MULTISTEP BRANCHED-MICROCHANNEL NETWORK FOR PURITY-CONTROLLED BLOOD PLASMA SKIMMING

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ABSTRACT

We propose a multistep branched-microchannel network structure to provide a useful technique for realizing purity-controlled blood cell/plasma separation in microfluidics-based separation devices. The present multi-step branching concept is aimed at mitigating the contamination problem due to the incomplete separation during the pre-treatment process in biochemical assays. In this report, a prototype is fabricated using PDMS and its effectiveness is examined through pig blood separation tests. It is successfully demonstrated that the blood plasma could be significantly purified with a series of branch-channel bifurcations.

KEYWORDS

Plasma skimming, Branched microchannel, Multistep, Purity

INTRODUCTION

Recently, microfluidics-based separation techniques [1, 2] have been widely employed in various biomedical applications including point-of-care (POC) diagnostic devices [3]. However, when the sample volume is minute in the order of micro liter, the sample contamination due to the incomplete separation during the pre-treatment process may often deteriorate and impede the biochemical assay. Therefore, in plasma skimming devices, there is a strong need for a remedy to control the purification of yielded plasma according to the required level especially in severe assays. In this report, we propose multistep branched-microchannel network to provide a useful technique for realizing purity-controlled blood cell/plasma separation. For optimizing the channel structure, we investigate the plasma layer thickness in straight PDMS microchannels, and show the measurement results presently obtained for various flow condition. Then, results of pig blood separation tests are presented, showing the effectiveness of our multi-step branching concept.

MULTISTEP BRANCHING CONCEPT

Figure 1 shows the present concept of blood plasma skimming with multistep branching mechanism. The whole blood is supplied from the inlet of the main channel and the plasma component is extracted from the outlet of the branch channels [4]. In microfluidic channels considered here, the Reynolds number is extremely low (below unity), so that the motion of fluids is characterized by steady laminar flow and small particles move along the streamlines. When small solid particles are considered, the particle trajectory at a bifurcation is determined by whether the particle's center is located in the inflow layer ("plasma layer") to the branch channel.

Conventionally, the branching structure is designed such that the plasma layer thickness (L_{pl}) is larger than the inflow layer thickness (L_{in}) at each bifurcation as shown in Fig. 1(a). With increasing the margin ($\Delta L = L_{pl} - L_{in}$), the

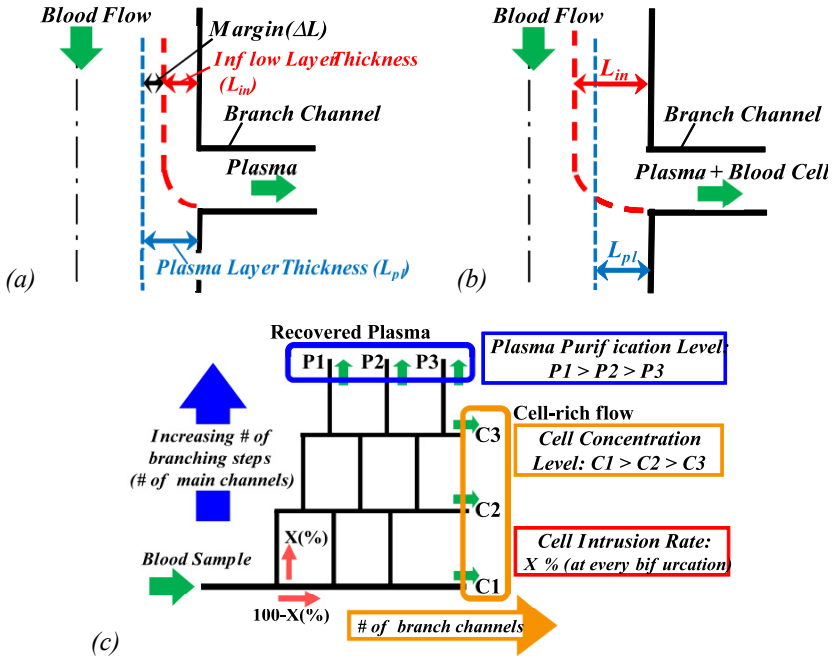

Figure 1: Present concept of blood plasma skimming with multistep branched-channel network: (a, b) relation between the inflow layer and plasma layer thickness, (c) schematic of the multistep branching mechanism.

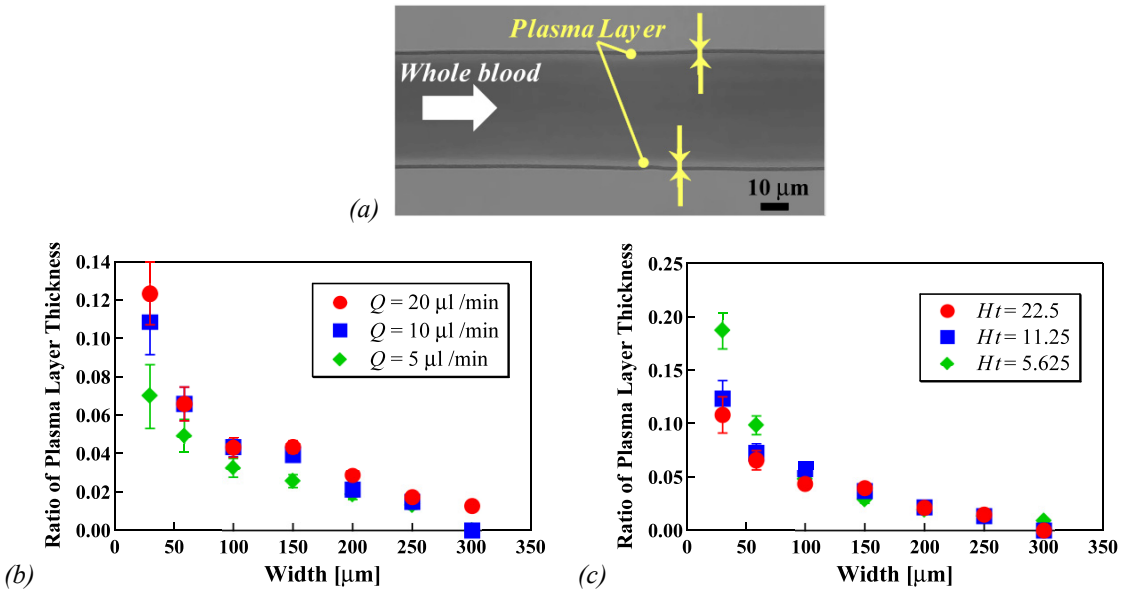

Figure 2. Measurement of the plasma layer thickness in straight microchannels: (a) Plasma layer formation in a PDMS microchannel (width $w = 30\,\mu m$, depth $h = 30\,\mu m$, $Ht = 11.25\%$, flow rate $Q = 10\,\mu l/min$), (b) Plasma layer ratio for different channel width and flow rates, (c) Plasma layer ratio for different channel width and hematocrit. The channel depth is kept constant at 30 μm.

risk of unexpected intrusion of cell components to the branch channel can be lowered at the penalty of reduced plasma recovery rate [2]. The present concept is shown in Fig. 1(b), where $L_{pl} < L_{in}$ at the bifurcation. The present multistep branching network as shown in Fig. 1(c) is based on the following concept: the cell intrusion is allowed in some portion at each bifurcation, but the purity of the plasma could be controlled with successive bifurcations of the branch channels. The cell intrusion rate at every bifurcation is assumed to be constant at X% in volume. By increasing the number of branch channels (N_{ch}), the total amount of plasma yield is increased. By increasing the number of branching steps (N_{step}), on the other hand, the plasma purity and cell concentration level at each outlet can be regulated in a more robust way if compared to the single-step structure.

RESULTS AND DISCUSSION

For efficient use of the Fåhræus effect (cell aggregation into the channel core) [5] in microfluidics-based plasma skimming, we examine the effect of the channel size and flow condition upon the plasma layer formation in PDMS channels. The sample solution is pig blood with varied hematocrit values. The blood flow distribution inside the channel is observed with a high-speed camera, and the plasma layer thickness is obtained by processing a series of gray-scale images. Figure 2 (a) shows a typical snapshot captured in the measurement. The measured plasma layer thickness versus the channel width for different flow rates and hematcrit values is shown in Figs. 2(b) and 2(c). The plasma layer is clearly formed along both sides of the channel wall when the channel width (w) is less than 300 μm. It is seen that the plasma layer thickness is increased for the larger flow rate and smaller hematocrit value. Most importantly, the ratio of the plasma layer thickness to the channel width is found to become increasingly large for smaller channel width. In the meantime, the channel width should be larger than some critical value in order to prevent the hemolysis. Here, we set as w = 58 μm for the first main channel from the inlet, based on the estimated shear rate under the present condition.

Figure 3 shows the experimental results of pig blood separation using the present multistep branched-channel network. The present prototype is fabricated with soft lithography using PDMS. In the present design, the number of branch channels and branching steps is defined as $N_{ch} = N_{step} = 2$ (Fig. 3a). The cell intrusion rate at each bifurcation is set as X = 1%. The width of the branching channels is set equally to ~ 20 μm, and the length of each channel is designed based on an electrical circuit model. In the separation test, the flow rate is chosen as $Q = 10\,\mu l/min$, and as the sample solution, the pig blood diluted with saline solution is prepared ($Ht \sim 22.5$, close to the human blood).

The snapshots at Spot I and Spot II during the separation test are shown in Figs. 3(b) and 3(c). The cell distributions at $Outlet_{c1}$ and $Outlet_{p2}$ are also shown in Figs. 3(d) and 3(e). The number density of blood cells at each outlet is summarized in Fig. 3(f). We performed the separation test 3 times, and the averaged value is presented in the figure. The tendency of the present experimental result is in reasonable agreement with our design strategy. At $Outlet_{c1}$ and $Outlet_{c2}$, the cell component is concentrated due to the plasma skimming to the branch channels. The cell concentration is highest at $Outlet_{c1}$. On the other hand, at $Outlet_{p1}$ and $Outlet_{p2}$, the plasma is expectedly purified after 2-step bifurcation. The purification level is highest at $Outlet_{p1}$. The present technique is quite simple and would be useful in providing purity-controlled blood plasma for the subsequent diagnostics and biochemical assays.

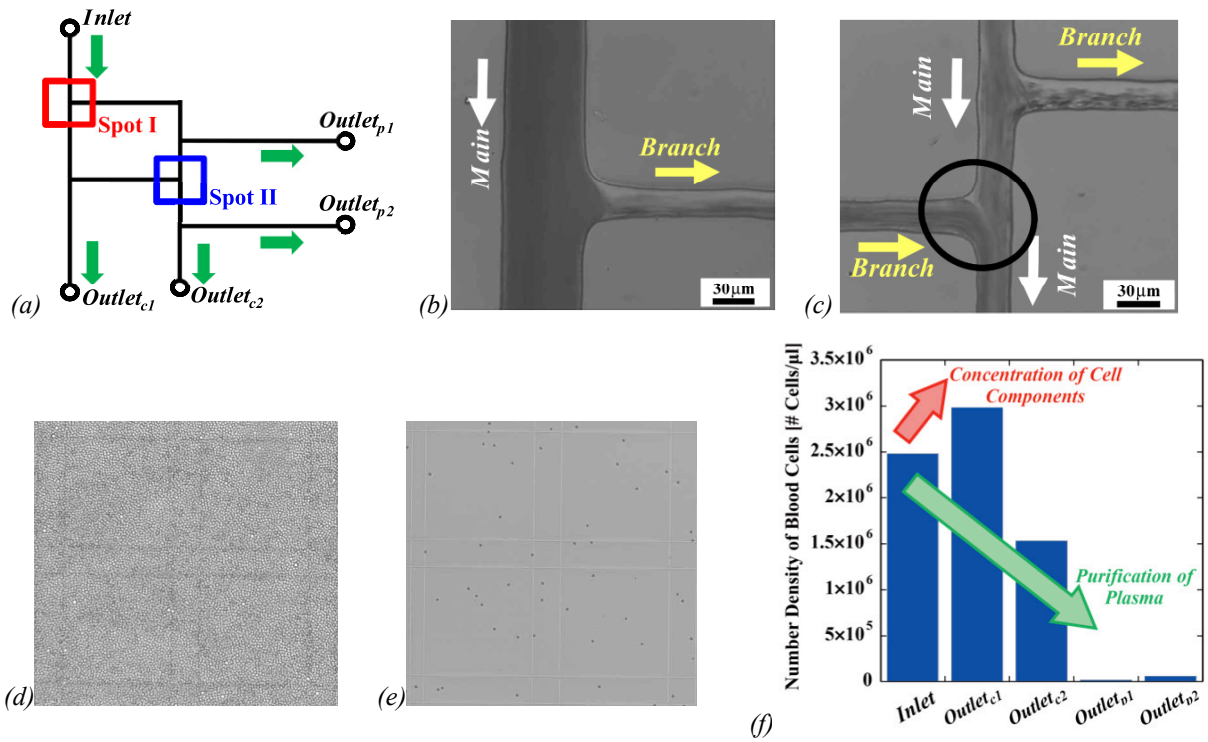

Figure 3: Experimental results of blood plasma separation for the inlet sample of pig blood: (a) Present design with 2-step bifurcation of 2-line branch channels (X =1%), (b) bifurcation at Spot I, (c) bifurcation and merging at Spot II, (d) cell distribution at Outlet$_{c1}$, (e) cell distribution at Outlet$_{p2}$, (f) number density of blood cells at each outlet.

CONCLUSION

A multistep branched-microchannel network structure is proposed in order to provide a useful solution for realizing purity-controlled blood cell/plasma separation. A prototype is fabricated using PDMS and its effectiveness is examined through pig blood separation tests. It is successfully demonstrated that the blood plasma could be significantly purified with a series of branch-channel bifurcations. The present technique is believed to be a viable tool for furthering the conventional plasma skimming technique and blood analysis systems.

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