SUPPORTING INFORMATION FOR

Propagation of Blood Clotting in the Complex Biochemical Network of Hemostasis is Described by a Simple Mechanism

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MATERIALS

All solvents and salts used in buffers were purchased from commercial sources and used as received unless otherwise stated. Poly(dimethylsiloxane) (PDMS, Sylgard Brand 184 Silicone Elastomer Kit) was purchased from Dow Corning. 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC), L-α-phosphatidylserine from porcine brain (PS), and L-α-phosphatidylcholine (Egg PC) were purchased from Avanti Polar Lipids. Texas Red® 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine (Texas Red® DHPE), rhodamine B 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (rhodamine DHPE), and FluoSpheres (sulfate microspheres, 1.0 μm, yellow-green fluorescent (505/515), 2% solids) were purchased from Molecular Probes/Invitrogen. Normal pooled plasma (human) (NPP) was purchased from George King Bio-Medical, Inc. *t*-butyloxycarbonyl-β-benzyl-L-aspartyl-L-prolyl-L-arginine-4-methyl-coumaryl-7-amide (Boc-Asp(OBzl)-Pro-Arg-MCA) and *t*-butyloxycarbonyl-Leu-Ser-Thr-Arg-4-methylcoumaryl-7-amide (Boc-Leu-Ser-Thr-Arg-MCA) were purchased from Peptides International. Albumin from bovine serum (BSA) was purchased from Sigma. Human recombinant tissue factor (TF), human thrombin, and corn trypsin inhibitor (CTI) were purchased from Calbiochem. Anhydrous methyl sulfoxide (DMSO, 99.7 % purity) was purchased from Fisher Scientific. Norland Optical Adhesive 81 was purchased from Norland Products Inc.

METHODS

Data acquisition. Images were acquired using a Leica DMI 6000B epi-fluorescence microscope with a 10x 0.3 NA or 5.0x 0.15 NA objective coupled to a cooled CCD camera ORCA ERG 1394 (12-bit, 1344x1024 resolution) (Hamamatsu Photonics, K.K.) with a 1.0x coupler. Lighting was provided by a 75W Xe light source. DAPI/Hoechst/AMCA (λ_{ex}=320-400 nm, λ_{em}=435-495) (chroma #31000v2) was used to detect MCA, and Texas Red (λ_{ex}=530-590 nm, λ_{em}=600-680) (chroma #41004) was used to detect the Texas Red DHPE lipid dye. Bright field microscopy (illumination from halogen lamp) was also used to detect the formation of fibrin during clotting. MetaMorph® Imaging System (Universal Imaging Corp) was used to collect images. Images were processed using MetaMorph® Imaging System, MATLAB®, and Adobe Photoshop.

Data analysis. The original grayscale fluorescence images of clotting and the phospholipid coated surfaces were false colored in MetaMorph®. The color was set by the emission wavelength of the filter cube. For all fluorescence images, the levels of intensity were adjusted to the same value. These images were copied and pasted directly from MetaMorph® into a new Adobe Photoshop document set

to RGB mode. All transformations were applied uniformly to every image, and all images were processed in an identical fashion.

Preparing human blood plasma samples. Human citrated normal pooled plasma (NPP) was purchased from George King Bio-Medical, Inc. and was stored in 1 mL aliquots at -80ºC until needed. When needed, the plasma was thawed by incubating at 18ºC. A stock solution of corn trypsin inhibitor (CTI, 6.27 mg mL^{-1}) was added to the blood plasma immediately after the plasma was thawed and incubated for approximately 1 hr at 18ºC prior to each experiment.

Fabricating microfluidic devices. All devices were fabricated using rapid prototyping in PDMS[46].The devices were sealed using a Plasma Prep II (SPI Supplies) and baked overnight at 110 ºC. The devices were then placed into a solution of NaCl (150 mM) and kept under vacuum to completely saturate the PDMS and to remove all air from the channels. Devices remained soaked in this NaCl solution throughout the experiment to aid in coating the surfaces with lipids, and to reduce convective flow during experiments in the absence of flow.

Flowing solutions into the microfluidic devices. Solutions where flowed into the microfluidic devices using previously described methods.[47] Flow rates were controlled using syringe pumps (Harvard Apparatus PHD 2000 Infusion Pumps). Syringes were connected to the microfluidic devices using 30 gauge Teflon tubing. For all solutions except blood plasma, the Teflon tubing (Weico Wire & Cable) was connected to 27 gauge needles on the syringes (Hamilton 1700 series RN). For blood plasma, a 30 gauge Teflon needle (Hamilton) was connected directly to the syringe (Hamilton 1700 series TLL). All syringes and Teflon tubing used to flow in blood plasma were blocked with BSA prior to filling with blood plasma.

Coating the surfaces of microfluidic devices with phospholipids and reconstituted Tissue Factor. Microfluidic channels were coated with phospholipids by flowing in two streams of lipid vesicles. The vesicles used to activate clotting (lipid-TF) consisted of DLPC/PS/Texas Red® DHPE (79.5/ 20/ 0.5 mole percents) with reconstituted tissue factor (Lipid:Tissue Factor mole ratio = $2x10^5$) at a concentration of 1.25 mg mL^{-1}, and the inert vesicles (inert lipids) consisted of EggPC at a concentration of 1.25 mg mL^{-1} (for the detailed procedure for making vesicles and reconstituting tissue factor see ref. 2 in the main text). To coat the channels with lipids, vesicles were flowed into the device for 15 min. The lipid-TF vesicles were flowed in at 0.5 µL min^{-1}, and the inert lipids were flowed in at 1.0 µL min^{-1}. Excess vesicles were removed from the channel by flowing in a solution of NaCl or TRIS buffer (20 mM TRIS-HCl, 100 mM NaCl, pH 7.5) (150 mM) for 15 min at 1.0 µL min^{-1}.

Experimental protocol for clot propagation through a junction in the presence of flow

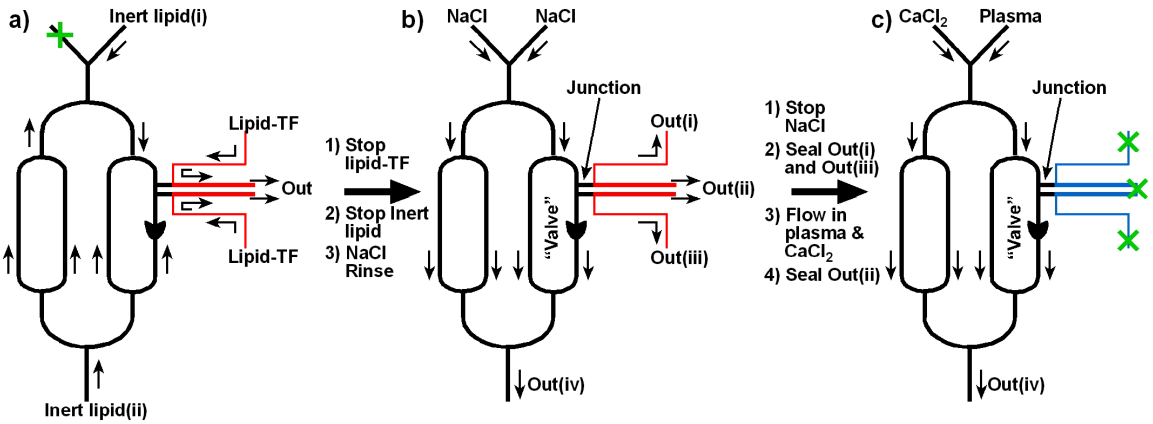

Figure S1. Schematic of the experimental procedure for monitoring clot propagation through a junction in the presence of flow. (a) Two types of phospholipid vesicles (lipid-TF and inert lipid) were flowed into a PDMS device that was soaked in a solution of NaCl (150 mM). Each lipid-TF stream (red) was flowed at 0.5 μL min^{-1}, and each inert lipid stream (black) was flowed at 2.0 μL min^{-1} for 15 min. To ensure that lipid-TF did not flow through the junction, the lipid vesicles were stopped in sequence. First, lipid-TF was stopped and inert lipid continued to flow for approximately one minute. To stop the inert lipid, the plugged inlet (green cross) was unplugged, and a solution of NaCl (150 mM) was started at 1.0 μL min^{-1} in this inlet. Next, the flow of inert lipid (i) was stopped, and a solution of NaCl (150 mM) was started at 1.0 μL min^{-1} in this inlet. Finally, the flow of inert lipid (ii) was stopped. (a) The excess lipid vesicles were removed by allowing the solutions of NaCl to flow for 20 min at 1.0 μL min^{-1} each. This procedure left a coating of lipids on the channel walls. After the solution of NaCl was stopped, the device was removed from the solution of NaCl and Out (i) and Out (iii) were sealed (top and bottom green crosses). To seal the outlets, a small amount (25-50 μL) of Norland Optical Adhesive 81 was applied to the PDMS and exposed to UV light (λ = 320-400 nm) for 15-20 sec. Next, blood plasma was re-calcified on chip by flowing in blood plasma and a solution of $CaCl_2$ ($CaCl_2$, 40 mM; NaCl, 90 mM; and Boc-Asp(OBzl)-Pro-Arg-MCA, 0.4 mM) at a 3:1 volumetric flow rate ratio (blood plasma:solution of $CaCl_2$). These solutions were allowed to flow for approximately one min and then Out (ii) was sealed as above (middle green cross). Finally, the device was submerged into a solution of EDTA (50 mM). (c) Clotting initiated (blue) where the channel walls were coated with lipid-TF. This clot propagated up to the junction, and clotting was monitored in the "valve".

Geometry and dimensions of the devices used in experiments where clot propagation at a junction in the presence of flow was monitored

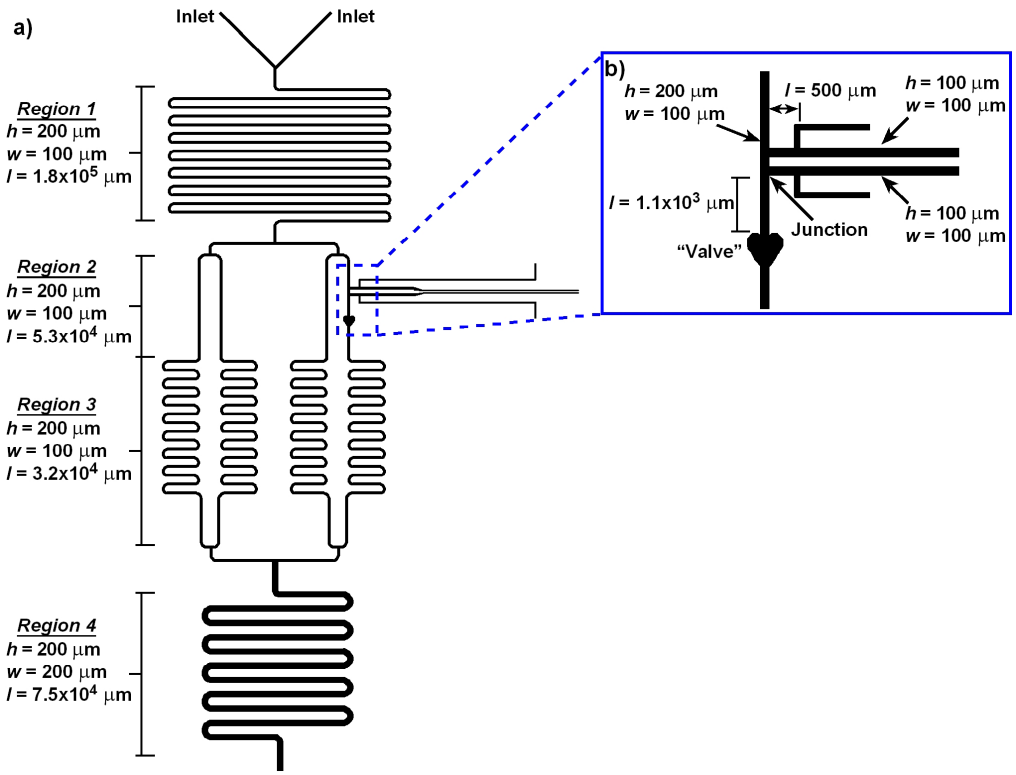

Figure S2. Schematic drawing showing actual geometry and dimensions of the devices used for clot propagation through a junction in the presence of flow. (a) Basic design for all devices. (b) Enlarged view of the junction region.

Determining that shear rate by the "valve" changes the re-circulation in the "valve".
To determine that shear rate, $\dot{\gamma}$ [s^{-1}] by the "valve" changed the re-circulation in the valve, a solution of FluoSpheres (sulfate microspheres, 1.0 μm diameter, yellow-green fluorescent (505/515), 2% solids) was flowed into the device shown in Figure S2 at varying volumetric flow rates (μl min^{-1}). The stock solution of FluoSpheres was diluted with a solution of NaCl (150 mM) 1:10 by volume. This solution was flowed into both inlets. The volumetric flow rate in each inlet was half the desired volumetric flow rate. For the experiments shown in Supporting Movie 1. the high $\dot{\gamma}$ of 340 s^{-1} was obtained by flowing the FluoSphere solution into each inlet at 10.4 μl min^{-1} (final flow rate = 20.8 μl min^{-1}), and the low $\dot{\gamma}$ of 17 s^{-1} was obtained by flowing the FluoSphere solution into each inlet at 0.52 μl min^{-1} (final flow rate = 1.04 μl min^{-1}).

Supporting Movie 1. Comparing the re-circulation in the "valve" at high a $\dot{\gamma}$ of 340 s^{-1} and a low $\dot{\gamma}$ of 17 s^{-1}. Re-circulation in the "valve" was observed using a solution of FluoSpheres. Movie is shown at four times actual speed. See Methods earlier in this Supporting document.