**Supplementary information**

Device Design

The geometry of the microdevice channels were designed in autoCAD and modeled to simulate the microvasculature of the human body. In order to emulate physiologic conditions, the smallest channel dimensions (30 x 30 um) and the ratio of total channel surface area to total daughter channel surface area (1:1.4) were set to approximate the anatomy of postcapillary venules in humans (Fung, Biomechanics: Circulation, 1997). Although the channels have square cross-sections, the lining of endothelial cells on the channels' inner walls round out the sharp edges and thus resulting in more circular, and therefore more physiologic, cross-sections.

Device Fabrication

A combination of standard photolithographic and soft lithographic techniques were utilized to fabricate the device. The autoCAD design was transferred onto a mask via laster photoplotting (CAD/Art Services, Inc.) which in turn was patterned onto 6" silicon wafers using SU-8 photoresist (MicroChem, Inc.) and subsequent UV exposure, post-baking, and developing steps per the manufacturer's specifications. The patterned wafers were then treated with (tridecafluoro-1,1,2,2-tetrahydrooctyl) trichlorosilane (Gelest) to prevent permanent adhesion of the device material, polydimethylsiloxane (PDMS). PDMS, mixed at a 10:1 ratio (w/w) of polymer to curing agent, was then poured onto the silicon wafers and cured at 60° C for about 24 h. The cured PDMS device was then excised and holes for the inlet, outlet, and bypass were formed using a 1.5 mm hole puncher. The complementing bottom portion of the device was created by pouring a thin layer of PDMS (approximately 1 mm in thickness) onto a standard disposable petri dish, curing the PDMS under the same conditions as above, and cutting out a rectangular piece large enough to encompass the surface area of the top portion of the device.

Once the top and bottom portions of the device were removed from their respective molds, transparent tape was used to remove any foreign particles from their surfaces. Both were then placed in a beaker filled with 100% ethanol and sonicated for 10-15 minutes. After rinsing the surfaces thoroughly with DI water, the two pieces were placed carefully, with bonding sides facing up, onto a clean petri dish, then air dried at 60° C in the oven.

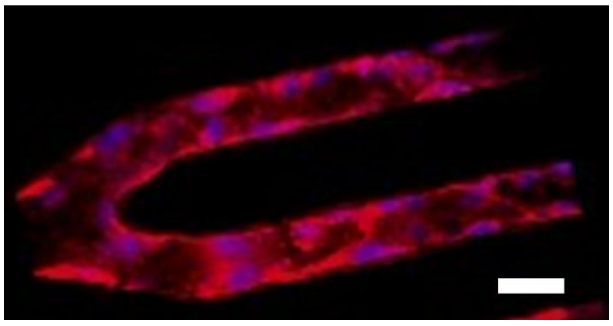
In the final step, after the PDMS was cleaned and dried, the top and bottom potions were bonded together using 15 second exposure to oxygen plasma using an oxygen plasma cleaner (Plasmod).

Cell Seeding and Perfusion

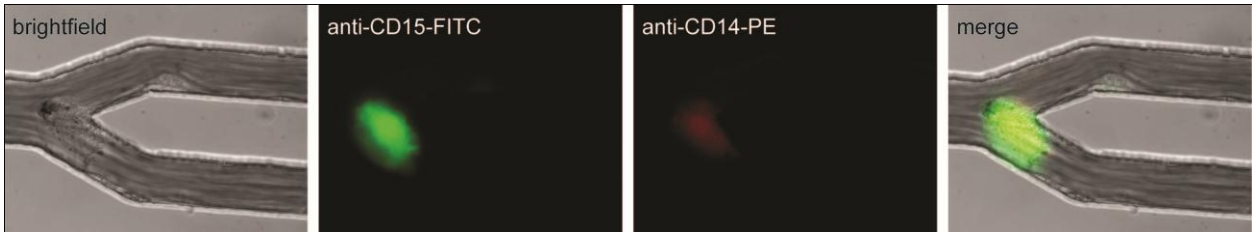
The microchannels were then coated with 50 µg/ml fibronectin from human plasma (Sigma) using a syringe and PTFE tubing (0.012"ID x 0.030"OD, Cole Parmer) inserted at the inlet and outlet. The device was incubated at 37°C, 5% CO2 for 45 min-1h then rinsed with phosphate buffered saline (PBS) to remove any excess fibronectin.

Cells were prepared at a concentration of 500,000 cells/mL in Endothelial Growth Medium-2 (EGM-2, Lonza) for HUVECs or EGM-2MV (Lonza) for microvascular endothelial cells with 8% mass/vol of dextran 500 (Sigma), which increases the viscosity of the cell suspension, a crucial step for successful seeding of the endothelial cells. The cells were then manually pre-loaded into the inlet tubing and then infused into the channels with a syringe pump at 1.25 uL/min for 2 hours, after which the syringe was replaced with a new syringe filled with pure EGM-2 (or EGM-2MV) and perfused through the channels at the same rate for 2-5 days.

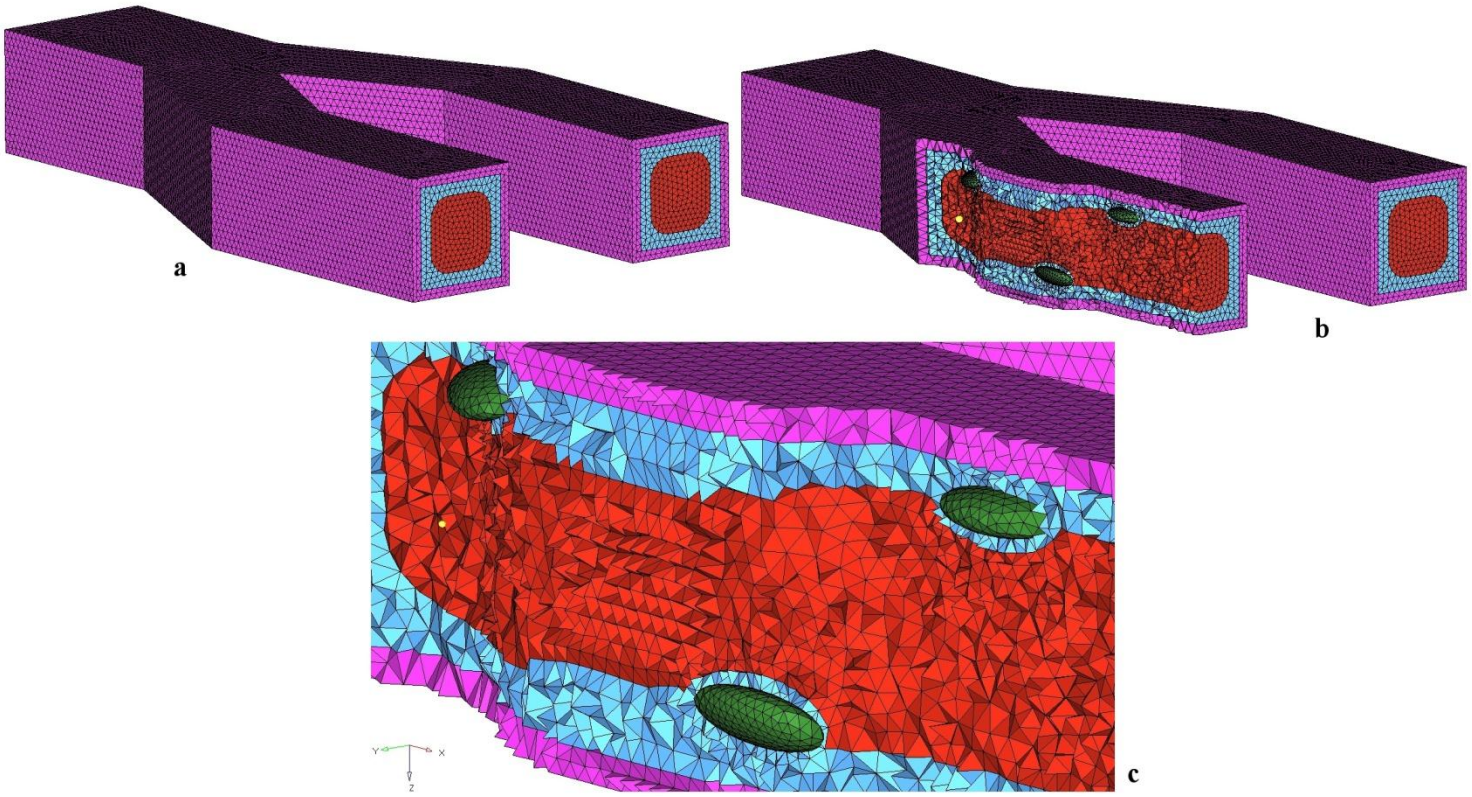
**Supplementary Figures**



Supplementary Figure 1. Human lung microvascular endothelial cells (HLMVECs) are successfully cultured to confluency and line the entire inner 3D surface of the microfluidic device. Using fluorescent cell membrane (red) and cell nuclear (blue) dyes, 3-D renderings of z-series acquired by a spinning disc confocal microscope showing that the endothelial cells line the entire inner surface of the microfluidic channels in 3-D. Scale bar = 30 µm.




Supplementary Figure 2. Leukocyte subsets involved in STX2-mediated thrombi. Flowing whole blood containing anti-CD15-FITC (green), which stains for neutrophils, and anti-CD14-PE (red), which stains for monocytes, over STX-activated HUVECs in our microdevice revealed that both types of leukocytes were involved with thrombi formation, with more neutrophils present than monocytes.




Supplementary Figure 3. 3D computational mesh for fluid dynamic simulations. red = blood, green = nuclei, blue = cytoplasm, purple = PDMS channel. **a)** Full channel geometry. **b)** Cutaway showing cytoplasmic and nuclear layers. **c)** Close-up view showing incorporation of nuclei in cytoplasmic layer. 3D CFD simulations were run on the blood domain using a 2x increase in mesh density for accuracy; 2D FSI simulations were completed on a representative slice through the 30 μm channel section.

**Supplementary Movies**

1. Confocal cell membrane cell nuclei dye movie 1
2. Confocal cell membrane cell nuclei dye movie 2

Supplementary Movies 1 and 2. Human lung endothelial cells are successfully cultured to confluency and line the entire inner 3D surface of the microfluidic device. Using fluorescent cell membrane (red) and cell nuclear (blue) dyes, 3-D reconstructions of z-series acquired by a spinning disc confocal microscope are rotated for a full 3-D view. These movies demonstrate that the endothelial cells line the entire inner surface of the microfluidic channels in 3-D.

3. Normal whole blood – brightfield
4. Normal whole blood – epifluorescence (rhodamine)

Supplementary Movies 3 and 4. Brightfield (Supplementary Movie 3) and epifluorescence (Supplementary Movie 4) movies reveal that. Using rhodamine-6G (R6G), a fluorescent dye that preferentially stains leukocytes and platelets, revealed that when whole blood is flowed into the microdevice cultured with HLMVECs at postcapillary venular flow conditions, flow is steady overall with occasional rolling but few adherent leukocytes.

5. TNF-α activated HUVEC + normal whole blood – epifluorescence (rhodamine)
6. TNF-α activated HUVEC + normal whole blood – brightfield

Supplementary Movies 5 and 6. TNF-α activated endothelial cells with non-activated whole blood. When endothelial cells in the microsystem were pre-activated with TNF-$\alpha$ before whole blood was flowed in, epifluorescence videomicroscopy using R6G staining (Supplementary Movie 5) and brightfield videomicroscopy (Supplementary Movie 6) revealed a slight decrease in overall flow velocity with occasional microchannel obstructions as well as an increase in adherent leukocytes

7. TNF-α-activated HUVEC + TNF-α-activated whole blood – epifluorescence (rhodamine)
8. TNF-α-activated HUVEC + TNF-α-activated whole blood – brightfield

Supplementary Movies 7 and 8. TNF-α activated endothelial cells with TNF-α-activated whole blood. Epifluorescence videomicroscopy using R6G staining (Supplementary Movie 7) and brightfield videomicroscopy (Supplementary Movie 8) revealed that TNF-$\alpha$ activation of both the endothelial cells and whole blood led to a dramatic increase in microchannel obstruction with subsequent decrease in overall flow, likely due to a combination of increased adhesion and cell stiffness.

9. sickle whole blood on hydroxyurea – brightfield
10. sickle whole blood not on hydroxyurea – brightfield

Supplementary Movies 9 and 10. Brightfield videomicroscopy of sickle cell whole blood. Whole blood from a sickle patient receiving hydroxyurea (Supplementary Movie 9) and from a sickle patient not receiving hydroxyurea (Supplementary Movie 10) both exhibited flow that was less steady and slower than whole blood from a healthy control. However, over time (~20 minutes in these cases), blood from patients not receiving the drug revealed a slower velocity and lead to more microchannel obstruction than blood from patients receiving the drug.

11. STX2-activated HUVEC + whole blood – low shear – brightfield
12. STX2-activated HUVEC + whole blood – low shear – epifluorescence (rhodamine)

Supplementary Movies 11 and 12. STX2-activated HUVEC + whole at low shear (1-4 dyne/cm$^2$). Brightfield videomicroscopy (Supplementary Movie 11) and epifluorescence videomicroscopy using R6G staining (Supplementary Movie 12) revealed that at low shear (wall shear stress: 1-4 dyne/cm$^2$), thrombi consisting of leukocytes and platelets formed within and occluded the endothelialized microchannels cultured with STX2-activated endothelial cells.

13. STX2-activated HUVEC + whole blood – high shear – brightfield
14. STX2-activated HUVEC + whole blood – high shear – epifluorescence (rhodamine)

Supplementary Movies 13 and 14. STX2-activated HUVEC + whole at high shear (10-40 dyne/cm$^2$). Brightfield videomicroscopy (Supplementary Movie 13) and epifluorescence videomicroscopy using R6G staining (Supplementary Movie 14) revealed that increasing shear (wall shear stress: 10-40 dyne/cm$^2$), also increased thrombi size, the percentage of obstructed microchannels, and the rate of obstruction.

15. HUVEC + whole blood with FITC-beads

Supplementary Movie 15. Calibration of pump settings using whole blood with fluorescent beads. During the control conditions of each experiment, whole blood mixed with 0.5 μm fluorescent beads was flowed into the device and tracked with epifluorescence videomicroscopy. Pump settings were adjusted so that beads flowing in the centerline of the smallest microchannels were consistently measured to maintain centerline velocities appropriate for the physiologic condition. Here, whole blood with fluorescent beads is flowing into four 30 μm endothelialized microchannels.