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## Supporting Information for

# **Hemolysis-Free Blood Plasma Separation**

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#### Fabrication of microfluidic blood plasma separation device

Fig. S1. (a) Design of top and bottom PDMS layers. (b) Fabrication process of filter-in-t op microfluidic blood plasma separation device. First, top and bottom PDMS layers wer e bonded together after  $O_2$  plasma treatment. The inlet and vertical up-flow channel wer e created with a Harris Uni-Core<sup>TM</sup> punch. The PDMS layer was attached to bottom plas

tic substrate, and a membrane filter (5 mm) was placed on top of the vertical up-flow ch annel. Finally, the top cover plastic was bonded to the top PDMS layer.

The microfluidic channels were fabricated using standard soft lithography replica moldi ng techniques. Briefly, a mold for the top layer was created through a double-layer proc ess using negative photoresist and a silicon wafer. The 5  $\mu$ m-thick first layer (blue circle in Fig. S1a) for embedment of membrane filter was fabricated with SU8-3005 (Microch em U.S.A). The diameters of the first layer and membrane filter were 5.5 and 5 mm, res pectively. To prevent leakage of RBCs, the thickness of first layer was designed to be lo wer than that of membrane filter (~8  $\mu$ m). The 80  $\mu$ m-thick second layer, which include s ruler markings to measure the separated plasma volume, was fabricated with SU8-303 5 (Microchem U.S.A). The width of the second layer channel was 200  $\mu$ m. For the botto m layer, a mold was created through a 100  $\mu$ m-thick single-layer process using negative photoresist, SU8-2100 (Microchem U.S.A.) on a silicon wafer. PDMS (Sylgard 184, Do w Corning) was prepared with a 10:1 mass ratio (base to cross-linker); degassed in a vac uum chamber for 30 min; then poured on the SU8 mold to a thickness of ~ 0.9 mm for e ach layer; then cured in an oven at 60 °C for at least 2 h. The PDMS was then carefully peeled off the mold. Fig. S4b shows the fabrication process of filter-in-top device.

#### Calculation of drag coefficient



Fig. S2. (a) Dimensions and (b) 3-dimensional schematic illustration of RBCs used for c alculation of drag coefficient. (c) Calculated drag coefficient along x-, y-, and z- directio ns.

The drag coefficient of single red blood cells (RBCs) was calculated by fluidic simulati on with a 3-dimensional RBCs model as shown in Fig. S2. The sectional shape of the m odel was based on Ref. 1 and revolved along the z-axis.<sup>1</sup> A uniform flow was applied to x-, y-, and z-direction and each drag force was calculated. The average value of three dr ag forces normalized by flow velocity was taken as the drag coefficient, since drag force is linearly proportional to the drag coefficient in low Reynolds number.



Fig. S3. (a) Several microfluidic blood plasma separation devices with large filter (devic e B, E) and multiple filters (device C, D) for increased plasma volume. (b) Separated pla sma volume measured 20 min after blood drop with different device design.



Fig. S4. Measured plasma volume (left axis) and changes of plasma volume  $(v_2-v_1)$  per unit time (right axis) with vertical up-flow channel diameters of (a) 2.5 mm, (b) 3.0 mm,

(c) 3.5 mm, and (d) 4.0 mm in filter-in-top blood plasma separation device.

### References

1. O. Shardt and J. J. Derksen, Int. J. Multiphase Flow, 2012, 47, 25–36.