ELECTRONIC SUPPLEMENTARY INFORMATION

Particle Sorting Using a Porous Membrane in a Microfluidic Device

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A. Soft lithography optimization

As discussed, the porous membrane is an essential component of the particle sorter, serving as a filter to separate particles by their size. The main requirement for forming the porous PDMS membrane on the POM-mold is that the thickness of the membrane should be less than the height of the photoresist posts in order to ensure completely through pores. However, the membrane must also be thick enough to withstand peeling from the membrane as well as the fluidic pressure generated during the chip's use. In order to achieve a sufficiently thin membrane, the PDMS prepolymer was diluted in cyclohexane to decrease the viscosity. The cyclohexane was expected to have evaporated quickly during the curing step in an 80 °C oven. However, the dilution of the PDMS prepolymer negatively impacts the toughness of the membrane.

Figure S1 presents a plot of the thickness of the PDMS membrane versus the dilution factor of PDMS prepolymer in cyclohexane as well as the spin-coating speed. First, PDMS prepolymer in varying dilutions of cyclohexane was spin-coated at 500 rpm for 18 s followed by 3000 rpm for 60 s. The data show that a mixture with a dilution factor of 0.2 or less generated membranes with thicknesses larger than 20 μm, which is the height of the photoresist posts on the POM-mold. Considering the toughness and 20 μm limit of the membrane, the thickest membrane under 20 μm, which was obtained from a dilution factor of 0.5, was adopted. Further experiments determined the optimum spin-coating speed under a dilution factor of 0.5. The data show that at a spin-coating speed higher than 2600 rpm, the resulting thickness of the PDMS membrane is less than 20 μm. Again considering toughness and the 20 μm limit, the spin-coating condition for fabricating all the porous PDMS membranes was set to 500 rpm for 18 s followed by 3000 rpm for 60 s. To summarize, these experiments led us to adopt a dilution factor of 0.5 and a spin-coating speed of 3000 rpm to obtain the thickest possible membrane under 20 μm. To make the porous membrane, the PDMS prepolymer mixture was spin-coated on the POM-mold according to the above settings and cured in an oven at 80 °C for 20 min. To peel off the porous membrane without damaging either the membrane or the photoresist posts, specially designed PDMS structures were used, the requisite height and width of which was determined according to the dimensions of the region containing the photoresist posts. Two alternative approaches were used to peel off the porous membrane from the POM-mold. First, a specifically fabricated PDMS support frame was used to bond with the membrane outside of the area containing the photoresist posts. The PDMS frame was cut off after bonding the membrane with other layers. Second, one of the flow layers could be aligned and bonded to the porous membrane directly. The chamber on the flow layer should be sufficiently tall and wide so as to not contact the photoresist posts. By these means we were able to peel the membrane from the POM-mold without removing the photoresist posts.

B. Controlling the pore size

Although more expensive transparency or chrome masks that provide higher resolution are commercially available, a transparency mask with 40,640 dpi resolution was adopted in our experiments, which is much cheaper and more convenient for fabricating the mask for the POM-mold.

When choosing a photoresist for fabrication of the POM-mold, we considered two main requirements. First, photoresist posts with large aspect ratios were needed in order to produce a thick membrane (tall photoresist posts) with small pores (narrow photoresist posts). However, there is an upper limit for the aspect ratio that a given photoresist material can achieve. Second, the photoresist posts should adhere firmly to the wafer surface so that they survive photolithography and repeated uses in soft lithography. With these requirements in mind, we tested the abilities of two available photoresists, SU-8 2015 (a negative resist) and SPR 220-7 (a positive resist readily supplied by the Stanford Nanofabrication Facility), to form the desired structures 20 μm in height. To enhance the adherence of the photoresist to the wafer surface, silicon wafers were cleaned with standard piranha solution and treated with HMDS prior to spin-coating. We determined that SPR 220-7 demonstrates superior surface adhesion compared SU-8 2015. However, SPR 220-7 was not capable of forming a 20 μm thick layer in a single spin-coating run, which meant that two coats were required.

Table S1 presents a comparison of expected pore sizes, their printed sizes on the masks, and the actual pore sizes obtained on the PDMS porous membranes. We printed masks for positive and negative photolithography with four expected pore sizes: 8, 9, 10, and 17 μm. It is important to note that our masks had a limited resolution that impacted the size of the printed images. For the negative mask used with SU-8 2015, where the pore image was transparent and the mask background was black, pore images were always smaller than the expected sizes. Conversely, the pore images on the positive mask required by SPR 220-7, with black pore images printed on a transparent background, were larger than the expected sizes.

Following photolithography and subsequent fabrication of porous PDMS membranes, we determined that the size distribution of pores generated from molds composed of negative photoresist appeared to be more regular than that of pores generated from positive photoresist molds. The reasons for this finding are: (1) due to the different light sensitivities of the two photoresists, a much longer exposure time is required for the positive photoresist, which induces diffraction and over-exposure, thus decreasing the size of the photoresist posts and the pores that are made from them, which turn out uncontrollable size transfer from the mask to the photoresist pattern on the mold; and (2) the low viscosity of positive photoresist meant that two coats had to be applied to obtained a 20 μm-thick layer, which results in a rougher surface. Additionally, the data in Table S1 show that, for positive photoresist, the magnitude of the error increases for smaller pores. Briefly, the character of positive photoresist determined the limit of usage for the fabrication of smaller posts. Therefore when our experiment called for pores with a regular controllable size under 15 μm, negative photoresist SU-8 2015 was selected to fabricate the POM-mold. However, in order to obtain pores larger than 15 μm, positive photoresist SPR 220-7 was an alternative option because it exhibited stronger adherence to the surface substrate.

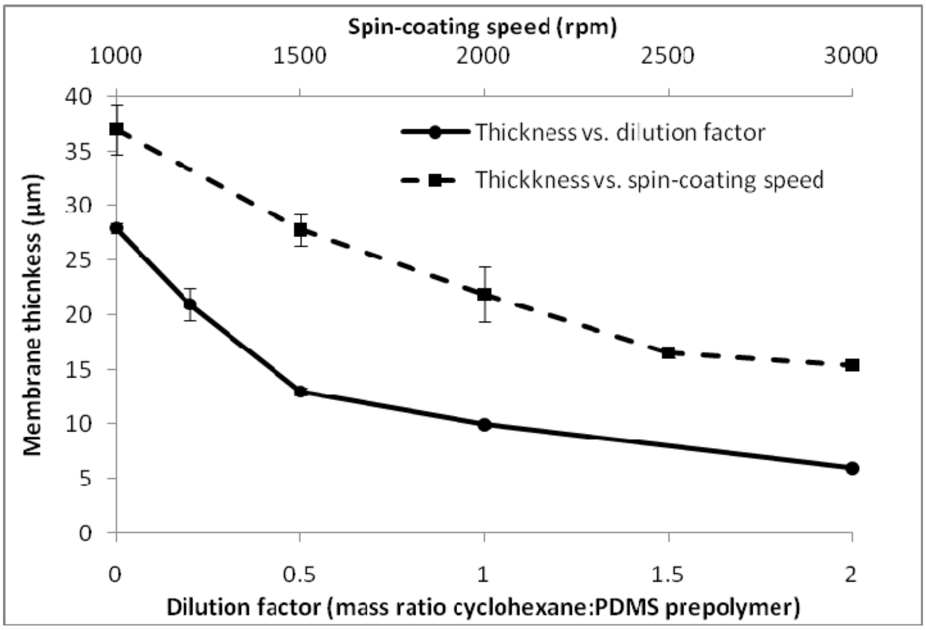

Fig. S1. Plot of the thickness of spin-coated PDMS membranes at various cyclohexane dilutions and spin-coating speeds. The solid line represents membrane thickness versus dilution factor of PDMS prepolymer in cyclohexane, spun at 500 rpm for 18 s followed by 3000 rpm for 60 s. The dashed line represents thickness versus the spin-coating speed, using a dilution factor of 0.5 and a coverage spin speed of 500 rpm for 18 s.

Table S1. Comparison of negative and positive photoresists for the fabrication of different pore sizes.

Photoresist	Expected pore ize (μm)	Printed pore size (μm) on the mask	Measured pore size (μm) on the PDMS membrane
SU-8 2015 negative	8	6.3	6.4±0.3
	9	7.5	7.8±0.3
	10	9.3	9.5±0.4
	17	16.3	16.6±0.3
SPR 220-7 positive	8	8.9	3.8±1.9
	9	9.7	5.6±1.6
	10	10.5	7.1±0.7
	17	17.1	15.7±0.4

C. Reproducibility of the sorting efficiency of whole blood samples

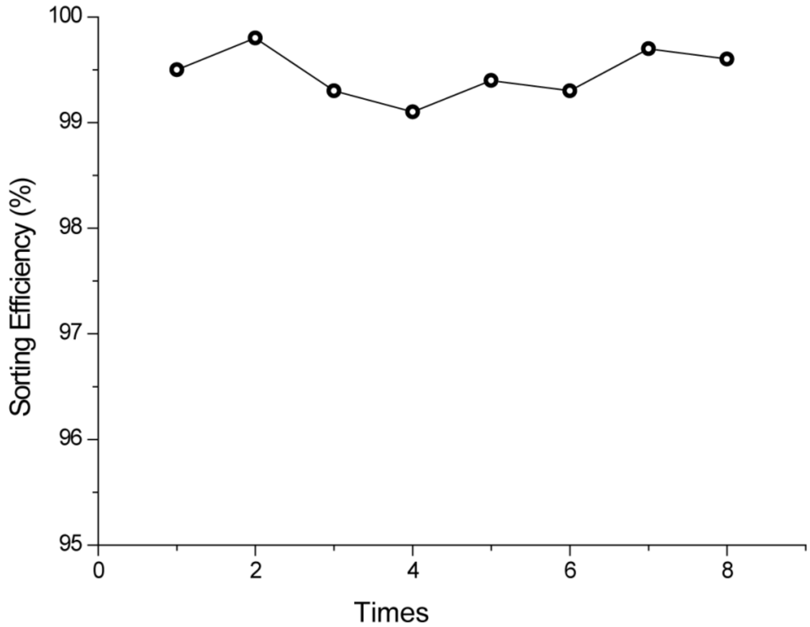

Fig. S2. The whole blood sorting experiment was performed 8 times and demonstrated good reproducibility with an average sorting efficiency of 99.7%.

D. Membrane filter free of clogging

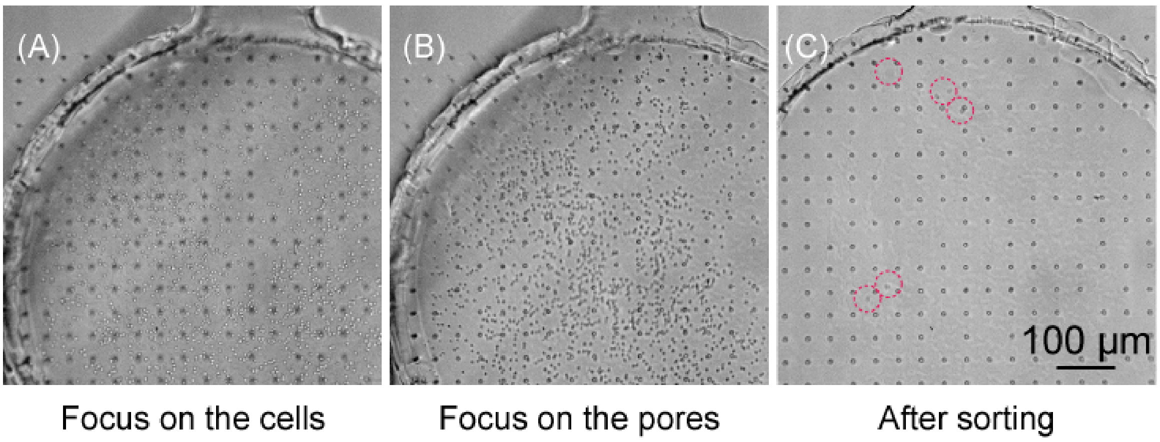

Fig. S3. Images of the same chamber during and after a sorting experiment with whole blood are shown. (A) The chamber filled with sample, with the camera focused on the cells. (B) The same as A), but focused on the pores. (C) The chamber after sorting a sort and collection cycle without an extra flushing step. Red circles mark the few REH cells which adhered to the membrane. These were removed with an extra flushing step.

E. Cell viability after sorting

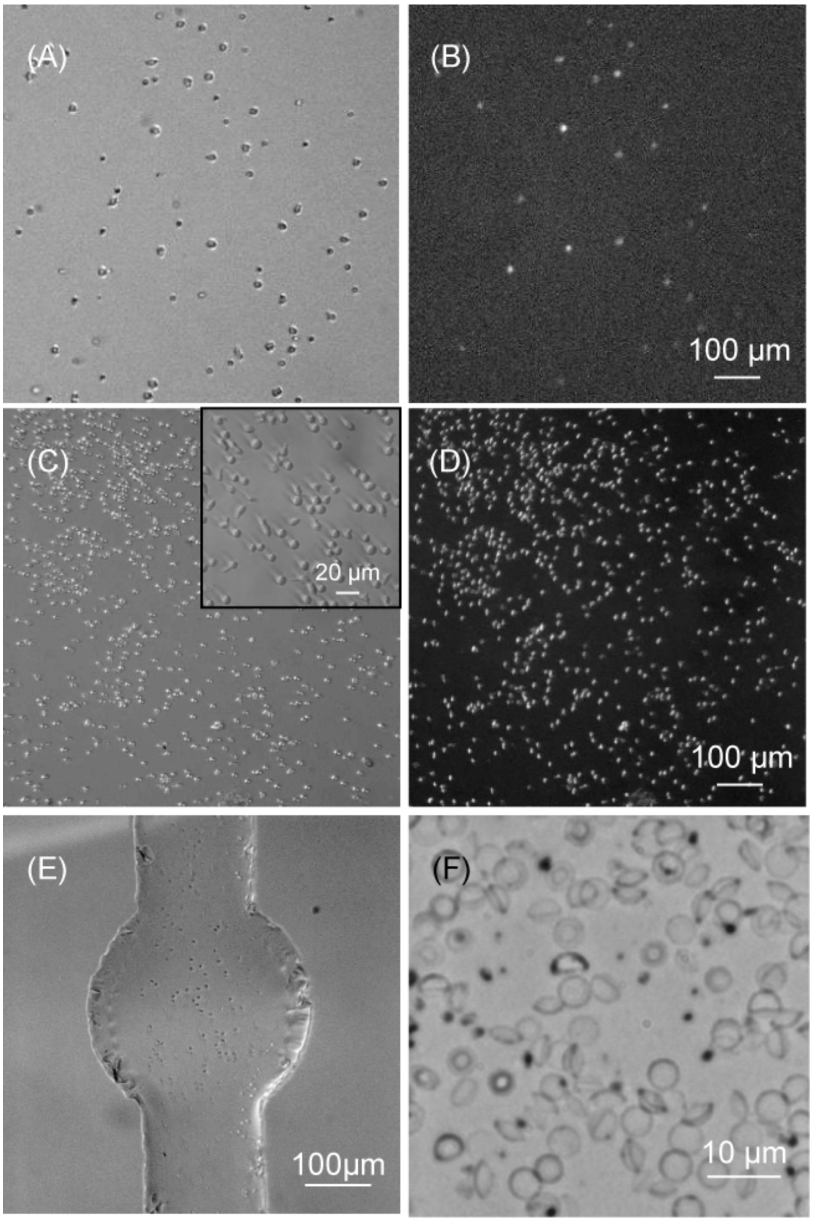

Fig. S4. Images of cell samples before sorting and after collection. REH cells were premarked with CellTracker Orange CMTMR, which can visualize the viability of cells. (A) Original sample containing mouse whole blood and marked REH cells. (B) Fluorescent image of the original sample. The marked REH cells can be observed. (C) Collected REH cells and WBCs from the whole blood sample. The inset shows the enlarged image of the cells. (D) Fluorescent image of the collection. The fluorescence intensity confirmed the viability of the REH cells was still high after being sorted from the whole blood sample. (E) Image of the channel that collects RBCs and platelets. (F) Enlarged image of collected RBCs and platelets.