Supplementary Information

Injury-On-A-Chip for Modelling Microvascular Trauma-Induced Coagulation

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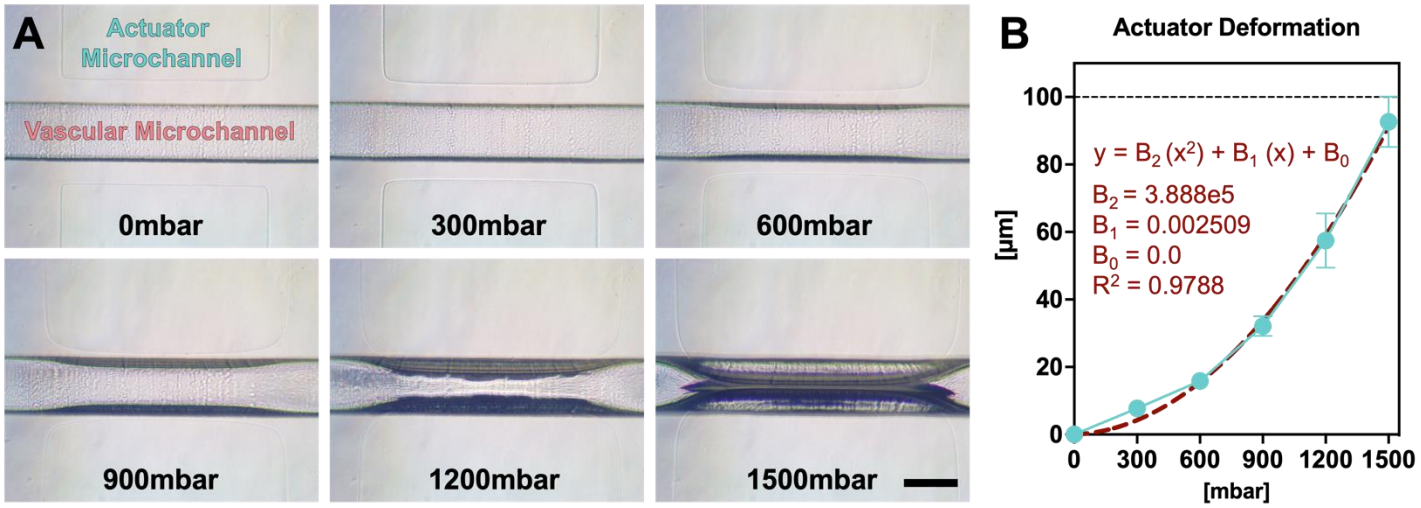

Figure S1. **A**) Top-down brightfield micrographs depicting compression of cell-free vascular microchannels with lateral actuator microchannels (Scale: 200 µm). Actuator microchannels have been primed with 1:1 glycerol-water mix. Compression induces an apparent wrinkling in vascular microchannel ceilings. **B**) A second order polynomial curve fit (dashed line) was constrained to the origin of actuator deformation data.

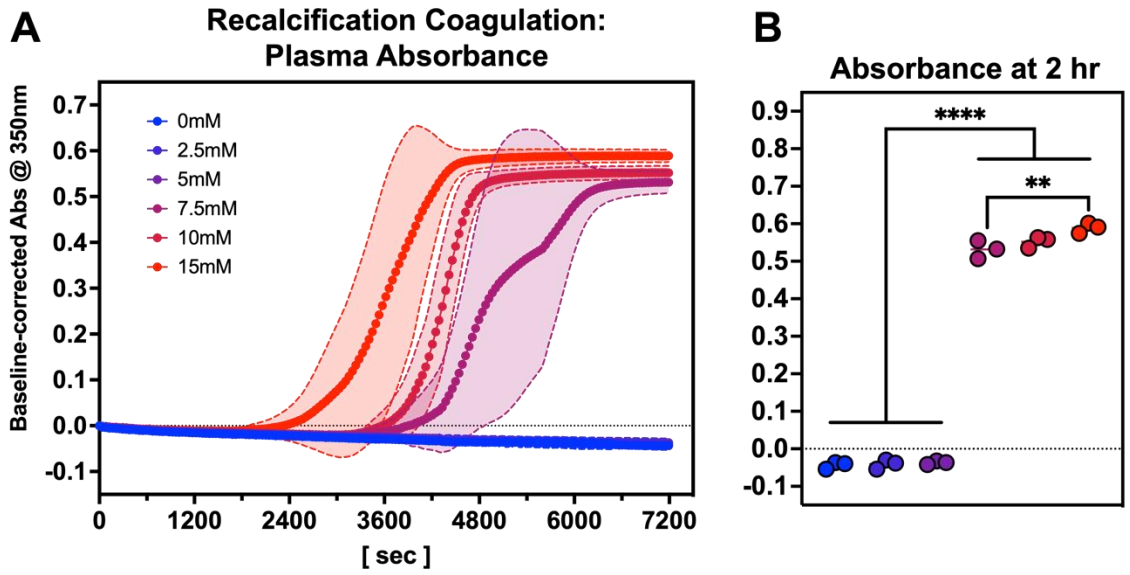

Figure S2. Recalcification of citrated plasma induces coagulation under static conditions. **A**) Baseline-corrected light absorbance with a microplate reader demonstrates that mixture of citrated plasma with various concentrations of $CaCl_2$ induces coagulation, seen as an increase in plasma light absorbance. **B**) Sufficient $CaCl_2$ concentration– above 0.025 M– is required to induce coagulation. Greater $CaCl_2$ concentration induces more rapid initiation of coagulation and a more densely associated clot as higher maximum absorbance. (One-way ANOVA; ****p<0.0001, **p=0.004).

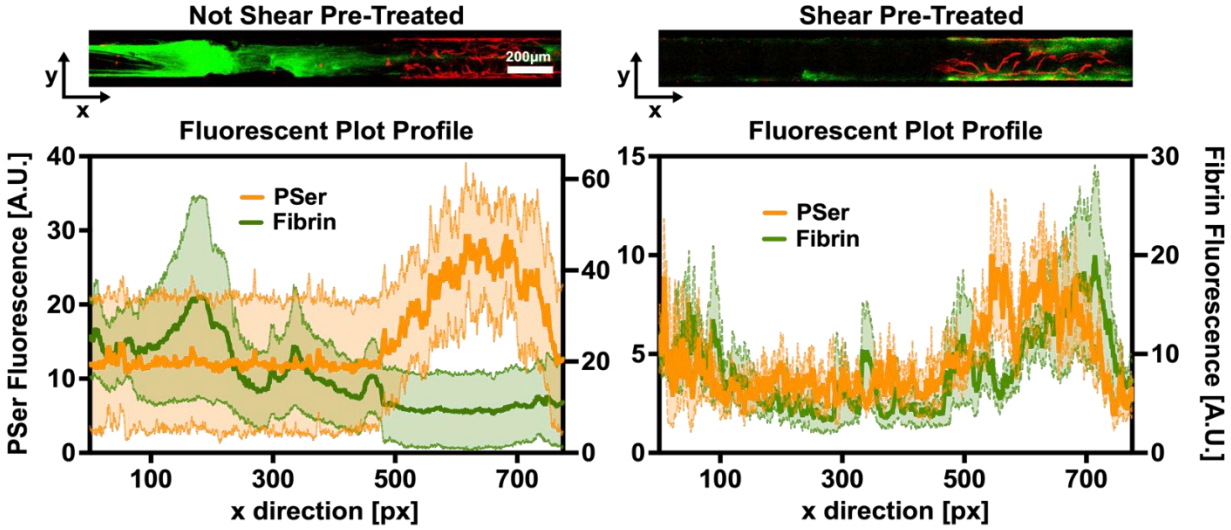

Figure S3. Example confocal micrographs and fluorescent plot profiles of (n = 4–5) PINCH vascular microchannels that did (right) or did not (left) receive extended fluid shear pre-treatment before injury and plasma perfusion. Plasma perfusion was from left to right, in the positive x direction. These graphs clearly depict a greater tendency of coagulation to occur upstream of injury sites in vascular microchannels that did not receive shear pre-treatment.

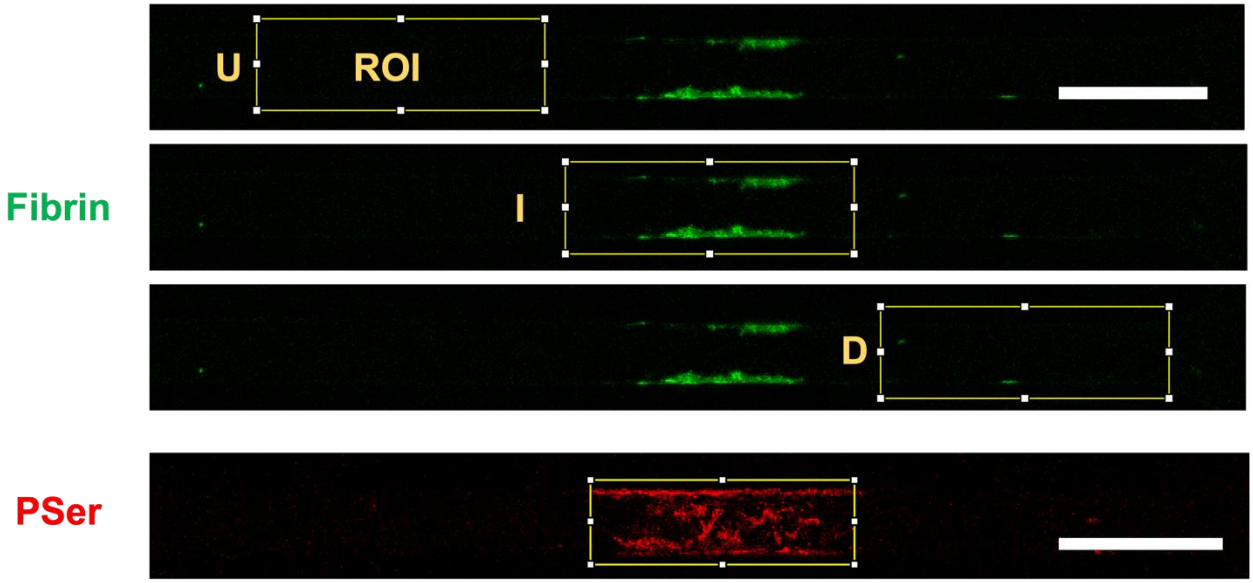

Figure S4. Schematic depicting quantification of coagulation site specificity. ROI are placed at, upstream, or downstream of injury sites. Fluorescent intensity is measured for each region before plotting. Within ImageJ (FIJI), image channels can be separated and ROI pasted into identical locations such that phosphatidylserine (PSer) and fibrin are quantified at identical locations for a given device-location combination. U, I, and D are 'upstream,' 'injury,' and 'downstream.' Injury is defined by the presence of a large amount of PSer staining with annexin V. (Scale: 500 μm).

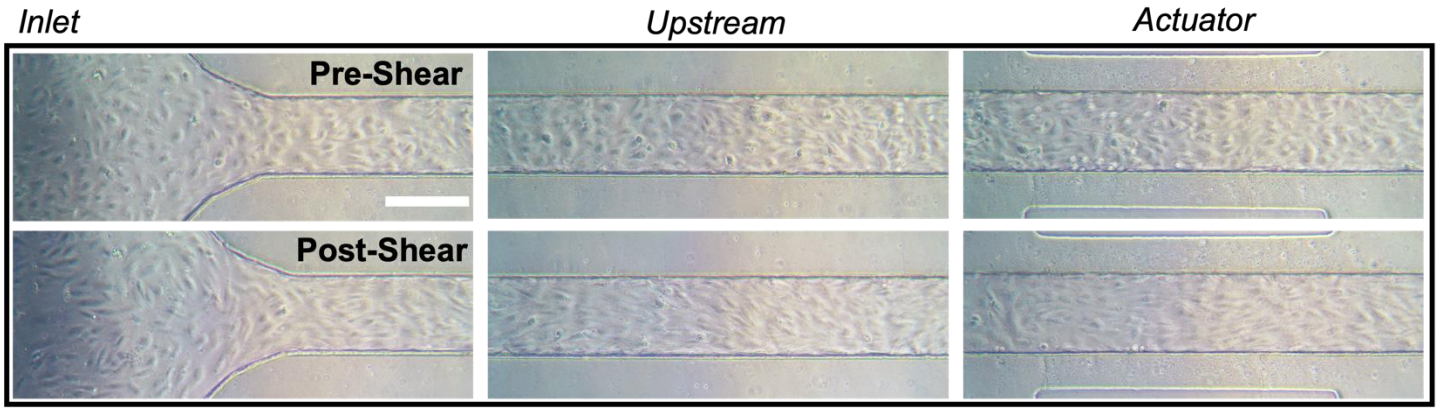

Figure S5. Example phase contrast micrographs of HUVEC-lined microchannels before and after application of 10 dyne/cm^2 of fluid shear stress. Reduction in unattached cells and enhanced alignment is visible. (Scale: 200 μm).

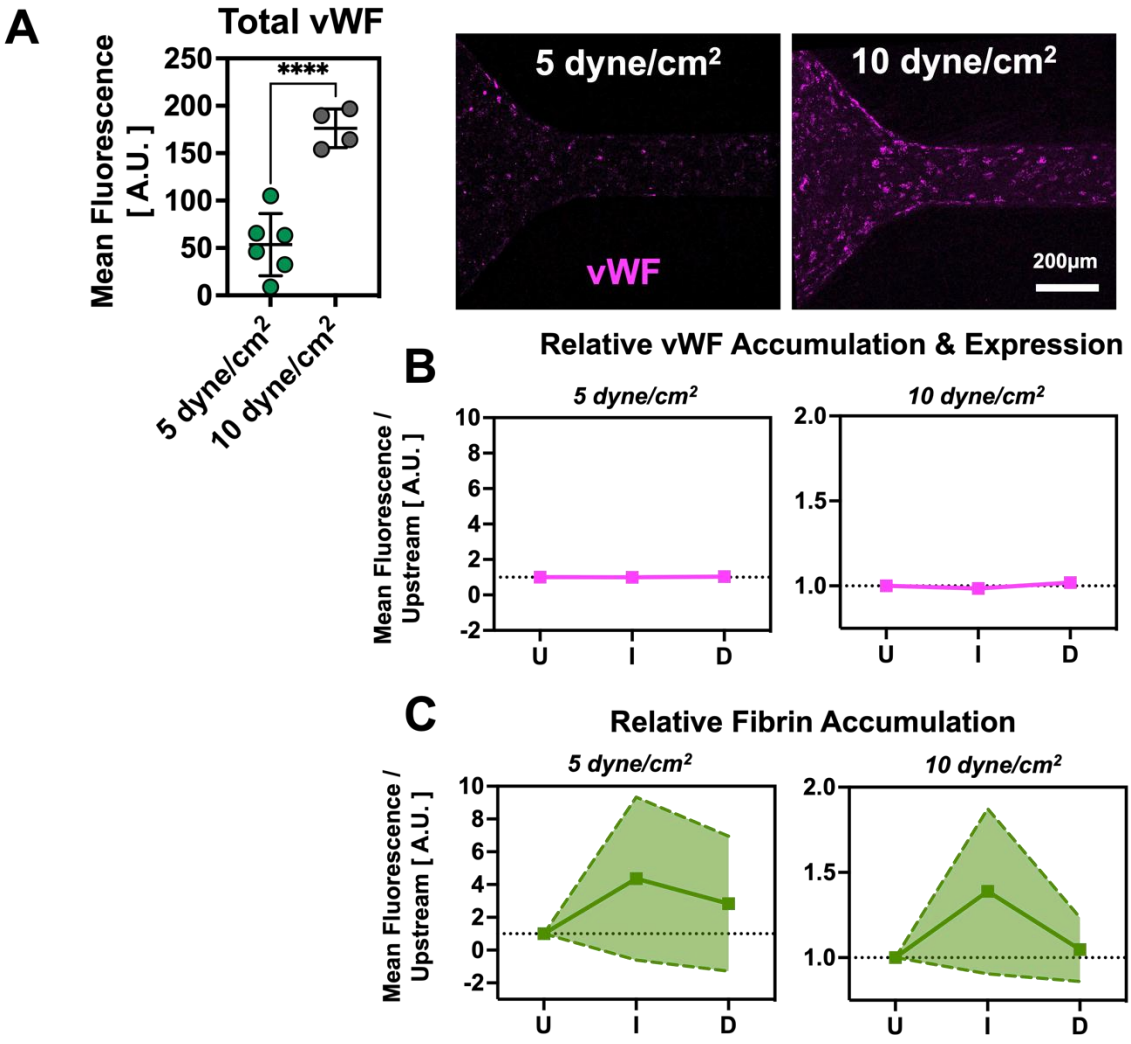

Figure S6. vWF expression and accumulation & fibrin accumulation in shear pre-treated vascular channels. **A**) HUVEC were immunostained for vWF (Alexa Fluor 647 mouse IgG; magenta) after injury and plasma perfusion at ~2.5 dyne/cm^2. The total mean fluorescence along the length of the vascular microchannels treated with 10 dyne/cm^2 was higher than the channels pre-treated with 5 dyne/cm^2 (Student's t-test with Welch's correction: ****p<0.0001, *n* = 4-6). **B**) Despite the higher mean vWF production, no change was detected according to injury. Mean vWF fluorescence levels were normalized to the upstream value. **C**) Normalization of mean fibrin fluorescence depicts a more pronounced injury response than vWF.

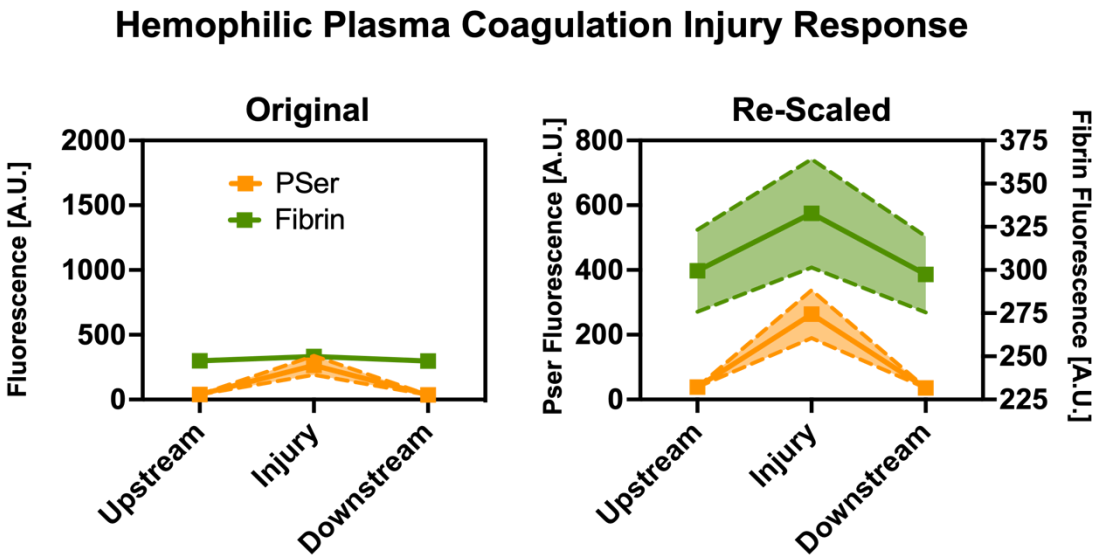

Figure S7. An injury response of hemophilic plasma (n = 4). Re-scaling the coagulation location charts reveals that hemophilic plasma elicits an injury response, despite its overall limited coagulation level.

Video 1. Microvideo of actuation in an empty PINCH device. As pressure is applied to the actuator channel, PDMS walls deform towards and compress the horizontal channel.

Video 2. Video of actuation of an empty PINCH device. Deformation of the actuator channel is visible without a microscope.