Enabling the recirculation of leukocytes in a high-throughput microphysiological system (MPS) to study immune cell-vascular tissue interactions

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Supplementary Information

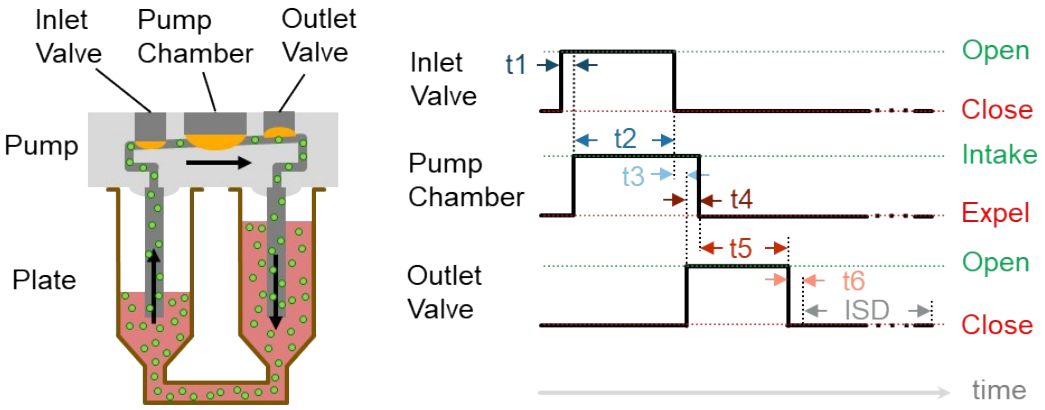

Figure S1. **Pump event timing diagram.** Left: Schematic of the flow circuit and pump features. Right: The timing diagram shows the layout of parameters t1 – t6 and interstroke delay (ISD) used to actuate the pump. Table S3 in the supplemental section provides the values for specific configurations used in this study.

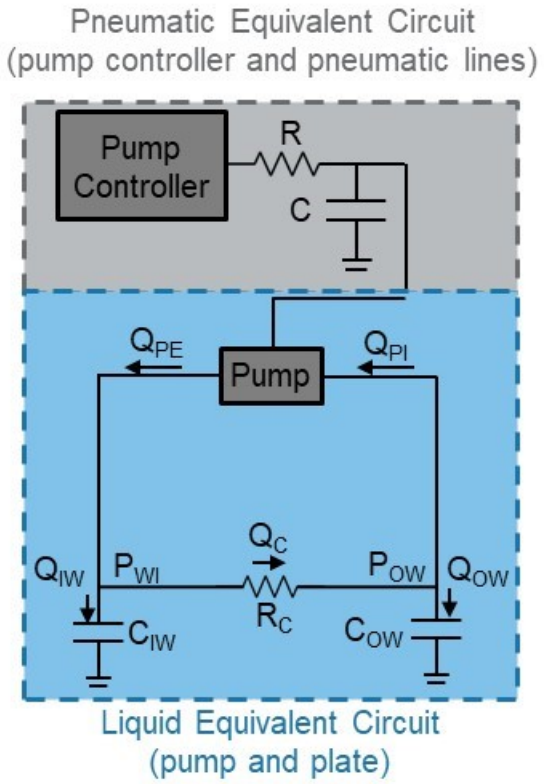

Figure S2. **System equivalent circuit for a single pump device and plate channel of PREDICT96.** The fluid equivalent circuit used to derive the analytical model of flow through PREDICT96 plate channels driven by the pump. Q_{PI} and Q_{PE} are the respective intake and expulsion flow rates produced by the pump. Q_{IW} and Q_{OW} are the respective flow rates into the inlet well and out of the outlet well. C_{IW} and C_{OW} are the respective capacitances of the inlet well and outlet well. P_{IW} and P_{OW} are the respective hydrostatic pressures at the inlet well and outlet well. Q_C is the flow rate through the tissue model channel with resistance R_C.

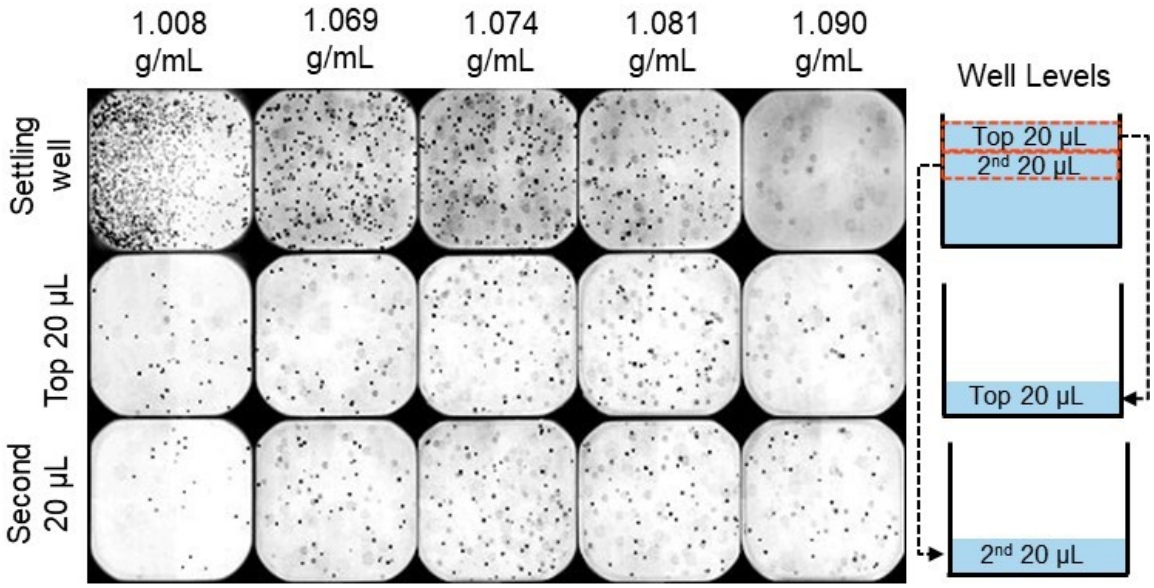

Figure S3. **Neutrophil distribution across the height of the neutrally buoyant suspensions after 2 h.** Representative contrast-adjusted images of neutrophil suspensions sampled from different fluid heights in a 384 well plate after 2 h. All images were acquired at the bottom of the wells. The top row of images shows the original suspension samples after 2 h. 20 µL aliquots of neutrophil suspensions were sequentially sampled from the top of the wells in the first row. The middle row shows the first 20 µL aliquot and the bottom row shows the second 20 µL aliquot. The different fluid densities tested are shown by column. For a given density (column), the more evenly the neutrophils are distributed across fluid heights (rows), the more homogenous the suspension after 2 h.

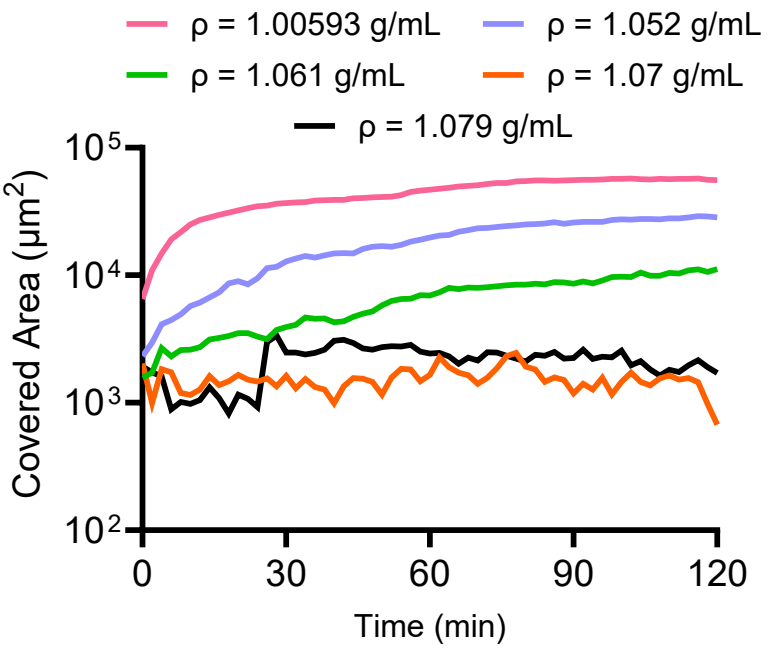

Figure S4. **Neutrally buoyant media experiments for primary human monocytes.** The area coverage on the bottom of a well measured overtime for monocytes in media suspensions with different densities (HBSS and Optiprep™).

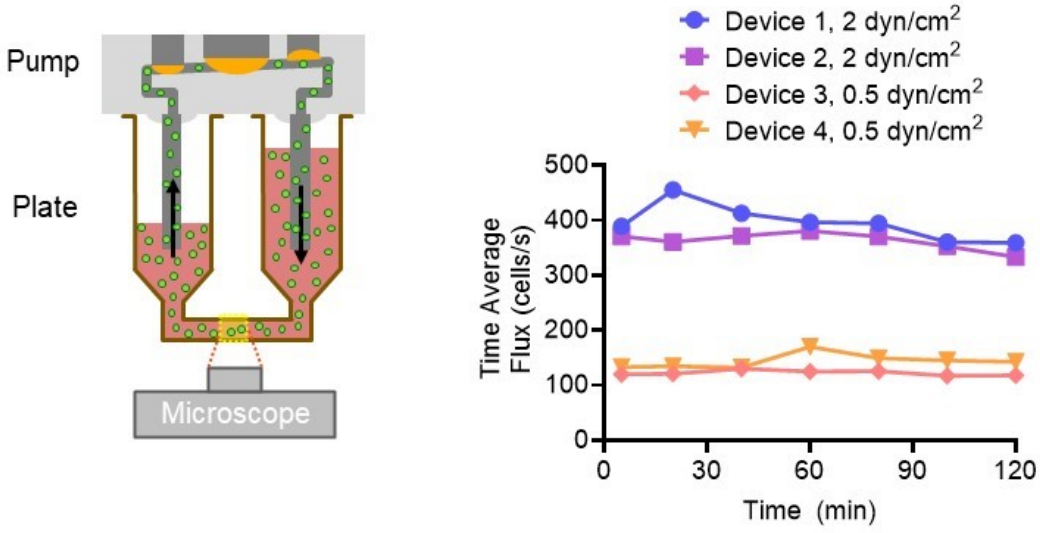

Figure S5. **Neutrophil flux was stable through microfluidic tissue channels of PREDICT96 during recirculation.** The flux of neutrophils through N = 2 individual PREDICT96 devices at two different channel shows stable neutrophil flux over 2 h of recirculation.

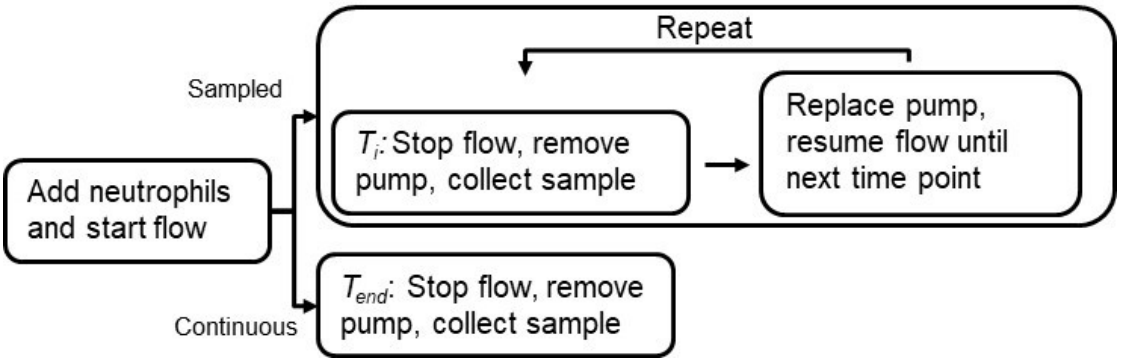

Figure S6. **Schematic illustrating the difference between the two sampling techniques used during cell recirculation experiments.** Sampling the recirculating cell suspension for measurements at multiple discrete time points required stopping flow, removing the pump, collecting the sample, replacing the pump and resuming flow until the next time point, at which point the process was repeated for the remaining samples. This differed from continuous flow where flow was only stopped and sampled at the end of the experiment.

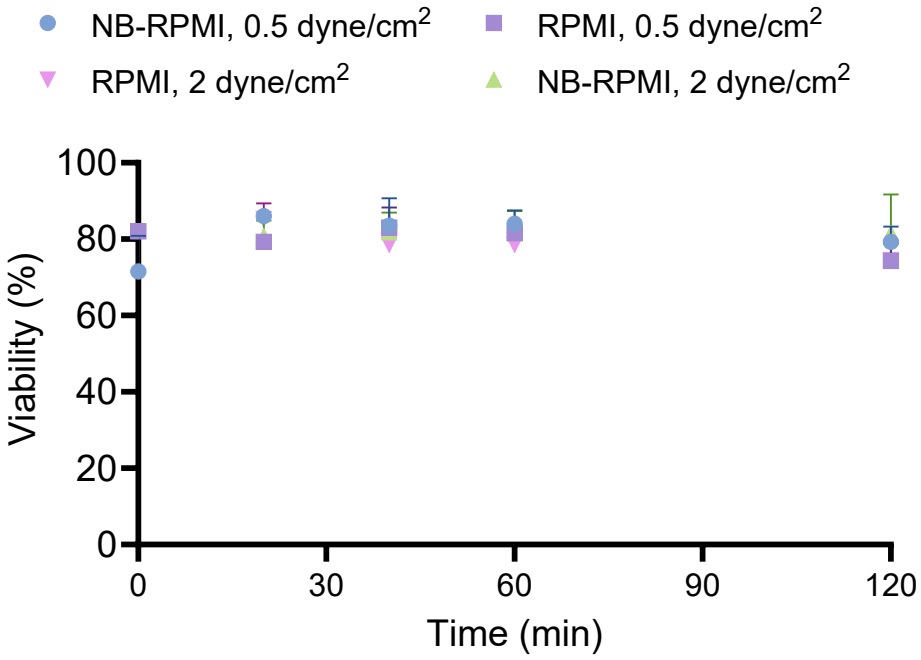

Figure S7. **PBMC viability was stable during 2 h of recirculation through PREDICT96.** The viability of PBMCs recirculated through PREDICT96 was measured at discrete intervals across 2 h. N = 4 devices, mean ± standard deviation. PBMC viability was not significantly affected by repeated starting and stopping of the pump during sampling.

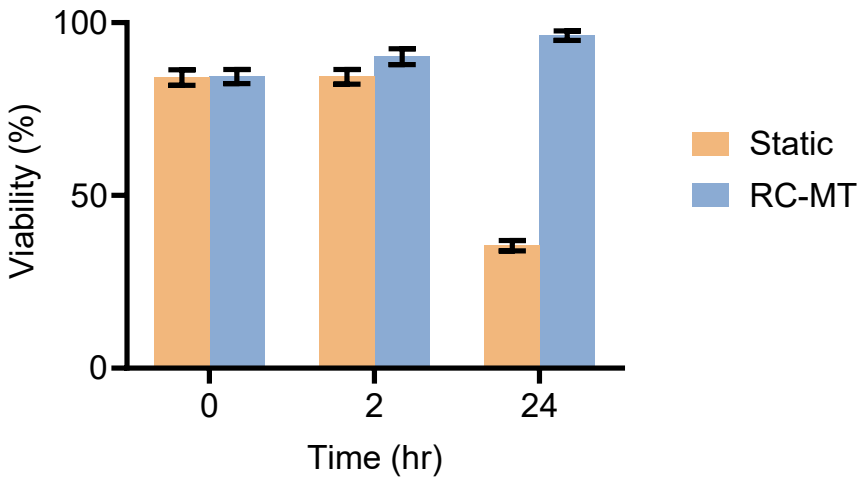

Figure S8. **Viability of monocytes recirculated through PREDICT96 with vascular tissue up to 24 h**. Viability for monocytes suspended in neutrally buoyant media (Lonza and OptiPrep™) under static and recirculating flow (140 µL/min) conditions in PREDICT96 operated with the RC-MT pump configuration. Human primary monocytes were isolated from an apheresis product or leukopak and isolated using a negative selection kit (STEMCELL Technologies, StemEasySep Direct Human Monocyte Isolation Kit). Isolated monocytes were cryopreserved using cryopreservation media (STEMCELL Technologies, CryoStor CS10). On the day of experiment, monocytes were thawed and used immediately. Static control: neutrophils suspended with no flow in a well plate. N = 4 devices, mean ± standard deviation.

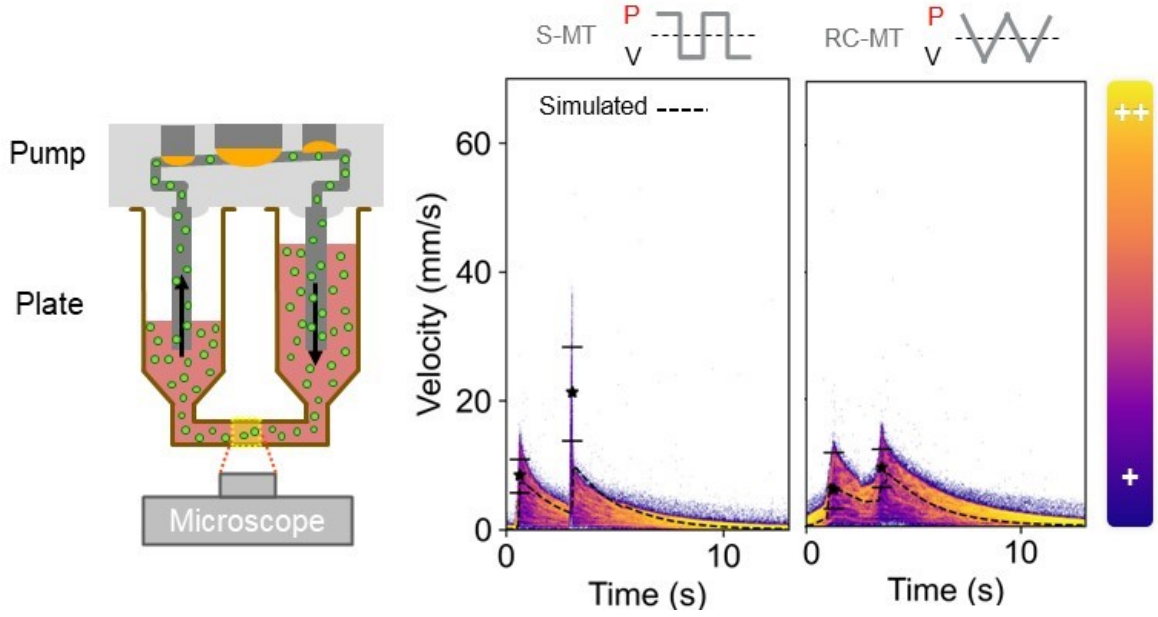

Figure S9. **Simulated and measured tissue model channel flow dynamics for a pressure waveform with reduced amplitude for pump actuation**. The measured and simulated velocity through the plate channel for a pump intake and expulsion cycle using the S-MT and RC-MT pump configuration using a reduced

pressure and vacuum amplitude (± 2 psi). The heat maps shows the density of particles with a given velocity.

Table S1. **Neutrophil donor characteristics.** Characteristics of the neutrophil donors and the figures where the donor cells are represented. All blood samples were collected by Charles River Laboratories Cell Solutions, Inc.

Age	**Gender**	**Height**	**Weight**	**BMI**	**Ethnicity**	**ABO/ Rh**	**Figure**
23	Male	6’1” (1.85 m)	232 lbs (105 kg)	30.61	Caucasian	O-POS	2C-F, 5B
21	Male	6’1” (1.85 m)	177 lbs (80 kg)	23.35	Black/White	O-POS	5B
44	Female	5’8” (1.73 m)	230 lbs (104 kg)	34.97	Caucasian	O-POS	5D
33	Male	5’8” (1.73 m)	235 lbs (107 kg)	35.73	Hispanic/ Latino	O-POS	5, 6A-B
27	Female	5’3” (1.60 m)	160 lbs (73 kg)	28.34	Filipino	O-POS	6C-D
31	Male	5’9” (1.75 m)	218 lbs (99 kg)	32.19	Hispanic	O-POS	6C-D
53	Male	5’10” (1.78 m)	195 lbs (88 kg)	27.98	Black	O-POS	6C-D

Table S2. **Neutrophil flow cytometry panel.** The panel used for neutrophil flow cytometry.

Neutrophil Purity Panel			
Marker	Fluorophore	Clone	Catalog #
CD3	Pacific Blue	HIT3a	300330
CD14	PE	M5E2	301806
CD16	FITC	3G8	302006
CD19	APC	4G7	392504
CD45	BV711	H130	304050
CD66b	PE-Cy7	G10F5	305116
Dead	Zombie NIR	N/A	423105
Neutrophil Activation Panel			
Marker	Fluorophore	Clone	Catalog #
CD62L	Pacific Blue	DREG-56	304826
CD45	PE	2D1	368510
CD16	FITC	3G8	302006
CD177	APC	MEM-166	315808
CD11b	SB702	ICRF44	67-0118-42
CD66b	PE-Cy7	G10F5	305116
Dead	Zombie NIR	N/A	423105

Table S3. **Pump event timing values.** The values used for each parameter in the standard timing (ST) and modified timing (MT) configuration. Refer to Supplemental Figure 1 for details.

	t1 (ms)	t2 (ms)	t3 (ms)	t4 (ms)	t5 (ms)	t6 (ms)	ISD (ms)
Standard Timing (ST)	100	300	100	100	300	100	4000
Modified Timing (MT)	50	2400	50	50	2400	50	0

Table S4. **Peak dynamic shear in tissue model channels for different pumping configurations.** The peak dynamic shear within microchannels for different pumping configurations.

Pump Configuration	**Peak Dynamic Shear (dyn/cm^2)**
S-ST	13
S-MT	6.5
RC-MT	5.7

Table S5. **Simulated channel velocity shows agreement with measurements across pumping configurations.** The peak velocities of 15 µm fluorescent polystyrene particles recirculated through the PREDICT96 for different pumping configurations. Bold-italicized text indicates the simulated value falls within 1 standard deviation of the measured mean.

Pressure	**Flow Rate (µL/min)**	**Connection, Measured Point**	**Peak**	**Simulated (mm/s)**	**Measured (mm/s)**		
					Mean	**Pos. Std. Dev.**	**Neg. Std. Dev.**
± 2 psi	140	RC-MT, Plate	***Intake***	***9.4***	***10.8***	***3.3***	***-3.5***
			Expel	***9.8***	***12.0***	***3.5***	***-3.8***
	55	RC-MT, Plate	***Intake***	***6.6***	***5.8***	***5.8***	***-3.1***
			Expel	***8.3***	***9.2***	***2.9***	***-3.2***
		S-MT, Plate	***Intake***	***7.3***	***8.4***	***2.5***	***-2.8***
			Expel	9.8	21.3	7.0	-7.5
± 6 psi	140	RC-MT, Plate	***Intake***	***13.6***	***14.9***	***4.7***	***-5.4***
			Expel	***14.6***	***20.6***	***8.2***	***-8.0***
		S-MT, Plate	***Intake***	***15.1***	***17.7***	***5.4***	***-5.7***
			Expel	***16.2***	***20.9***	***21.3***	***-11.0***
	55	RC-MT, Plate	***Intake***	***8.9***	***7.5***	***4.2***	***-2.6***
			Expel	13.3	20.7	5.6	-6.4
		S-MT, Plate	Intake	10.3	15.4	3.5	-4.3
			Expel	***14.9***	***21.9***	***24.4***	***-14.1***
		RC-MT, Pump	***Expel***	***120***	***93.9***	***27.3***	***-30.3***
		S-MT, Pump	Expel	432	277.2	71.7	-65.7

Video S1. **Neutrophils settling in neutrally buoyant experiments.** Video of neutrophils settling in different density media across 120 minutes.

Video S2. **Neutrophils recirculating through tissue model channels in PREDICT96.** Video of neutrophils flowing through PREDICT96 channel.