Supplementary Information

Blood plasma separation in a long two-phase plug flowing through disposable tubing

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Experimental Section

Chemicals and equipment

The main hardware used for plasma separation is a 10-cm long PTFE tubing (203 µm i.d., 355 µm o.d., Zeus, USA, < 0.5 USD) which is used as a cartridge[S1] to load the blood plugs and a mineral oil (Sigma-Aldrich) carrier. The tubing is connected to a syringe (Gastight 1710, Hamilton, USA) via a Tygon tubing (250 µm i.d., 760 µm o.d., Saint-Gobain, USA). Standard photolithography procedures[S2] were used to fabricate a mold (SU-8 2100, MicroChem) and polydimethylsiloxane (PDMS) device with 200-µm channel in width and height. Whole blood from human sources, treated with Na-Citrate anticoagulant, was obtained from Lampire Biological Laboratories. The blood was serially diluted up to 20-fold with phosphate-buffered saline (PBS) prior to experimentation. The hematocrit in our experiments was varied from 2.25~45%, assuming that the hematocrit of whole blood is 45%. The fluids were driven with a Harvard PHD 2000 syringe pump and the ratio of length (L) to width (w) of the plug is typically, $L/w > 100$. Separation occurs as the oil carrier and blood plug move in the cartridge at certain flow rate. The Reynolds number is typically < 0.1, and capillary number is < 0.001.

To enable easy image-based quantification of the effect of control parameters on separation efficiency, the cartridge is plugged into a square cross-section microchannel, as the cylindrical tubing introduces unwanted optical effects. Images were taken with CCD or CMOS cameras (StreamView-LR, SVSi, USA; PL-B776F, PixeLINK, Canada) coupled with a stereo microscope (SZX-16, Nikon, Japan). To observe the sedimentation of cells in the tubing, we oriented a 10x objective and light source mounted on an inverted microscope perpendicular to gravity (CKX 41, Olympus, Japan) and images were obtained with a high-speed CMOS camera (pco.1200s, PCO, Germany). Differing viscosities of blood samples were prepared by mixing whole blood with 60%, 30% and 10% (wt%) glycerol (Sigma), corresponding to viscosities of 10.8, 2.50 and 1.31 mPa·s at 20 °C respectively,[S3] in a volume ratio of 1:4. Suspensions of polystyrene particles (10 µm diameter, Polysciences, USA) in different densities of the suspending liquid were made by mixing 2% suspensions with PBS (1:1), D_2O (Acros Organics, 1:1) and D_2O (1:2), corresponding to densities of 1.0, 1.05 and 1.07 kg/m^3 respectively. To detect the cholesterol in plasma, we mixed the extracted plasma with 300-µM Amplex Red (Life Technologies) at upstream of T-junction before droplet microreactors were produced.

Description of particle tracking velocimetry (PTV) experiments

PTV experiments were performed using a 250-fold dilution of 2-µm polystyrene microspheres (density 1.05 kg/m^3, Bangs Laboratories Inc., USA) in PBS. A 1-µL plug of the microsphere suspension was aspirated into a PTFE tubing prefilled with mineral oil, and mineral oil was continually aspirated following the aqueous plug. The tubing was embedded and cured in a mass of PDMS to approximately index-match the tubing sidewalls and enable particle visualization in flow. Experiments were visualized using a Phantom v310 CMOS camera coupled to an Olympus IX-70 microscope using a 40× objective. Typically exposure times of 15 µs and frame rates of 30 fps were used. The volumetric flow rate used was 0.5 µL/min. Particles were detected in the images using MATLAB image processing software. 50 images from the beginning to the end of the plug's traversal through the field of view were averaged to create a background of light transmission through the plug. This background image was then subtracted from each of the images of the plug, a constant grayscale threshold was applied, particles were identified using MATLAB's *bwlabel.m* command, and centroids were determined using the *regionprops.m* command. Once all of the centroids of the tracer particles were determined, a predictive particle-tracking algorithm[S4,S5] was employed to determine the trajectories of the tracer particles through the field of view. Horizontal and vertical velocity vectors were determined for each particle by calculating the frame-to-frame time derivative of the particles trajectories over 4 runs, binned and averaged at regular spatial intervals. The velocity vectors for each of the spatial bins were then rendered using MATLAB's *quiver* plot command with zero scaling of the vectors.

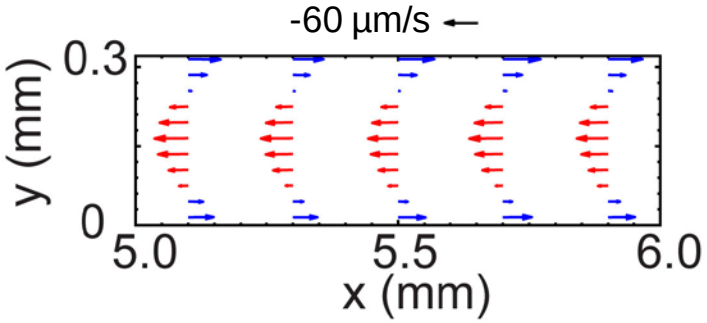

Figure S1: The flow field, at the center of a long flowing plug, determined using particle tracking velocimetry with ~2 µm diameter polystyrene spheres in the plug. The center line velocity is ≈ 60 µm/s, and the mean plug velocity is ≈ 80 µm/s.

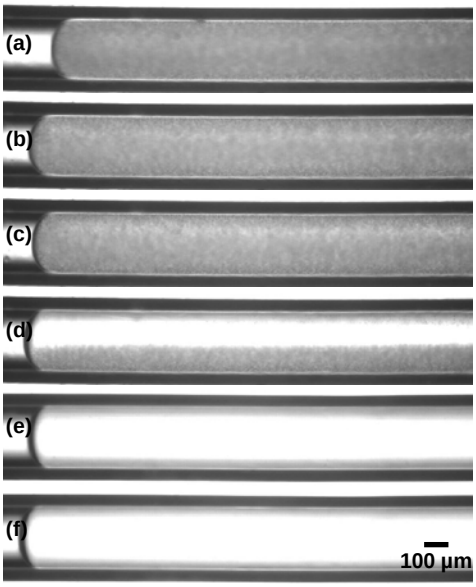

Figure S2: The influence of flow rate on 5-fold diluted blood cells sedimentation and collection. (a) t_{obs} = 15 s, t_{coll} = 12 s, t_{adv} = 12 s, Q = 4 µL/min. (b) t_{obs} = 30 s, t_{coll} = 24 s, t_{adv} = 24 s, Q = 2 µL/min. (c) t_{obs} = 60 s, t_{coll} = 48 s, t_{adv} = 48 s, Q = 1 µL/min. (d) t_{obs} = 120 s, t_{coll} = 96 s, t_{adv} = 96 s, Q = 0.5 µL/min. (e) t_{obs} = 300 s, t_{coll} = 240 s, t_{adv} = 240 s, Q = 0.2 µL/min. (f) t_{obs} = 600 s, t_{coll} = 480 s, t_{adv} = 480 s, Q = 0.1 µL/min. For all experiments t_s = 110 s.

Figure number	t_s (s)	t_{adv} (s)	t_{coll} (s)	t_{obs} (s)	t_s/t_{adv}	t_{coll}/t_{obs}	Sediment?	Clear front end?	Plotting color	Plotting symbol
2c (Blood dilution expts.)	110	120	120	360	0.9167	0.3333	y	y	red	open diamond (◊)
2d	75	120	120	360	0.625	0.3333	y	y	red	open diamond (◊)
2e	57	120	120	360	0.475	0.3333	y	y	red	open diamond (◊)
3a (Blood flow rate top view expts.)	75	300	300	900	0.25	0.3333	y	y	red	solid circle (●)
3b	75	120	120	360	0.625	0.3333	y	y	red	solid circle (●)
4a (Observation time expts.)	75	120	120	60	0.625	2	y	n	blue	open circle (○)
4b	75	120	120	120	0.625	1	y	y	red	open circle (○)
4c	75	120	120	240	0.625	0.5	y	y	red	open circle (○)
4d	75	120	120	480	0.625	0.25	y	y	red	open circle (○)
6a (Density expts.)	37.3	96	96	120	0.3885	0.8	y	y	red	open triangle (Δ)
6b	533	96	96	120	5.5521	0.8	n	n	black	open triangle (Δ)
6c	87	96	96	120	0.9062	0.8	y	y	red	open triangle (Δ)
7a (Viscosity expts.)	417	240	240	300	1.7667	0.8	n	n	black	solid diamond (♦)
7b	157	240	240	300	0.4083	0.8	y	n	blue	solid diamond (♦)
7c	120	240	240	300	0.2167	0.8	y	y	red	solid diamond (♦)
7d	110	240	240	300	0.1625	0.8	y	y	red	solid diamond (♦)
S2a (Flow rate expts.)	110	12	12	15	9.1667	0.8	n	n	black	open square (□)
S2b	110	24	24	30	4.5833	0.8	n	n	black	open square (□)
S2c	110	48	48	60	2.2917	0.8	n	n	black	open square (□)
S2d	110	96	96	120	1.1458	0.8	y	n	blue	open square (□)
S2e	110	240	240	300	0.4583	0.8	y	y	red	open square (□)
S2f	110	480	480	600	0.2292	0.8	y	y	red	open square (□)
S3a (Diluted blood)	75	300	300	300	0.25	1	y	y	red	cross (+)
S3b (Whole blood)	392	600	600	1800	0.6533	0.3333	y	y	red	cross (+)

Table S1: Data collection for the behavior diagram in Fig. 8.

Figure S3: Stitched images of the separation of plasma from diluted and whole blood. (a) Plasma separation in a 1-µL 10-fold diluted blood plug at a flow rate of 0.2 µL/min behind 1-µL of oil. ~790 nL (79% v/v) plasma obtained. (b) Plasma separation in a 1-µL whole blood plug at a flow rate of 0.1 µL/min behind 3-µL of oil. ~70 nL (7% v/v) plasma obtained.

Movies

Movies showing the separation of plasma from blood cells in 1-µL 10-fold diluted blood plugs at flow rate of 0.5 µL/min. Movie S1, top view; Movie S2, side view.

References

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