig. 1A, D) in the incubator overnight with 10 ml of endothelial growth media. Endothelial cells were introduced by pipetting 20 μl of cell suspension at a concentration of 10 million cells per ml into the inlet Teflon reservoir after disconnecting the device from the flow setup. Slight differences in liquid heights between the inlet and outlet reservoirs would ensure low flow permitting cells to settle and adhere to the surface of the channel. Endothelial cells would actively adhere to the surface of conditioned gels, and devices were tilted on their sides and upside-down to encourage adhesion of the cells on all surfaces of the channels. Channels typically yielded 50,000 cells per cm^2 of coverage after seeding and would be confluent within 24 h.

Vessel perfusion and flow control

Vessels were perfused using a custom gravity flow setup (Fig. 1A). Hydrostatic pressure on the device was maintained by keeping the lower reservoir at 10 cm of height. Flow was induced by elevating the liquid level of the inlet reservoir 5 cm of higher than that of the lower reservoir. Precise flow rates were achieved by automatically recirculating a small volume, typically 250 μl, from the lower reservoir to the upper reservoir at a set interval. Height differences between the

lower and upper reservoirs would equilibrate to the necessary heights in order to match the time-average flow rate within the channel to that of the pump. We used a programmable single syringe pump NE-1000 (New Era Pump Systems, Inc., Farmingdale, NY) to withdraw fluid from the lower reservoir and inject it into the upper reservoir. A 3-way solenoid pinch valve (Bio-Chem Fluidics, Boonton, NJ) enabled electronically controlled switching of the syringe pump's connection between the two reservoirs. The syringe pump and solenoid valve were controlled by a programmable microcontroller (Arduino, Ivrea, Italy) and serially interfaced with a laptop to change pump frequencies as needed.

Immunofluorescence staining

All solutions for fixing, washing, and staining were perfused through the vessels with low flow. Samples were fixed with 4% formaldehyde in PBS for 10 min. After washing with 10 ml of PBS, samples were blocked with 5% donkey serum (Life Technologies) in PBS for 30 min. Primary anti-human antibodies against PECAM-1 (mouse mAb WM-59) (Sigma-Aldrich, St. Louis, MO) and thrombospondin-1 (rabbit pAb ab85762) (Abcam, Cambridge, MA) were diluted to 1:200 in blocking solution and incubated with the sample for 1 hr at room temperature by manually recirculating a 200 µl volume through each channel. After washing with 10 ml of PBS, anti-rabbit and anti-mouse secondary antibodies (Life Technologies) were similarly used at 1:200 and recirculated through the channel for 1 hr. Channels were washed again and incubated with DAPI (Life Technologies) diluted 1:2500 in PBS for 30 min. Following another wash step, samples were either left in the microfluidic device or removed and submerged in PBS for imaging.

Scanning Electron Microscopy (SEM)

Samples were prepared by cross-linking with 4% formaldehyde in PBS for 1 h. After three 10 min washes in PBS, samples were further fixed with 4% osmium tetroxide for 1 h. Following three 10 min washes with ddH_2O, samples were serially dehydrated in 25%, 50%, 75%, 100% ethanol. Dehydrated samples underwent critical point drying. SEM images of the collagen ECM were obtained by sputter coating a thin layer of platinum on fixed and dried samples. Images are

obtained using a high-resolution field emission SEM (JEOL 6700F) in high vacuum at 15kV accelerating voltage to obtain secondary electron images.

Calibration and detection limits of vessel permeability measurements

Fluorescence intensity was calibrated to ensure a linear relation between camera pixel intensities and concentration of BSA that extended 2 orders of magnitude above and below the experimentally used concentration. 2 min intervals for image capturing resulted in less than 1% reduction in fluorescence intensities due to photobleaching of background and solute over a 30 min imaging period; this limited our detection to permeability values above approximately 1.0 x 10^{-7} cm s^{-1}. The lag time in introducing an approximately constant concentration of fluorescent BSA into the vessels was typically 6 min, which limited our detection to permeability values below 5 x 10^{-5} cm s^{-1}.