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Biomimetic postcapillary expansions for enhancing rare blood cell separation on a microfluidic chip†

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Abstract

Blood cells naturally auto-segregate in postcapillary venules, with the erythrocytes (red blood cells, RBCs) aggregating near the axis of flow and the nucleated cells (NCs)—which include leukocytes, progenitor cells and, in cancer patients, circulating tumor cells—marginating toward the vessel wall. We have used this principle to design a microfluidic device that extracts nucleated cells (NCs) from whole blood. Fabricated using polydimethylsiloxane (PDMS) soft lithography, the biomimetic cell extraction device consists of rectangular microchannels that are 20–400 μm wide, 11 μm deep and up to 2 cm long. The key design feature is the use of repeated expansions/contractions of triangular geometry mimicking postcapillary venules, which enhance margination and optimize the extraction. The device operates on unprocessed whole blood and is able to extract $94 \pm 4.5\%$ of NCs with 45.75 ± 2.5 -fold enrichment in concentration at a rate of 5 nl s^{-1} . The device eliminates the need to preprocess blood *via* centrifugation or RBC lysis, and is ready to be implemented as the initial stage of lab-on-a-chip devices that require enriched nucleated cells. The potential downstream applications are numerous, encompassing all preclinical and clinical assays that operate on enriched NC populations and include on-chip flow cytometry

A Introduction

Blood contains cells that are useful for diagnosis, but they are often present in relatively small numbers. Erythrocytes (RBCs) constitute the vast majority of all blood cells and are normally ~ 1000 times more abundant than leukocytes. Other cells such as circulating tumor cells (CTCs) and circulating progenitor cells (CPCs) are even less abundant than leukocytes.^{1–4} The concentrations of these cells in the circulation are dynamically regulated in various diseases and could therefore be used as diagnostic indicators or surrogate markers for therapy.^{5–14} An important feature that distinguishes RBCs and platelets from the less abundant cell populations is their lack of a cell nucleus. Without genetic material, their diagnostic utility is limited compared with nucleated cells (NCs), which include leukocytes, CTCs and CPCs. Current methods for isolating NCs in the clinic require complicated, expensive equipment. In general, several millilitres of whole blood are collected and centrifuged. Plasma, platelets, white and red cells can be separated using this procedure, which takes advantage of differences in cell densities and sedimentation rates. This

†Electronic supplementary information (ESI) available: Movie S1 shows the operation of an optimal NC separation device. See DOI: 10.1039/c1lc20401g

procedure is a labor-, energy- and time-intensive and relies on special equipment and trained operators.^{15,16} Another common approach is to expose the whole blood to hypotonic solution, lysing the RBCs which are more sensitive to osmotic shock than nucleated cells.^{17–19} In this case, centrifugation is still necessary to remove the lysed RBC debris. Moreover, even though the nucleated cells survive the osmotic shock, it is likely that this harsh treatment affects their phenotype and/or gene expression.^{20,21} Unfortunately, these separation methodologies are still “state-of-the-art,” in use in clinics and laboratories around the world, and are even used to pre-enrich cell populations for on-chip analyses.

Existing microfluidic approaches to rare cell sorting²² are based on filtration,^{15,24–27} lateral displacement,²⁸ pinched flow fractionation,²⁹ RBC lysis,^{30,31} dielectrophoresis (DEP)³² or require preliminary cell modification steps such as fluorescent labeling^{33–35} or tagging with magnetic beads.^{32,36,37} These methods typically have low target cell yield,³⁸ purity^{25,26} or concentration,²⁷ and require sample predilution^{25,28,29,32} and washing of lysates.^{30,31}

Our goal was to provide an on-chip unit that can replace centrifugation and lysis, allowing development of bona fide point-of-care devices that can operate on whole blood directly from a patient. Such a unit would robustly and conveniently prepare the blood sample directly on the chip, minimizing cost and potential user-induced errors.^{39–41} An on-chip enrichment unit would also reduce the required sample size. Most current on-chip analysis devices process only sub-microlitre sample volumes, but rely on off-the-chip sample preparation *via* centrifugation or RBC lysis, which require several millilitres of blood.^{15,16}

To develop such a separation device, we take advantage of the natural flow properties of blood. It is well-documented that leukocytes preferentially localize near the vessel wall in post-capillary venules.^{42–44} Although leukocyte rolling is mediated by adhesion molecules expressed by the vessel wall in these regions, NC margination and their initial contact with the wall are mediated by the unique rheology of blood at expansions.^{42,43,45–50} Experiments and computational modeling have shown that when blood enters expansions, RBCs exclude NCs from the main core, forcing them to the wall. The process is affected by RBC aggregation, hematocrit and conduit geometry^{42,45–48,50–54} and has been validated in vivo^{43,49,55} and demonstrated in rectangular channels.⁵⁶ The underlying principle is that RBCs reversibly adhere to each other, but not to leukocytes; this homotypic adhesion is dynamically activated as shear stresses decrease in expansions, resulting in exclusion, or “squeezing out” of NCs from the bulk flow. A core of loosely-aggregated RBCs forms at the axis of flow, and the majority of NCs marginate to the wall, where they reside in a plasma-rich layer.

In previous work, Shevkoplyas *et al.* achieved a 32-fold increase in NC concentration by extracting margined NCs from whole blood samples.⁵⁷ Here we describe a device that uses biomimetic postcapillary venule expansions to optimize NC margination in such a device, significantly enhancing purity and yield. The key novel design feature is the inclusion of multiple triangular expansions upstream from the extraction channel, which mimic the effect of postcapillary venules and maximize NC margination while excluding the majority of contaminating RBCs. The resulting device is able to collect $94 \pm 4.5\%$ of NCs with 45.75 ± 02.5 fold enrichment.

B Methods

Microdevice design and fabrication

Our design for the operational unit is shown in Fig. 1A. All experiments were conducted with channels that were 11 μm high. Microfabrication was carried out at Whitaker Micro/Nano Biosystems Fabrication Facility of Boston University. The details of the

microfabrication are explained elsewhere.⁵⁸ Briefly, the devices were fabricated using soft lithography of PDMS.^{58,59} Sylgard 184 PDMS prepolymer (Dow Corning, Midland, MI) was cast on the silanized master which had the positive relief of the channel features formed by the SU8 photoresist. The PDMS was then cured at 80 °C in a convection oven for 50 min. The cured PDMS was peeled off the master and permanently bonded to a 500 μ m high PDMS coated glass slide after treating both with oxygen plasma (Harrick Plasma, Ithaca, NY). The blood flow experiments were conducted immediately after plasma treatment and bonding. This ensured that the PDMS surface was hydrophilic when it was contacted by the blood, thus preventing air bubble accumulation and entrapment in low shear regions.⁶⁰ A syringe pump (Nanomite, Harvard Apparatus, Holliston, MA) with 1 ml syringe (Hamilton Company, Reno, NV) and 23 gauge needle (0.5" long, type 304, ID 0.017", OD 0.025") was used to push the samples through the microfluidic devices. Fluid entered and exited the device through Tygon tubing (ID 0.02", OD 0.06", 0.02" wall; VWR, Brisbane, CA) connected to stainless steel tubes (0.025" OD $\times$ 0.017" ID, 0.500" length; New England Small Tube, Litchfield, NH) inserted into the PDMS ports.

Blood preparation

Peripheral human blood apheresis by-products were collected at the Massachusetts General Hospital blood bank. The hematocrit was measured using an Autocrit Ultra3 hematology analyzer (Becton Dickinson, Franklin Lakes, NJ). NCs were collected using a pipette from the top layer after centrifuging blood at 3000 rpm for 10 min in a 50 ml tube. NCs were stained using the fluorescent dye Carboxyfluorescein succinimidyl ester (CFSE; Invitrogen, Carlsbad, CA) as per the product instructions. RBCs and NCs were re-suspended in autologous plasma after staining to achieve a hematocrit of 40% and NC concentration of 10000–20000 cells/ μ l.

Computational model

COMSOL Multiphysics (COMSOL Inc, Burlington, MA) was used to simulate blood flow in devices with various channel geometries (see the ESI for details[†]). The 2D model assumed homogenous, but non-Newtonian blood rheology. A finite velocity was assigned as the inlet boundary condition, and constant pressure was imposed at the outlets. The non-Newtonian constitutive equations were applied and parameters were chosen based on published values⁶¹ (ESI, Fig. S1, S2[†]). We analyzed the distribution of streamlines in the resulting simulations and computed the effective resistance imposed by the various candidate geometries for the extraction section. This procedure allowed us to optimize the extraction channel to minimize RBC contamination.

Video microscopy

Images were acquired using the 20x, NA 0.5 objective of an epifluorescence microscope (Nikon Diaphot) and a CMOS Camera (PCO1200s, Cooke Corp., Germany). To quantify yield and purity, images were acquired at 30 frames per second (fps); long exposure times (10 fps) were used to visualize NC streaks and quantify distances between the NC centroids and the wall.

A combination of epifluorescence and transmitted illumination was used to visualize the channel walls and fluorescent cells simultaneously (Fig. 1 and 2). Analyses were performed using MATLAB. The images were binarized and merged to obtain the mean centroid position of an NC in the channel.

[†]Electronic supplementary information (ESI) available: Movie S1 shows the operation of an optimal NC separation device. See DOI: 10.1039/c1lc20401g

Assessment of yield and purity

To compare the performance of different designs, we quantified two standard performance metrics—yield and NC enrichment. The yield (Y) is defined as the percentage of total NCs that are collected into the extraction channel:

$$Y = \frac{100N_{\text{extraction}}}{N_{\text{drain}} + N_{\text{extraction}}}$$

where $N_{\text{extraction}}$ is the number of nucleated cells entering the extraction channel, and N_{drain} is the total number that are lost through the drain channel. Some RBCs will inevitably enter the extraction channel along with the margined NCs and therefore contaminate the collected cell sample, decreasing purity. In this work, we define the purity in terms of NC enrichment (E). By fluorescently labelling 5% of the RBCs in the sample and counting the numbers of NCs and RBCs in drain and extraction channels we can calculate the NC enrichment (E) as

$$E = \frac{\left(\frac{N}{R_{bc}}\right)_{\text{extraction}}}{\left(\frac{N}{R_{bc}}\right)_{\text{sample}}}$$

where N/R_{bc} is the ratio of NCs to RBCs. This ratio is roughly equal to 1/1000 in the original whole blood sample.

Measurement of margination and plasma layer thickness

To quantify performance of the various candidate geometries for the pre-extraction units, we focused on the point where the flow stopped diverging and began converging (*i.e.*, the central angle in all designs) and determined the mean distance between the NC centroids and the nearest wall (w_c ; Fig. 2C) and the plasma thickness (w_p). We define the difference $w_c - w_p = \lambda$, a quantity that determines the RBC exclusion: for best RBC exclusion, the plasma layer should be as large as possible compared with w_c .

Ideally, if λ is less than zero, then $w_c < w_p$, indicating that the NC centroids lie inside the plasma layer; this implies that the RBC centroids follow streamlines farther from the wall than those of the NCs, and can therefore be excluded with appropriate placement of the extraction channels.

C Results and discussion

A schematic of the device is shown in Fig. 1A. Our goal was to collect the NC cell population at high yield while excluding the majority of RBCs. Thus, we separately optimized two different sections of the device—the pre-extraction and extraction regions (Fig. 1B, C). The pre-extraction region consists of a series of expansions/contractions for enhancing NC margination, while the extraction region includes two symmetrical extraction channels for collecting margined cells from both sidewalls as well as a central drain channel which collects the bulk of the RBCs.

Empirical optimization of the pre-extraction section

The purpose of the pre-extraction section is to encourage NC margination and maximize the plasma rich layer near the channel sidewall. Exclusion of RBCs from the extraction channel is best obtained when the NCs reside in a thick, plasma-rich, RBC-poor zone near the wall

where they can easily be extracted *via* “plasma skimming” with minimal collection of contaminating RBCs. To identify the channel expansion geometry with best margination and most extensive plasma layer,⁵⁶ we fabricated microchannels with repeated expansions-contractions in various diamond and triangular geometries of different internal angles as shown in Fig. 1B and Fig. 2. In these experiments, we did not yet include an extraction channel, but instead focused on margination and plasma layer enhancement.

All geometries tested were capable of marginating more than 97% of the NCs, with no statistical difference between the designs (Fig. 2; ESI, Movie S1[†]). This is not surprising, since leukocyte margination in postcapillary venules is robust, even though vessel geometry *in vivo* can vary significantly. However, there were differences in plasma layer thickness (Fig. 2A). Compared with the diamond shapes, the triangle produced the most rapid convergence of streamlines as the flow exited the unit. This caused maximal spreading of streamlines at the corners, and consequently, the thickest plasma-rich zones. The aggregation of RBCs in the bulk flow near the channel axis contributes to this effect. As the flow expands, RBCs tend to remain aggregated near the center of the channel, while the streamlines near the wall diverge, populated by low viscosity plasma. During the contraction, the RBCs converge rapidly, reinforcing existing RBC aggregation, and bringing additional, lone RBCs into the core. This is why repeated expansions increase margination and plasma thickness—in each unit, the RBC core is fortified, packing in the RBCs and widening the plasma layer. This effect plateaus after a few expansion/contractions, however, as the RBC core saturates. Note that practical considerations limited the maximum width of the expansion: the ratio of width/height of a PDMS channel cannot typically exceed thirty without risk of collapse,⁵⁸ and our channel height was 11 μm everywhere.

Thus, the triangular shaped microchannel produced the largest plasma rich zone (w_p)—more than 30 μm (or three times an NC diameter) after 10 expansions (Fig. 2C), and was chosen as the geometry for the pre-extraction units. In this geometry, λ levels off by the 10th expansion-contraction unit, but is always greater than zero, indicating that there will be some level of RBC contamination due to the presence of RBCs closer to the wall than some of the NCs (Fig. 2D). Based on these results, we designed the pre-extraction section of the device using 15 triangular expansion-contractions. The extraction channels were situated at the acute angles within the 15th triangular expansion (Fig. 1A). Note that the lower flow rate of 5 nl s^{-1} resulted in lower λ (Fig. 2C) and hence should lead to more RBC exclusion/NC enrichment (E).

Computational modeling for optimization of the extraction channels

The goal for design of the extraction section was to balance NC extraction and RBC exclusion (Fig. 3A). In reality, the plasma-rich zone varies greatly, and λ fluctuates with time. When it is less than 0, NCs in the extraction channel are pure, but with higher λ , RBCs frequently enter the extraction channel with the NCs. (Fig. 1C). Thus, to a first approximation, we can optimize the extraction channel by adjusting flow distribution to precisely define the laminar flow stream lines that are allowed into the extraction channel. For optimal E and Y , we designed the extraction region such that the streamline situated one cell radius (5 μm) away from the wall upstream of the expansion is contiguous with the point of flow separation near the extraction (Fig. 3A). In theory, any NC closer to the wall than this critical streamline will be captured while those farther away will exit through the drainage channel (Fig. 3B). To estimate the correct dimensions for establishing the separation point at the desired location, we modeled blood flow through the device using non-Newtonian constitutive equations (Carreau⁶² and Generalized Power Law;⁶³ see Methods and the ESI[†]). This approach assumes that blood is a homogenous fluid, so does not explicitly reproduce plasma skimming. However, as a first approximation, it allows determination of the streamlines (Fig. 3A). Since the model does not account for the

discontinuity of the plasma layer, it always predicts non-zero RBC concentrations near the wall, and thus would provide artificially low, conservative estimates of E . The location of the streamline terminating at the point of flow separation is dictated by the distribution of flow between the drain and extraction channel, which in turn is determined by the relative flow resistances in the drain and extraction sections (see the ESI, eqn 5[†]). Therefore, to adjust the relative resistance we varied the ratio of extraction channel length to drain channel length (ω). As ω is increased, the relative channel resistance in the extraction region is increased resulting in fewer fluid streamlines entering the extraction. We tested various designs with ω ranging from 0.2 to 3.3 and computed ϕ , the distance between the critical streamline and the wall, measured upstream of the expansion. For two different non-Newtonian models, the optimal length ratio (the point at which ϕ was 5 μm) was close to 1 (Fig. 3B). The dramatic influence of the non-Newtonian rheology of blood is apparent in these microchannels: compared with the Carreau and Power law models, the Newtonian model significantly overestimates the number of streamlines entering the extraction. This is expected, since the Newtonian model underestimates the viscosity and overestimates the flow in the small extraction channel at larger length ratios (ESI, Fig. S2[†]).

Experimental validation of design

We next constructed devices similar to those simulated in the model and measured enrichment and yield as a function of ω (Fig. 4). As the length ratio ω increases from unity, enrichment (E) trends higher as predicted by the non-Newtonian computational models (Fig. 3B), and the yield (Y) decreases sharply as ω exceeds ~ 1.5 , indicating that ϕ has fallen below the NC radius and cells are being lost into the drainage channel. This agrees reasonably well with the model prediction of ~ 1 , considering the limitations of the model, which considers the fluid to be homogenous (although with variable viscosity). Hence, while more RBCs are excluded, yield suffers. This analysis can be used to achieve a desired compromise between enrichment and yield. For integrated microfluidic assays that may tolerate some RBC contamination, we can sacrifice enrichment for better yield and construct a device with ω of 1.17 by making the drain channel 3.4 mm and extraction section 4 mm. At a throughput of 5 nL s^{-1} , this device enriches the sample NC : RBC ratio from 1 : 1000 at the inlet to 1 : 21 in the extraction channel—a 46 fold increase. Most importantly, while the NC concentration of whole blood ($\sim 1 : 1000$) is too low for most assays, 1 : 21 is sufficiently pure for integrating with assays that require DNA or RNA extraction. The performance at various flow rates is shown in Fig. 5. While yield does not vary dramatically with flow rate, purity suffers at very low or very high flow rates. Approximately 5 nl s^{-1} leads to the highest combination of E and Y in this device, as also predicted from Fig. 2A–B.

D Conclusions

We have developed and optimized a biomimetic microfluidic device to enrich the nucleated cell population starting from a sample of whole, unprocessed blood using only appropriately-designed microchannels. The device consists of a series of triangular expansions that reproduce the blood dynamics seen in post-capillary venules. The current device extracts $94\% \pm 4.5\%$ of the sample NCs with 45.75 ± 2.5 -fold enrichment in concentration at the rate of 5 nl s^{-1} . Assuming whole blood contains 10 000 NCs per ml of blood, we can retrieve approximately 100 NCs in 15 min with a single flow path. The traditional method of nucleated extraction is centrifugation. Blood centrifugation can recover 80–90% of nucleated cells with an absolute purity of approximately 90%. Our device is able to extract up to 99% of nucleated cells from a sample. We do, however, sacrifice absolute purity, achieving 5% in the current configuration. Note, however, that this level of purity is sufficient for many clinical assays. Within a few minutes, this device can

isolate a sufficient number of cells for extraction of genetic material and PCR analysis. Furthermore, being very simple in design and operation, this device can be easily scaled up to increase the throughput to practically any required amount and can be integrated with various downstream microfluidic bioassays including nucleic acid extraction, PCR, CTC collection and leukocyte analyses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Notes and references

1. Swartz MA, Kristensen CA, Melder RJ, Roberge S, Calautti E, Fukumura D, Jain RK. Br. J. Cancer. 1999; 81:756–759. [PubMed: 10555742]
2. Bockhorn M, Roberge S, Sousa C, Jain RK, Munn LL. Cancer Res. 2004; 64:2469–2473. [PubMed: 15059900]
3. Rafii S, Lyden D, Benezra R, Hattori K, Heissig B. Nat. Rev. Cancer. 2002; 2:826–835. [PubMed: 12415253]
4. Butler TP, Gullino PM. Cancer Res. 1975; 35:512–516. [PubMed: 1090362]
5. Stoll BR, Migliorini C, Kadambi A, Munn LL, Jain RK. Blood. 2003; 102:2555–2561. [PubMed: 12775571]
6. Beerepoot LV, Mehra N, Linschoten F, Jorna AS, Lisman T, Verheul HM, Voest EE. Cancer Lett. 2004; 213:241–248. [PubMed: 15327840]
7. Beerepoot LV, Mehra N, Vermaat JS, Zonnenberg BA, Gebbink MF, Voest EE. Ann. Oncol. 2004; 15:139–145. [PubMed: 14679134]
8. Del Papa N, Colombo G, Fracchiolla N, Moronetti LM, Ingegnoli F, Maglione W, Comina DP, Vitali C, Fantini F, Cortelezzi A. Arthritis Rheum. 2004; 50:1296–1304. [PubMed: 15077314]
9. Dignat-George F, Sampol J. Eur. J. Haematol. 2000; 65:215–220. [PubMed: 11073162]
10. Monestiroli S, Mancuso P, Burlini A, Pruner G, Dell'Agnola C, Gobbi A, Martinelli G, Bertolini F. Cancer Res. 2001; 61:4341–4344. [PubMed: 11389057]
11. Popa ER, Kas-Deelen AM, Hepkema BG, Van Son WJ, The TH, Harmsen MC. Transplantation. 2002; 74:1320–1327. [PubMed: 12451273]
12. Zhang H, Vakil V, Braunstein M, Smith EL, Maroney J, Chen L, Dai K, Berenson JR, Hussain MM, Klueppelberg U, Norin AJ, Akman HO, Ozcelik T, Batuman OA. Blood. 2005; 105:3286–3294. [PubMed: 15618473]
13. Jain RK, Duda DG. Cancer Cell. 2003; 3:515–516. [PubMed: 12842078]
14. Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, Chung DC, Sahani DV, Kalva SP, Kozin SV, Mino M, Cohen KS, Scadden DT, Hartford AC, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Chen HX, Shellito PC, Lauwers GY, Jain RK. Nat. Med. 2004; 10:145–147. [PubMed: 14745444]
15. Cheng J, Kricka LJ, Sheldon EL, Wilding P. Microsystem Technology in Chemistry and Life Science. 1998; vol. 194:215–231.
16. Abramowitz S. Biomed. Microdevices. 1999; 1:107–112. [PubMed: 16281110]
17. Ridings J, Weedon H, Ioannou C, Flego L, Macardle PJ, Zola H. J. Immunol. Methods. 1996; 195:43–48. [PubMed: 8814318]
18. Szekeres E, Monostori E, Ando I, Petri I. Haematologia (Budap.). 1990; 23:229–232. [PubMed: 2101807]
19. Phillips WA, Hosking CS, Shelton MJ. Scand. J. Clin. Lab. Invest. 1983; 43:621–627. [PubMed: 6658377]

20. Humpe A, Beck C, Schoch R, Kneba M, Horst HA. *Transfusion*. 2005; 45:1208–1213. [PubMed: 15987368]
21. Lang KS, Lang PA, Bauer C, Duranton C, Wieder T, Huber SM, Lang F. *Cell. Physiol. Biochem*. 2005; 15:195–202. [PubMed: 15956782]
22. Gossett DR, Weaver WM, Mach AJ, Hur SC, Tse HTK, Lee W, Amini H, Di Carlo D. *Analytical and Bioanalytical Chemistry*. 397:3249–3267. [PubMed: 20419490]
23. Kricka LJ, Wilding P. *Anal. Bioanal. Chem*. 2003; 377:820–825. [PubMed: 12925867]
24. Yuen PK, Kricka LJ, Fortina P, Panaro NJ, Sakazume T, Wilding P. *Genome Res*. 2001; 11:405–412. [PubMed: 11230164]
25. Li JM, Liu C, Dai XD, Chen HH, Liang Y, Sun HL, Tian H, Ding XP. *J. Micromech. Microeng*. 2008; 18:7.
26. Sethu P, Sin A, Toner M. *Lab Chip*. 2006; 6:83–89. [PubMed: 16372073]
27. VanDelinder V, Groisman A. *Anal. Chem*. 2007; 79:2023–2030. [PubMed: 17249639]
28. Zheng SY, Liu JQ, Tai YC. *J. Microelectromech. Syst*. 2008; 17:1029–1038.
29. Bhagat AAS, Hou HW, Li LD, Lim CT, Han J. *Lab Chip*. 2011; 11(11):1870–1878. [PubMed: 21505682]
30. Schilling EA, Kamholz AE, Yager P. *Anal. Chem*. 2002; 74:1798–1804. [PubMed: 11985310]
31. Sethu P, Moldawer LL, Mindrinos MN, Scumpia PO, Tannahill CL, Wilhelmy J, Efron PA, Brownstein BH, Tompkins RG, Toner M. *Anal. Chem*. 2006; 78:5453–5461. [PubMed: 16878882]
32. Berger M, Castelino J, Huang R, Shah M, Austin RH. *Electrophoresis*. 2001; 22:3883–3892. [PubMed: 11700717]
33. Fu AY, Spence C, Scherer A, Arnold FH, Quake SR. *Nat. Biotechnol*. 1999; 17:1109–1111. [PubMed: 10545919]
34. Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, Smith MR, Kwak EL, Digumarthy S, Muzikansky A, Ryan P, Balis UJ, Tompkins RG, Haber DA, Toner M. *Nature*. 2007; 450:1235–1241. [PubMed: 18097410]
35. McClain MA, Culbertson CT, Jacobson SC, Ramsey JM. *Anal. Chem*. 2001; 73:5334–5338. [PubMed: 11721938]
36. Talasaz AH, Powell AA, Huber DE, Berbee JG, Roh KH, Yu W, Xiao WZ, Davis MM, Pease RF, Mindrinos MN, Jeffrey SS, Davis RW. *Proc. Natl. Acad. Sci. U. S. A*. 2009; 106:3970–3975. [PubMed: 19234122]
37. Blankenstein G, Larsen UD. *Biosens. Bioelectron*. 1998; 13:427–438.
38. Wilding P, Kricka LJ, Cheng J, Hvizhia G, Shoffner MA, Fortina P. *Anal. Biochem*. 1998; 257:95–100. [PubMed: 9514776]
39. Ramsey JM. *Nat. Biotechnol*. 1999; 17:1061–1062. [PubMed: 10545906]
40. Wilding P, Kricka LJ. *Trends Biotechnol*. 1999; 17:465–468. [PubMed: 10557158]
41. Vilkner T, Janasek D, Manz A. *Anal. Chem*. 2004; 76:3373–3385. [PubMed: 15193114]
42. Schmid-Schonbein GW, Usami S, Skalak R, Chien S. *Microvasc. Res*. 1980; 19:45–70. [PubMed: 7360047]
43. Pearson MJ, Lipowsky HH. *Am. J. Physiol.-Heart and Circulatory Physiology*. 2000; 279:1460–1471.
44. Sun CH, Migliorini C, Munn LL. *Biophys. J*. 2003; 85:208–222. [PubMed: 12829477]
45. Goldsmith HL, Spain S. *Microvasc. Res*. 1984; 27:204–222. [PubMed: 6708830]
46. Goldsmith HL, Spain S. *Kroc. Found. Ser*. 1984; 16:131–146. [PubMed: 6585479]
47. Colditz IG. *Surv. Synth. Pathol. Res*. 1985; 4:44–68. [PubMed: 3895348]
48. Nobis U, Pries AR, Cokelet GR, Gaetgens P. *Microvasc. Res*. 1985; 29:295–304. [PubMed: 3999988]
49. Pearson MJ, Lipowsky HH. *Microcirculation*. 2004; 11:295–306. [PubMed: 15280083]
50. Nash GB, Watts T, Thornton C, Barigou M. *Clin. Hemorheol. Microcirc*. 2008; 39:303–310. [PubMed: 18503139]
51. Bagge U, Blixt A, Strid KG. *Int. J. Microcirc. Clin. Exp*. 1983; 2:215–227. [PubMed: 6678848]

52. Bagge U, Karlsson R. *Microvasc. Res.* 1980; 20:92–95. [PubMed: 7412589]
53. Phibbs RH. *Am J Physiol.* 1966; 210:919–925. [PubMed: 5947268]
54. Munn LL, Dupin MM. *Ann. Biomed. Eng.* 2008; 36:534–544. [PubMed: 18188702]
55. Melder RJ, Yuan J, Munn LL, Jain RK. *Microvasc. Res.* 2000; 59:316–322. [PubMed: 10684738]
56. Jain A, Munn LL. *PLoS One.* 2009; 4:e7104. [PubMed: 19768109]
57. Shevkoplyas SS, Yoshida T, Munn LL, Bitensky MW. *Anal. Chem.* 2005; 77:933–937. [PubMed: 15679363]
58. Jain, A.; Munn, LL. *Lab-on-a-Chip Technology*. Herold, KE.; Rasooly, A., editors. Vol. vol. 2. Caister Academic Press; Norwich (UK): 2009.
59. Duffy DC, McDonald JC, Schueller OJA, Whitesides GM. *Anal. Chem.* 1998; 70:4974–4984. [PubMed: 21644679]
60. Whitesides GM, Ostuni E, Takayama S, Jiang XY, Ingber DE. *Annu. Rev. Biomed. Eng.* 2001; 3:335–373. [PubMed: 11447067]
61. Johnston BM, Johnston PR, Corney S, Kilpatrick D. J. *Biomech.* 2004; 37:709–720. [PubMed: 15047000]
62. Cho YI, Kensey KR. *Biorheology.* 1991; 28:241–262. [PubMed: 1932716]
63. Ballyk PD, Steinman DA, Ethier CR. *Biorheology.* 1994; 31:565–586. [PubMed: 7833458]

Fig. 1.

Design of nucleated cell separation unit. A. Schematic of the separation device. The device is made using PDMS soft lithography. B. Illustration of working principle. Whole blood enters the device at the inlet port. Nucleated cell margination is encouraged in the pre-extraction stage of the device. With the NCs segregated near the wall, they can then be collected into the extraction channels. Pure RBCs are also collected through the central drain channel. C. A snapshot of the extraction region of the device. Labeled NCs can be seen entering the extraction channels at top and bottom.

Fig. 2.

Empirical optimization of pre-extraction section. A. Thickness of plasma layer near the wall as a function of the number of expansion-contractions for various channel geometries. Measurements were made at the locations indicated in panel A. The plasma thickness is largest in the triangular channels. B. Margination in various geometries after 10 expansion-contractions. The dimensions of different channels are in microns. C. The plasma thickness and mean NC centroid position as a function of the number of triangular units. By 10 expansions, the size of the plasma layer and the NC positions plateau, indicating that 10 units are sufficient. D. Snapshots of NCs in whole blood traveling through a series of 15 expansion/contractions. The labeled NCs are distributed randomly initially (left). After 10 expansions, 98.3% NCs have margined to the sidewall (right).

Fig. 3. Optimization of the extraction section of device using computational modeling. A. Schematic of the region that is optimized. Also shown, streamline distribution computed in COMSOL software showing the point where extraction channel streamlines separate from drain channel streamlines. Lateral movement of this point, achieved by changing the relative flow resistance in the extraction and drain channels, affects how many RBCs are allowed into the extraction channel, and how many NCs are lost in the drain channel. To vary the flow resistance ratio, we adjust the lengths of the extraction and drain channels. B. The streamline farthest from the channel wall that still enters the extraction (situated a distance ϕ from the wall) is plotted against channel length ratio (ω).

Fig. 4. Yield and NC enrichment are measured against ω . Enrichment increases with ω but yield drops precipitously above 2.0 because NCs are lost in the drain channel. The error bars represent one standard deviation. $n = 4$.

Fig. 5.

Yield and NC enrichment are measured against the inlet flow rate. With this configuration, operation is robust, achieving maximum purity and yield at 5 nl s^{-1} . The error bars represent one standard deviation. $n = 4$.