SUPPORTING INFORMATION FOR

The Effects of Shear Rate on Propagation of Blood Clotting Determined Using Microfluidics and Numerical Simulations

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Methods

Determining front velocity in the absence of flow. For experiments in the absence of flow in PDMS channels, recalcified blood plasma (final [$CaCl_2$] = 10 mM) containing a thrombin-sensitive, peptide-modified fluorescent dye, Boc-Asp(OBzl)-Pro-Arg-MCA (100 μM), was flowed into the device from the inlet where egg phosphatidylcholine (Egg PC) was flowed in. Recalcified blood plasma was flowed in for two minutes at 5 μL min^{-1}, and then flow was stopped. Clotting initiated where the channel walls were coated with lipid-TF and propagated into the section of the channel coated with inert lipids. To measure the rate of propagation, the fluorescence intensity along the length of the channel (dashed red line, Figure S1a) was measured in 2.5 minute intervals, and the intensity profile in each image was measured and normalized by using MATLAB® (Figure S1b, also see Figure 1 of main text).

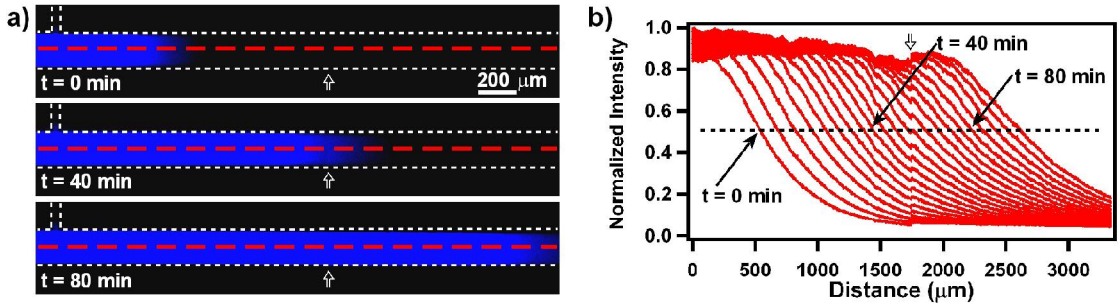

Figure S1.

Characterization of the velocity of propagation of clotting in the absence of flow. (a) Time-lapse images at two stage positions were acquired and aligned (open white arrow) by using MetaMorph®. The fluorescence intensities at each time-point were measured along the length of the channel (long-dashed red line). (b) Time-lapse fluorescence intensity profiles of propagation of clotting. Five minute intervals are shown here for clarity, while original data was acquired at 2.5 min intervals. The indicated time-points correspond to the images shown in panel a, and the open black arrow points out a minor defect in the intensity profile where the two images were aligned (corresponds to the open white arrow in panel a). These intensity profiles were converted into the distance vs. time plot shown in Figure 1d of the main text by measuring the distance between each intensity profile at 50 percent maximum intensity (dashed line).

Determining front velocity in the absence of flow in glass capillaries coated with thrombomodulin at 37 ºC. The rate of propagation of clotting in circular glass capillaries (530 μm inner diameter) coated with thrombomodulin (TM) at 37 ºC was also determined. The capillaries were first made hydrophilic by flowing a solution of "piranha" (3:1 sulfuric acid:hydrogen peroxide, WARNING: piranha reacts violently with organic substances, including all human tissues) for 20 min, followed by rinsing with deionized water. To coat glass capillaries with TM, 250 μL of the vesicles of reconstituted TM were pulled through the capillary for 5 min, and the capillaries were then rinsed by pulling through 500 μL of TRIS buffer (20 mM TRIS-HCl, 100 mM NaCl, pH 7.5). Lipid-TF was patterned on one end of the capillary to serve as the initiation site for clotting. The tip of the capillary was exposed to air, which removed the bilayer with reconstituted TM, and then the tip was touched to a drop of lipid-TF vesicles to fill in the exposed area. The capillary was rinsed with TRIS buffer. Lipid-TF (red fluorescence) could be seen on the glass surface only at the tip. The capillary was filled with recalcified plasma from the non-TF end of the capillary and sealed at the ends with silicon grease. Clotting initiated on the lipid-TF, and the rate of propagation was measured as in Figure S1.

Measuring the activity of reconstituted TM. To measure the activity of TM on the PDMS and glass surfaces, the rate of protein C activation in the presence of thrombin was measured (Esmon, N.L., DeBault, L.E. & Esmon, C.T. *J. Biol. Chem.* **258** 5548-5553, (1983)). The concentration of activated protein C (aPC) was determined by using an aPC-sensitive fluorogenic peptide dye, Boc-Leu-Ser-Thr-Arg-MCA (Ohno, Y. et al. *J. Biochem* **90,** 1387-1395 (1981)). A solution containing 5 nM human thrombin, 800 nM human protein C, 0.1% bovine serum albumin, 5 mM $CaCl_2$, 20 mM TRIS, and 100 mM NaCl (final pH 7.5) was prepared and immediately flowed into the PDMS channel or glass capillary. The PDMS channel was maintained at room temperature during the experiment, and the glass capillary was maintained at 37 ºC. The blue fluorescence intensity was measured every 3 min for up to 3 h. The aPC production was determined based on Michaelis-Menton kinetics and known kinetics for the aPC fluorogenic dye (Ohno, Y. et al. *J. Biochem* **90,** 1387-1395 (1981)) (Table S1). Thrombin is known to activate protein C directly, and to slowly activate the aPC-sensitive fluorogenic dye. Thus, the background fluorescence was measured in control samples containing no TM and was subtracted from the measured fluorescence intensity in samples containing TM.

Table S1.

Quantification of activated protein C (aPC) production from Egg PC lipid coated surfaces with reconstituted thrombomodulin (TM). Velocities of propagation of clotting (front velocity, F_v) are shown.

Surface	Temp. (°C)	TM:Egg PC ratio	Calculated Surface Density (pmol m^{-2})	aPC production (pmol min^{-1} m^{-2})	Front Velocity (μm min^{-1})
PDMS	25	N/A	N/A	N/A	20
PDMS	25	1:75600	4	10	24
PDMS	25	1:7560	40	5*	25
Glass	37	N/A	N/A	N/A	41
Glass	37	1:75600	4	0.3	ND
Glass	37	1:7560	40	10	50

*Saturation in TM concentration may have been reached (Tseng, P. Y., Jordan, S. W., Sun, X. L, & Chaikof, E. L. *Biomaterials* **27**, 2768-2775 (2006)).
N/A = Not applicable because no TM was present. Data is shown only for the comparison of front velocities.
ND = Not determined.

Performing numerical simulations of propagation of clotting in the absence of flow. All numerical simulations were performed by using Comsol Multiphysics® version 3.3 (Stockholm, Sweden). Two rate equations were used to numerically simulate the change in concentration of activator, C_{act}. These rate equations are based on previously described equations (Kastrup, C. J.; Runyon, M. K.; Shen, S.; Ismagilov, R. F. *Proc. Natl. Acad. Sci. U. S. A*. **103,** 15747-15752 (2006)). The rate of autocatalytic production of C_{act} was described by Eq. 1.

$$R_1 = k_1[C_{act}]^2[C'] / (k_{sat}[C]^2 + 14) + b \quad (1)$$

The rate of linear inhibition of C_{act} was described by Eq. 2.

$$R2 = k_2[C_{act}] \quad (2)$$

Transport of C_{act} was modeled by using the convection-diffusion equation. All solution phase species in the numerical simulation had diffusion coefficients of 5 x 10^{-11} m^2s^{-1} (Krasotkina, Y. V.; Sinauridze, E. I.; Ataullakhanov, F. I. *Biochim. Biophys. Acta-Gen. Subj.* **1474,** 337-345 (2000)).

A surface-bound inhibitor, I_{surf}, similar to thrombomoldulin was incorporated into the simulation according to the scheme shown in Figure S2. C_{act} binds to I_{surf} and the C_{act}:I_{surf} complex converts a solution phase precursor, I'_{sol}, to a solution phase inhibitor, I_{sol}, with a rate that was described by Eq. 3.

$$R_3 = k_3[I'_{sol}][I_{surf}][C_{act}] / [I'_{sol}] + Km_3 \quad (3)$$

I_{sol} inhibits C_{act} with a rate that was described by Eq. 4.

$$R_4 = k_4[I_{sol}][C_{act}] / [C_{act}] + Km_4 \quad (4)$$

The total rate of inhibition in the presence of I_{surf} is the sum of R_2 and R_4 and was described by Eq. 5.

$$R_5 = k_2[C_{act}] + k_4[I_{sol}][C_{act}] / [C_{act}] + Km_4 \quad (5)$$

The species and their initial concentrations are based on known proteases in the blood coagulation cascade. C' is similar to a proenzyme and is converted to its active form, C_{act}. The rate of removal of $C' = -R_1$. The initial [C'] = 1.4 x 10^{-6} M (Butenas, S.; van't Veer, C.; Mann, K. G. *Blood.* **94,** 2169-2178 (1999)) and the initial [C_{act}] = 5 x 10^{-12} M (Butenas, S.; Orfeo, T.; Gissel, M. T.; Brummel, K. E.; Mann, K. G. *J. Biol. Chem.* **279**,

22875-22882 (2004)). Propagation of clotting was initiated by setting the initial [C_{act}] = 35 x 10^{-9} M in a 200 x 200 μm^2 plane at the end of the channel (see Figure 2 of the main text). I'_{sol} is the precursor the solution phase inhibitor, I_{sol}, and had initial [I'_{sol}] = 100 x 10^{-9} M (Feistritzer, C.; Schuepbach, R. A.; Mosnier, L. O.; Bush, L. A.; Di Cera, E.; Griffin, J. H.; Riewald, M. *J. Biol. Chem.* **281**, 20077-20084 (2006)). I'_{sol} is removed with a rate = -R_3. I_{sol} had an initial [I'_{sol}] = 0 M. I_{surf} had an initial [I_{surf}] = 42 nM. The surface [I_{surf}] was estimated by assuming a surface density of I_{surf} = 42 pmol m^{-2} (Feistritzer, C.; Schuepbach, R. A.; Mosnier, L. O.; Bush, L. A.; Di Cera, E.; Griffin, J. H.; Riewald, M. *J. Biol. Chem.* **281**, 20077-20084 (2006) and Esmon, C. T. *Faseb J.* **9**, 946-955 (1995)), which corresponds to 3.3 x10 $^{-17}$ moles / wall. This number of moles was assumed to fill a 1 μm thick boundary layer at the wall (total volume = 8 x 10^{-10} L). The diffusion coefficient of I_{surf} was set as 1 x 10^{-50} m^{2}s^{-1} to localize I_{surf} to the channel walls during the timescale of the simulation.

The parameters used in the rate equations were approximated from literature values: k_1 = 1.93 x 10^{13} M^{-2} s^{-1} was derived by taking a known rate constant for an enzyme in the coagulation cascade (Kuharsky, A. L.; Fogelson, A. L. *Biophys. J.* **80**, 1050-1074 (2001)) and dividing by the initiation concentration of *C*'; k_{sat} = 1.5 x 10^{15} M^{-2} was chosen such that the maximum [C_{act}] generated was approximately 500 nM; b = 1 x 10^{-13} M s^{-1} was chosen as the baseline rate of production of C_{act}; k_2 = 0.02 s^{-1}(O'Brien, L. M.; Mastri, M.; Fay, P. J. *Blood.* **95**, 1714-1720 (2000)); k_3 = 3.4 x 10^8 M^{-1} s^{-1} was derived by multiplying a known rate constant k'$_3$ = 0.17 s^{-1} (Fogelson, A. L.; Tania, N. *Pathophysiol. Haemost. Thromb. 34*, 91-108 (2005)) and the equilibrium constant, K_{eq} = 2 x 10^9 M^{-1} (Esmon, C. T. *Faseb J.* **9**, 946-955 (1995)); Km_3 = 5 x 10^{-6} M (Esmon, C. T. *Faseb J.* **9**, 946-955 (1995)); k_4 = 0.04 s^{-1} (Marlar, R. A.; Kleiss, A. J.; Griffin, J. H. *Blood*. **59,** 1067-1072 (1982)); and Km_4 = 102 x 10^{-9} M (Varfaj, F.; Neuberg, J.; Jenkins, P. V.; Wakabayashi, H.; Fay, P. J. *Biochem. J.* **396**, 355-362 (2006)).

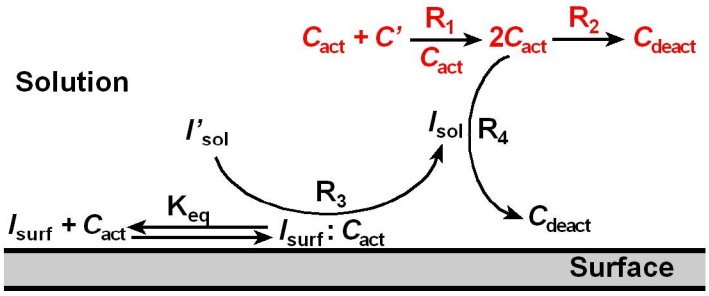

Figure S2.

Schematic drawing of the reactions used in the numerical simulations. Reactions shown in red were used to represent autocatalytic production and linear consumption. These are the only two reactions that take place in the absence of a surface-bound inhibitor. Reactions shown in black were added to mimic a surface-bound inhibitor similar to thombomodulin. R represents rate. The rates for R_2 and R_4 are for inhibition of one molecule of C_{act}. See supporting text above for details.

Performing numerical simulations of propagation of clotting in the presence of flow. To simulate front propagation in the presence of flow, the same two rate equations used to describe front propagation in the absence of flow and in the absence of a surface-bound inhibitor were used. Flow was modeled by using the incompressible Navier-Stokes equation, and transport of C_{act} was modeled by using the convection-diffusion equation. For the simulation with a shear rate above the threshold ($\dot{\gamma}$ = 170 s^{-1}), the initial flow velocity, V [m s^{-1}], was set to 2.2 x 10^{-3} m s^{-1}. For the simulation with a shear rate below the threshold ($\dot{\gamma}$ = 10 s^{-1}), the V was set to 1.3 x 10^{-4} m s^{-1}.

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

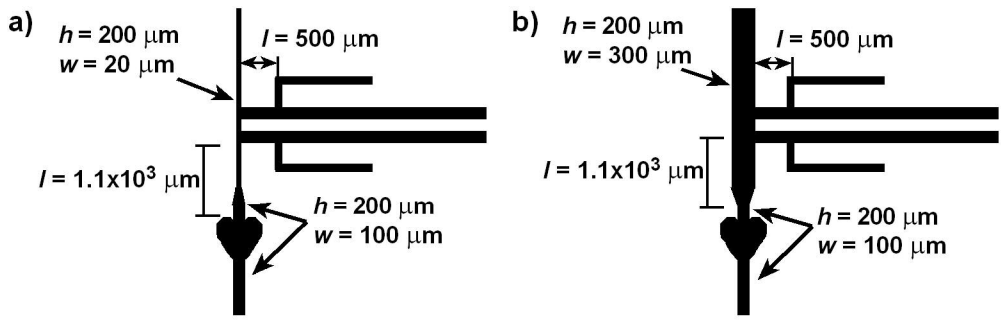

Figure S3. Schematic drawing showing the dimensions of the junction and "valve" regions in the devices used in experiments monitoring propagation of clotting through a junction in the presence of flow. (a) Channel dimensions for experiment with a shear rate above the threshold at the junction and a shear rate below the threshold at the "valve". (b) Channel dimensions for experiment with a shear rate below the threshold at the junction and a shear rate above the threshold at the "valve". The overall device geometry has been previously described (Runyon, M. K.; Johnson-Kerner, B. L.; Kastrup, C. J.; Van Ha, T. G.; Ismagilov, R. F. *J. Am. Chem. Soc.* **129**, 7014-7015 (2007)). All dimensions are given as height (*h*), width (*w*), and length (*l*).

Characterizing shear rate at the junction and the "valve".

Table S2. Flow rates and shear rates for experiments shown in Figure 4 of the main text.

Experiment	Volumetric flow rate* ($\mu L\ min^{-1}$)	Flow velocity junction ($mm\ s^{-1}$)	Shear rate junction** (s^{-1})	Flow velocity "valve" ($mm\ s^{-1}$)	Shear rate "valve"*** (s^{-1})
190/30	2.9	1.1	190	0.4	30
30/190	0.5	0.8	30	2.4	190

* This is the volumetric flow rate in the channel with the "valve". The input volumetric flow rate was four times larger.

** Shear rate was calculated at the midpoint of the wall for flow in a rectangular channel (Nataraja.N & Lakshman.S. *Indian Journal of Technology* **10**, 435-438 (1973)).

*** Shear rate at the "valve" corresponds to the shear rate in the rectangular channel just above and below the "valve" (see Figure S3).

Determining that clots at a junction remain active after a 10 min exposure to a shear rate above the threshold. To determine if clots remain active after a brief exposure to shear rate above the threshold, plasma was flowed into a device similar to that shown in Figure 5 of the main text, at a shear rate ($\dot{\gamma}$ = 140 s^{-1}) above the threshold, and clotting was initiated in the initiation channel as described previously (Runyon, M. K.; Johnson-Kerner, B. L.; Kastrup, C. J.; Van Ha, T. G.; Ismagilov, R. F. *J. Am. Chem. Soc.* **129**, 7014-7015 (2007)). This clot propagated in the initiation channel in the absence of flow up to the junction. After the clot reached the junction, a shear rate above the threshold ($\dot{\gamma}$ = 140 s^{-1}) was maintained for 11 min or 18 min (two independent experiments).

Then, the shear rate was reduced to below the threshold ($\dot{\gamma}$ = 30 s^{-1}), and propagation of clotting out of the junction was monitored.

In both experiments, propagation of clotting into the down stream "valve" was observed approximately 3 min after the shear rate was reduced, suggesting that the clot at the junction remained active. These experiments were performed under slightly different conditions than the rest of the experiments analyzing propagation of clotting in the presence of flow. These differences were: 1) platelet rich plasma was used instead of normal pooled plasma; 2) the device had multiple down stream "valves"; and 3) the dimension of the flow channel were h = 200 μm and w = 200 μm, instead of h = 200 μm and w = 100 μm. Regardless of these differences, these results demonstrate that the clot at the junction remained active after exposure to a shear rate above the threshold for 11 to18 min.

Determining that slowed front propagation through a junction after a D-phenylalanyl-L-prolyl-L-arginyl-chloromethyl ketone (PPACK) exposure is not due to PPACK adsorbing to and releasing from PDMS. To determine if PPACK adsorbs to and releases from PDMS, a solution of TRIS buffer (20 mM TRIS-HCl, 100 mM NaCl, pH 7.4) and a solution containing 40 mM $CaCl_2$, 90 mM NaCl, and 3 μM PPACK were flowed into a device at flow rates of 17.2 μL min^{-1} and 5.7 μL min^{-1}, respectively (Figure S4a). These solutions were flowed for 7.5 min and collected at the outlet (PPACK fraction). The PPACK fraction was stored at 4°C until later use. Next, the PPACK flow was stopped and a solution containing 40 mM $CaCl_2$ and 90 mM NaCl was flowed into the device (Figure S4a). This solution was collected at the outlet in 80 sec intervals (buffer fractions) and stored at 4°C until later use. Thrombin activity was determined by combining 50 μL of a solution of thrombin (40 mM $CaCl_2$, 90 mM NaCl, 1 mg ml^{-1} BSA, and 2 nM thrombin), 50 μL of a solution containing Boc-Asp(OBzl)-Pro-Arg-MCA (100 μM), and 10 μL of the fraction collected at the outlet of the device (Figure S4b). The rate of cleavage of Boc-Asp(OBzl)-Pro-Arg-MCA was measured on a Tecan SafireII^{TM} fluorimeter (λ_{ex} = 380 nm, λ_{em} = 440 nm).

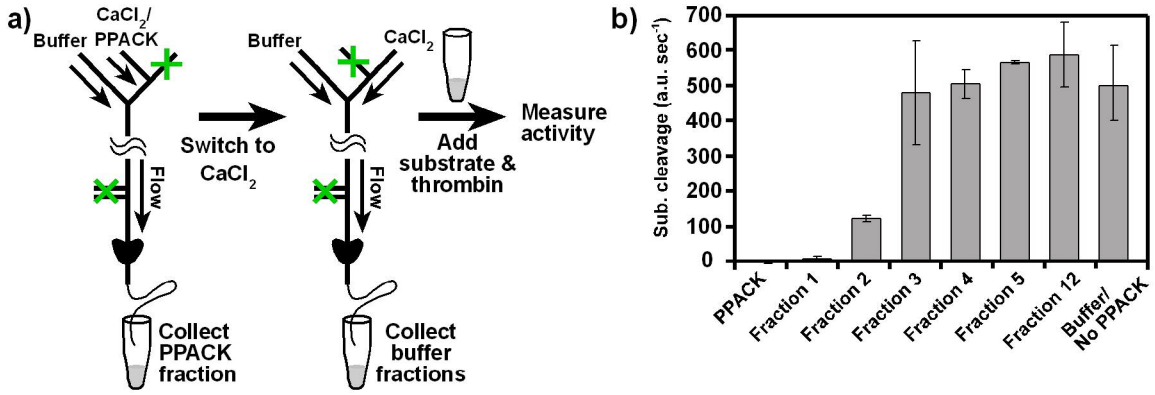

Figure S4.

Slowed propagation of clotting to the "valve" after PPACK exposure is not due to PPACK adsorbing to PDMS. (a) Schematic showing the experimental procedure for determining if PPACK adsorbs to and releases from PDMS. See supporting text above for details. (b) Characterization of thrombin activity as a function of fraction number. For each data point, the average is shown and error bars represent the minimum and maximum values (n = 2). Thrombin activity reaches 100 % by buffer fraction 3, which corresponds to 4 min after PPACK flow was stopped.