Supporting information

Humidity-enhanced microfluidic plasma separation on Chinese Xuan-papers

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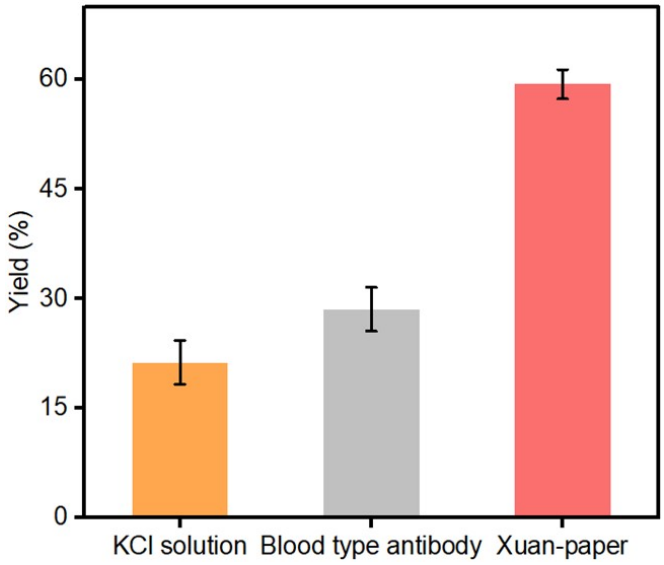

Fig. S1. Comparison of the yields of three methods for plasma separation. Here, we repeat the experiments of the two references through using their methods and materials and measure the plasma yields of three methods (KCl solution[1], blood type antibody[2] and our method). Overall, these two methods can only obtain an extremely low plasma yield on a paper (<30%).

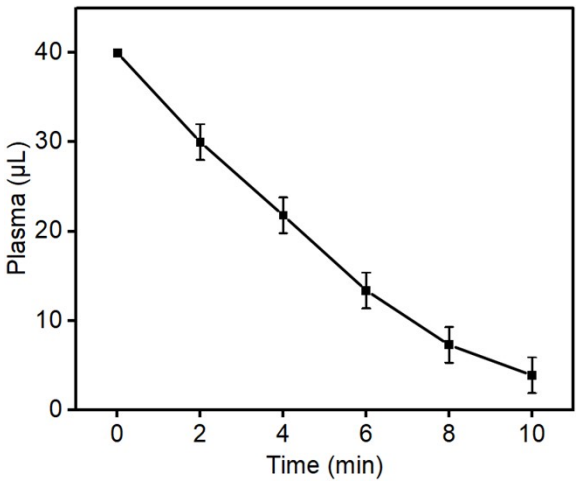

Fig. S2. Evaporation of plasma volume over time when the environment is in open-air mode. In particular, when the time increases to 5 minutes, the volume of plasma is only 45% of the initial volume. We conduct a plasma vaporization experiment (40 μL pure plasma) on a filter paper when the environment is in open-air mode (temperature: 23.2 ℃, humidity: 46.2% and windless). Firstly, 40 μL of plasma is dropped on the filter paper. Then, we put the filter paper with plasma in open-air mode and weigh it every 2 minutes.

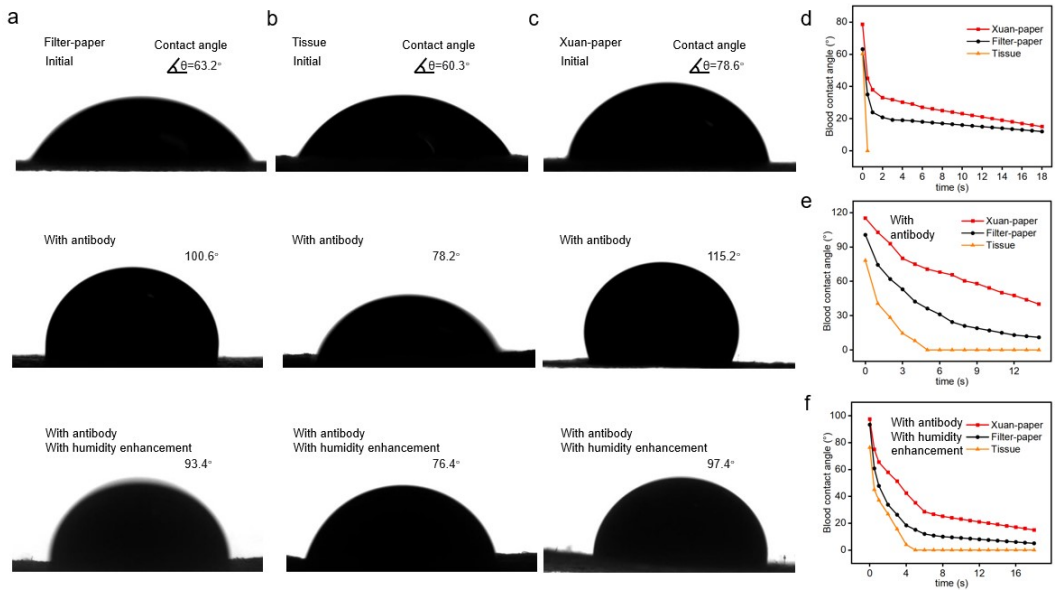

Fig. S3. Effect of antibodies and increased humidity on blood contact angles. (a) The contact angles of the filter paper, the filter paper with antibody, and the filter paper with antibody and humidity enhancement. (b) The contact angles of the tissue, the tissue with antibody, and the tissue with antibody and humidity enhancement. (c) The contact angles of the Xuan paper, Xuan paper with antibody, and Xuan paper with antibody and humidity enhancement. (d) The contact angle changes of the original three materials over time. (e) The contact angle changes of the three materials coated with antibody over time. (f) The contact angles changes of the three materials with antibody and humidity enhancement over time. The average of the five experimental results is defined as the final contact angle result.

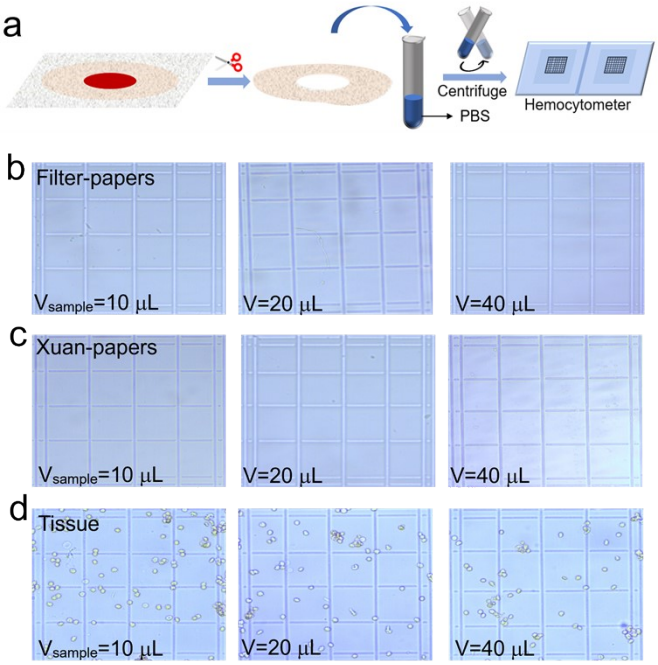

Fig. S4. The testing process of plasma purity. (a) The number of red blood cells is characterized using a blood cell counting plate. (b-d) Blood cell counting plate for plasma purity characterization of filter paper, Xuan paper and tissue.

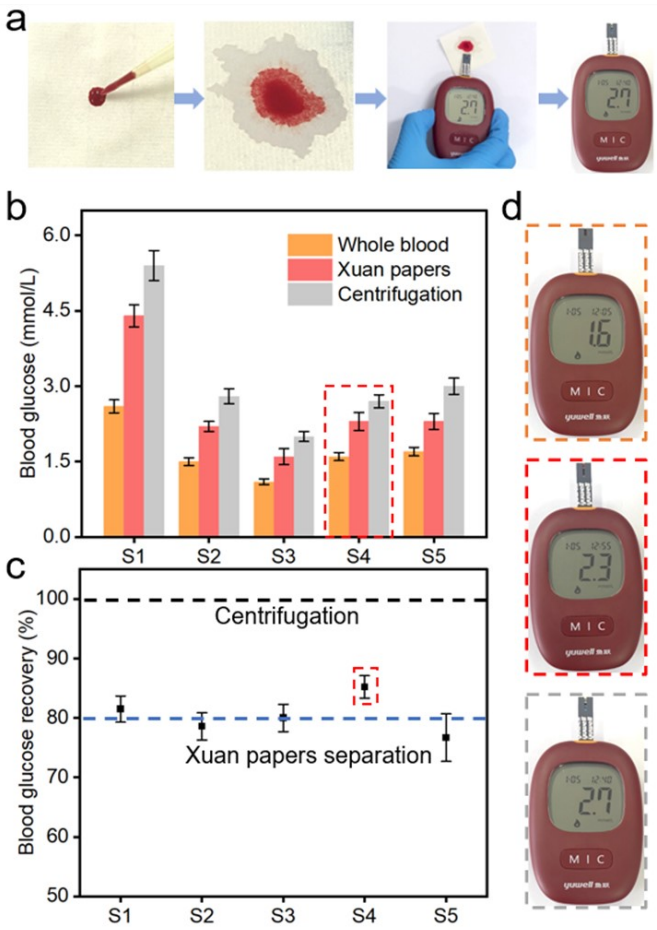

Fig. S5. Detecting the recovery rate of blood glucose. (a) Procedure for detection the value of blood glucose by blood glucose meters. (b) For five sets of samples, blood glucose values from whole blood, plasma separated by centrifugation and plasma extracted by our method are compared. (c) Consider the centrifuge as the gold standard and characterize the glucose recovery rate of Xuan-paper. We can see that the blood glucose recovery rates of Xuan-paper basically keep around 80%. (d) Blood glucose values of the sample 4 measured by the three methods.

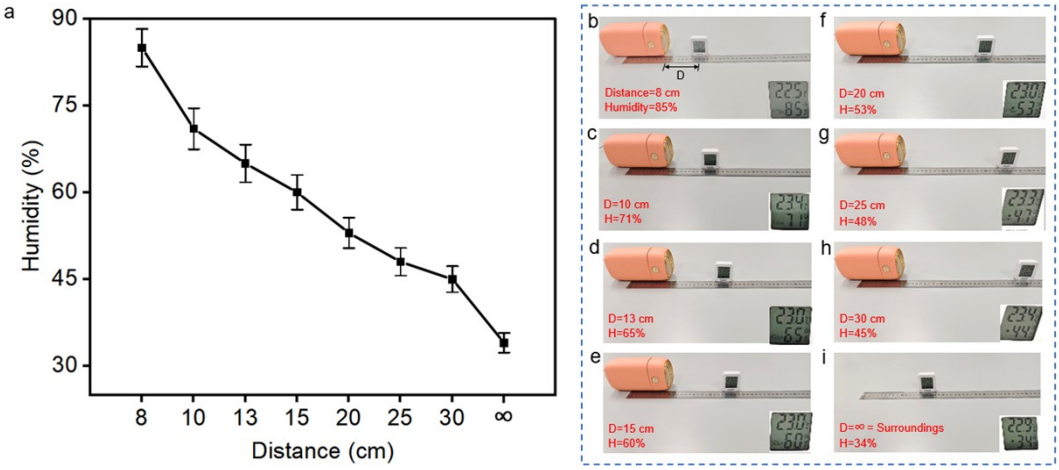

Fig. S6. Relationship between environmental humidity and distance to the humidifier (a) The relationship between ambient humidity and the distance from the humidity detector to the humidifier. (b-i) Real images of ambient humidity in different positions. The distance approaching infinity is the original environmental humidity.

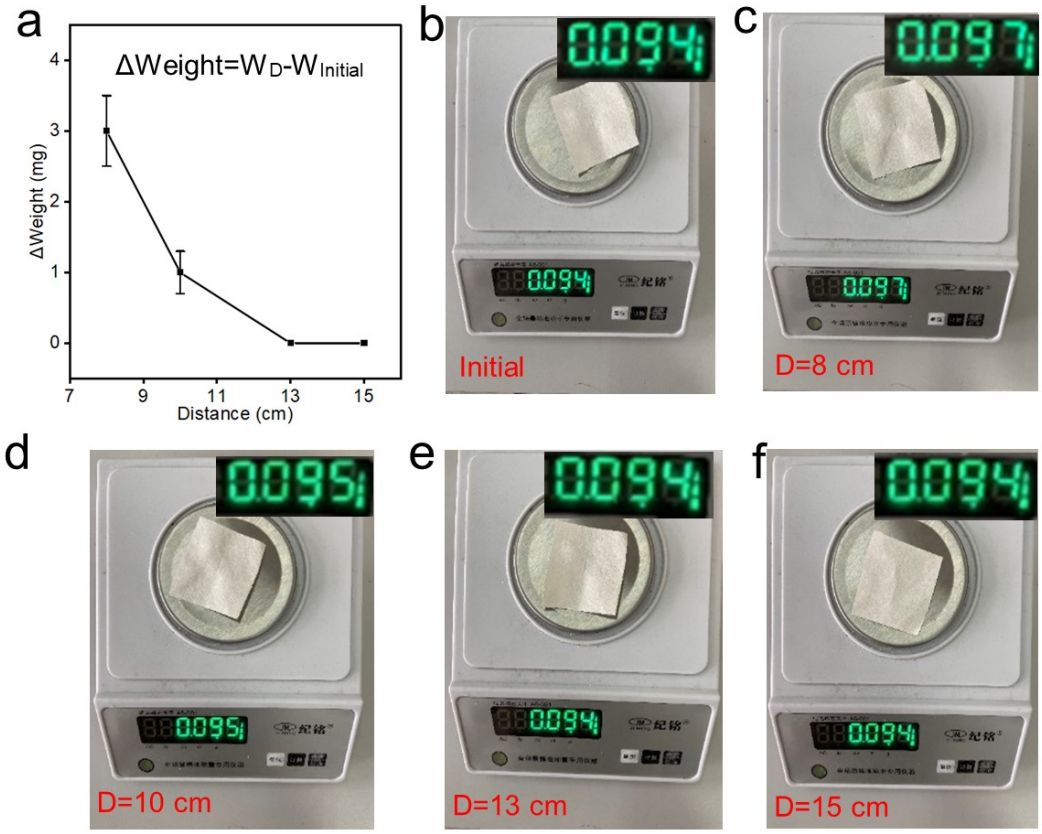

Fig. S7. Variation of the distance from the humidifier to the center of the paper in relation to the weight of the paper. (a) The change in the weight of the paper before and after the humidification experiments decreases as the distance from the humidifier to the center of the paper increases. (b) The original weight of the paper. (c-f) Weight of the paper at a distance of 8-15cm from the humidifier. Once the distance reaches 13 cm, the weight of the paper does not change after humidification. In other words, the paper will not absorb the water vapor from the humidifier when the distance is more than 13 cm. As a result, the presence of water vapor does not affect the paper weight or the following plasma volume within 5 minutes.

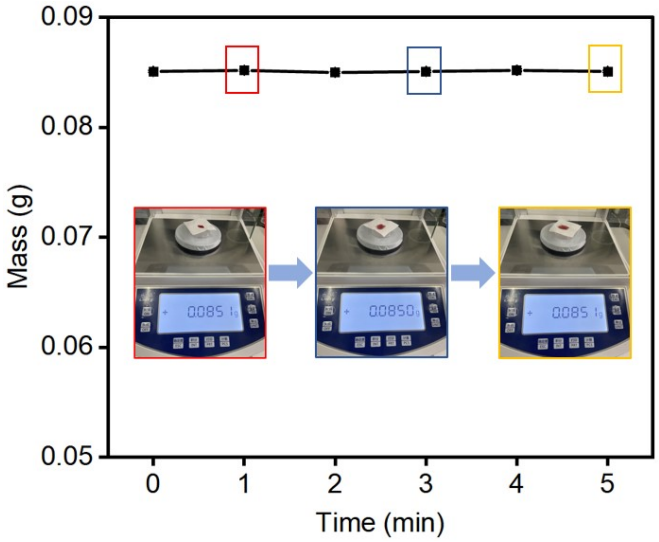

Fig. S8. The mass of Xuan paper during the 5 min experiments with humidity enhancement. The inset shows the real images of the mass of Xuan paper during 5 min. The error bars give the deviation values (N = 3). Firstly, 20 μL of whole blood drops is placed on the antibody-coated Xuan paper and the mass of the paper is 0.0851g. Then, the humidifier is positioned on the side of the paper parallel with the paper to create a relatively high humidity environment to improve the plasma separation. We measure the mass of paper per minute during the 5-minute plasma separation process. The stability of the mass of Xuan paper can prove that the collected volume is pure plasma and not influenced by absorbed ambient humidity.

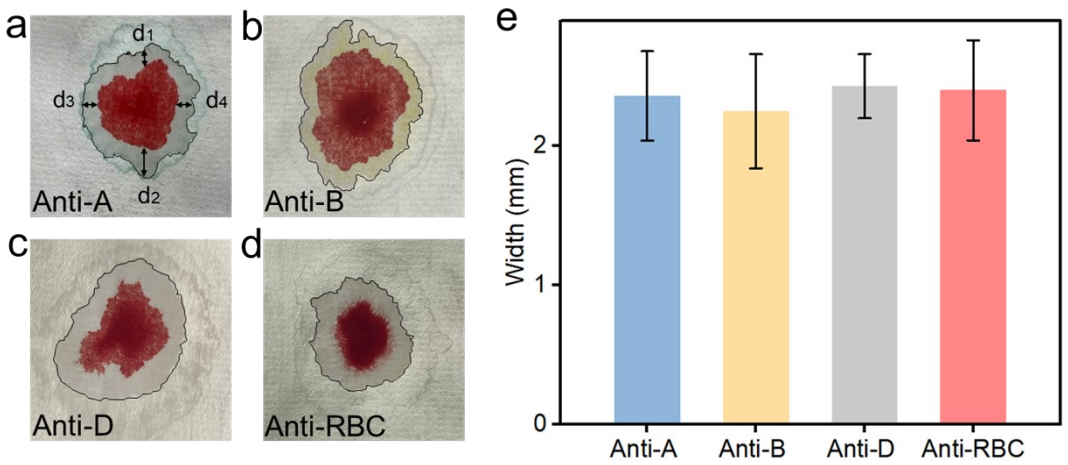

Fig. S9. (a-d) Plasma obtained by using different dry antibodies (Anti-A, Anti-B, Anti-D, and Anti-RBC) on Xuan-papers. (e) Statistical results of the average plasma separation widths. There is almost no difference in plasma separation effect.

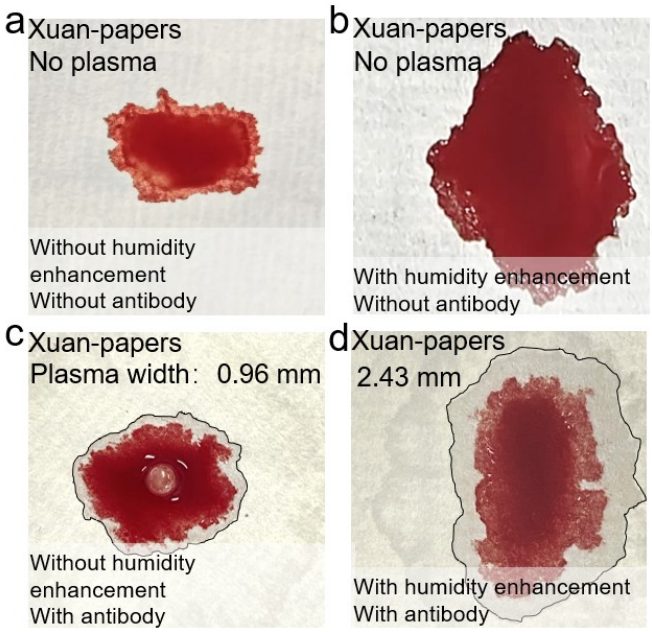

Fig. S10. Plasma separation effects on the conditions of with humidity enhancement/without humidity enhancement and with/without antibody on Xuan-papers. (a) Image of plasma separation on Xuan paper under the condition of without humidity enhancement/antibody. (b) Image of plasma separation on Xuan paper under the condition of with humidity enhancement and without antibody. (c-d) Images of plasma separation on Xuan paper under the conditions of without humidity enhancement and without antibody and with humidity enhancement and antibody. Through experimental comparisons, plasma can be effectively separated only in the case of with humidity enhancement and with antibody.

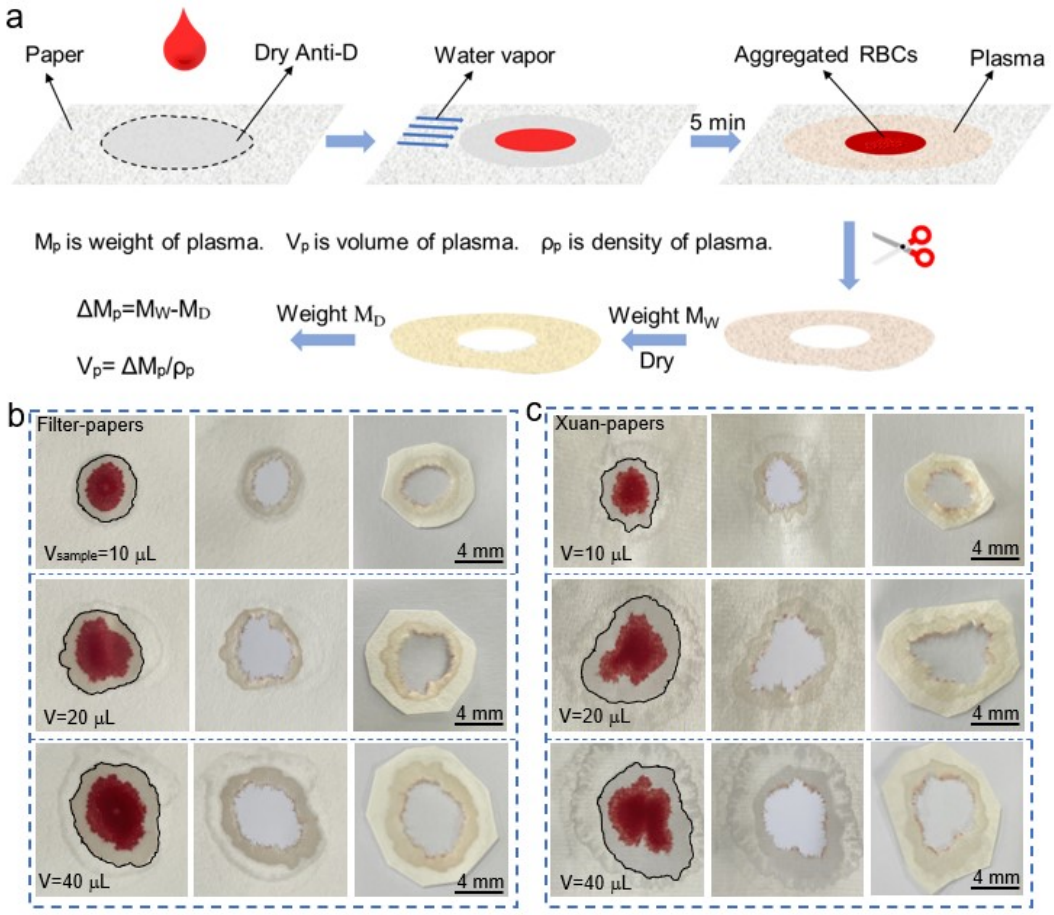

Fig. S11 (a) Procedure for measuring plasma volume. Firstly, the blood cells in the center of paper are cut off, then we cut off the peripheral part of the paper. We measure the weight of the prepared paper with plasma (M_W). Then, the paper is heated until it is completely dry and the weight is measured again (M_D). Then plasma volume can be calculated according to the equation $\Delta M_P=M_W-M_D$ and $Vp=\Delta M_p/\rho_p$ where M_p and V_P are plasma mass and volume of plasma, respectively. ρ_p is the density of plasma. M_W is the mass of wet PPSDs. M_W is the mass of dry PPSD. (b-c) Images of plasma separated by filter paper and Xuan paper. We can observe that the extracted plasma is yellow and pure.

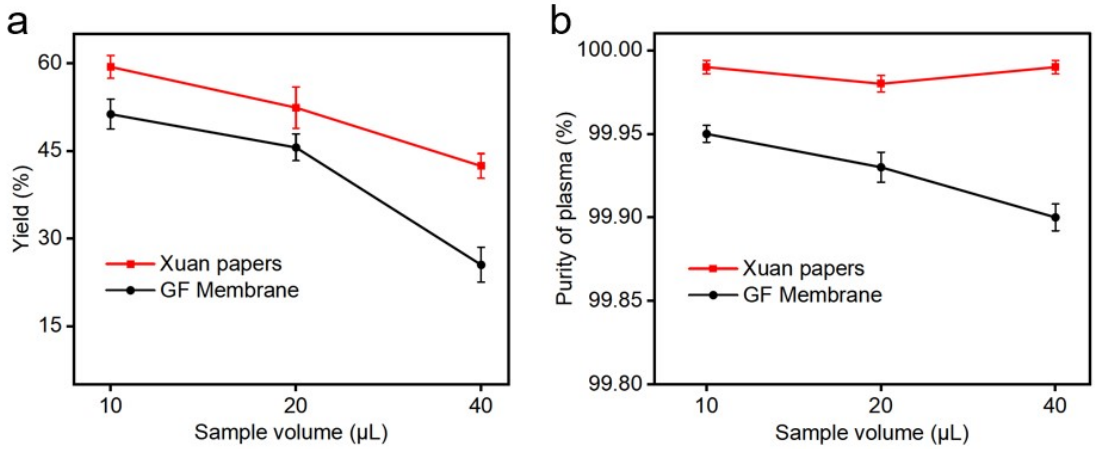

Fig. S12. Plasma yield and purity of Xuan paper and commercialized Vivid^{TM} plasma separation membrane. (a) Relationship between plasma yield and blood sample volume. (b) Plasma purity obtained from different whole blood volumes.We use a commercialized one-step plasma separation membranes (GF membrane, VividTM, Pall Life Sciences) for plasma separation experiments. The separation yield and purity of plasma are shown in Fig. S12. (the hematocrit was 45%). The plasma yield of Xuan paper is higher than that of GF membrane regardless of the volume of the sample (e.g., 10, 20, 40 μL). For example, when the sample volume is 40 μL, the plasma yield of GF membrane is only 25.5%, while the yield of Xuan paper is 42.4%. In addition, the purity of plasma separated by Xuan paper is better than that of GF membrane (Fig. S12b).

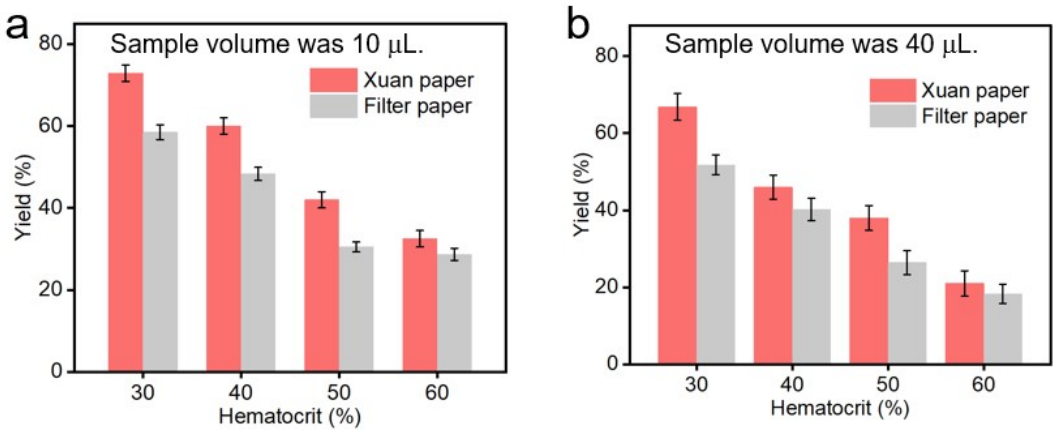

Fig. S13. Plasma yields in different hematocrit conditions for Xuan paper and filter paper. The red bars mark the yield of Xuan paper and the gray bars mark the yield of filter paper.

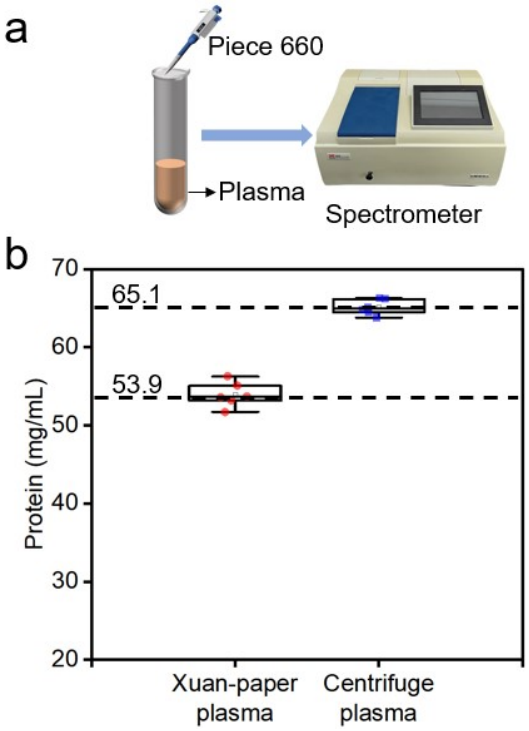

Fig. S14. Detecting the recovery rate of total protein of plasma. (a) Procedure for detection the value of total protein by spectrophotometer. (b) Consider the centrifuge as the gold standard and characterize the total protein recovery rate of Xuan-paper. It can be concluded that the total protein recovery rate of Xuan-paper keep around 82.7% when compared with traditional centrifugation method. We used the Pierce 660-nm protein assay to quantify total protein in plasma samples according to an established protocol[3]. Firstly, we prepare a calibration curve using bovine serum albumin (BSA) solutions over a linear range from 0.05-2 mg/mL. Then, we add 1500 μL of the Pierce 660-nm reagent into a colorimetric dish followed by 100 μL of diluted plasma (1:100 in 1X PBS). Finally, the colorimetric dish is incubated for 5 minutes at room temperature before reading at 660 nm using a spectrophotometry (N4, INESA, China).

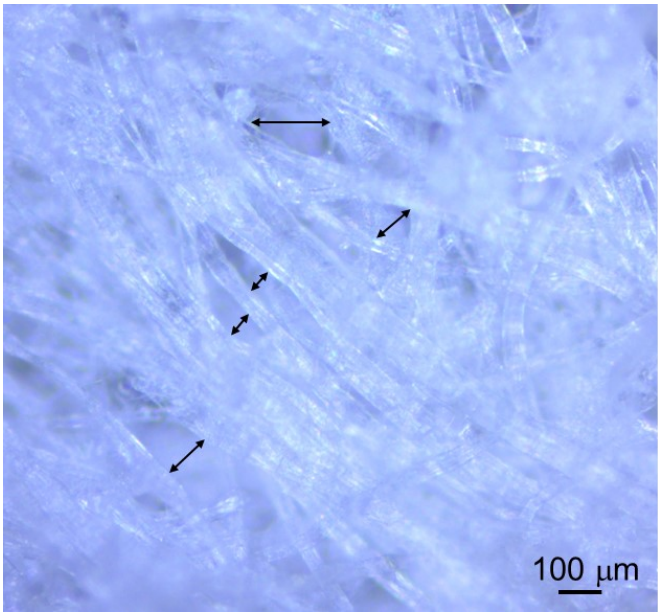

Fig. S15. Micrograph of the pores of Xuan paper. It can be observed that the pore size of Xuan paper is about 100 μm.

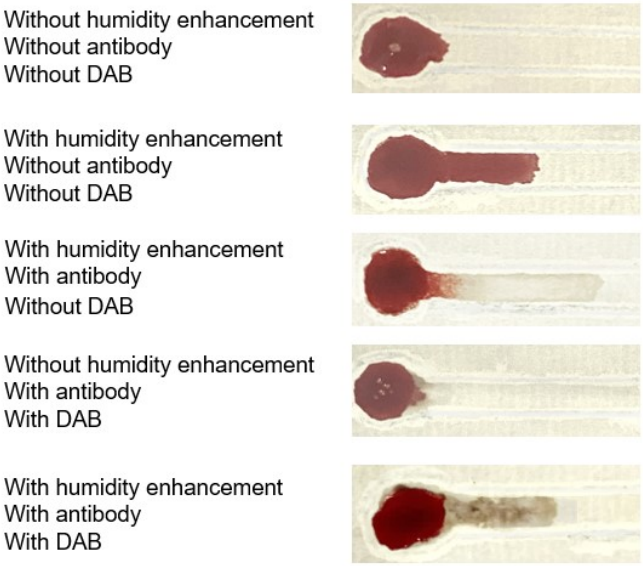

Fig. S16. The real images of Xuan-PADs in the presence or absence of humidity enhancement, antibody and DAB. We can confirm that only in the presence of DAB, with humidity enhancement/antibody, a brown precipitate can be produced along the channel.

Reference

1. A. Nilghaz and W. Shen, *RSC Advances*, 2015, 5, 53172-53179.

2. X. Yang, O. Forouzan, T. P. Brown and S. S. Shevkoplyas, *Lab Chip*, 2012, 12, 274-280.

3. Pierce 660-nm protein assay instructions, Thermo Scientific, 2018, accessed December 18, 2019.