MULTI-COMPONENT SEPARATION CHIP UTILIZING MICROPILLAR ARRAYS IN SPLITLEVEL SPIRAL CHANNEL

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ABSTRACT

A microchip with multiple rows of micropillar arrays along the spiral-channel, in which plasma and various blood cells with different sizes could be separated or enriched simultaneously, has been achieved. The proposed method combines crossflow, centrifugation and size-selective separation approaches. Specially, a 40μm-high step is designed between the inner and middle channel, which can enhance the separation efficiency. In the chip with the splitlevel channels, the number of RBC gathered from inner outlet is decreased significantly, and the collected plasma volume is increased, compared to those in the isobathic one.

KEYWORDS: Microfluidic, Separation, Spiral channel, Blood

INTRODUCTION

Blood tests are burgeoning into irreplaceable clinical trials methods, which are easily accessible and representative of complex patientpathologic states. However, depending on different diseases, a series component analysis of blood requires constituents' separation before testing and diagnosing. For the sake of speedy, precise and reagent-saving separation, a wealth of emphasis is put on microfluidics technologies, and various miniaturized separation devices have been developed for clinical analysis and biological experiments [1]. The reported methods include pinched flow injection [2], hydrodynamic filtration [3], bifurcation [4], centrifugation [5], etc. In our former work [6], the barrier of blood cell and plasma separating has been removed. Drawing on that, a device, in which red blood cells (RBCs), white blood cells (WBCs) and plasma can be separated in the blood, is presented.

APPROACH

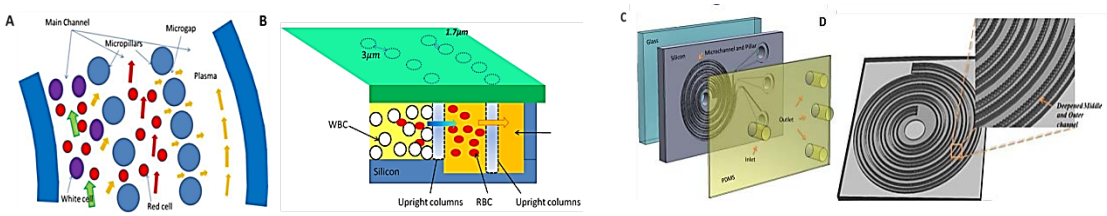

Figure 1: The Schematic diagram of the separation chip. (A) The top view of the separation chip working principle; (B) the cross-section of the separation microchannel, which is divided into three microchannels by upright micropillars;(C) the assembled schematic diagram of the separation chip; (D) the step located between inner and middle channel.

The schematic view of the prototyping separation device for separating plasma, RBCs and WBCs, is shown in Fig. 1. The main spiral-channel is divided into three sub-channels (inner, middle and outer channels) by pillar arrays. The height of the first and second micropillar arrays are 40μm and 80μm, respectively. The width of the main channel is 270μm. The inner, middle and outer channels are 40, 80 and 80μm, respectively. The gap of the first row pillar arrays is 3μm and that of the second row pillar arrays is 1.7μm. The whole size of packaged device is 10mm×5mm×5mm. With the aid of two parallel pillar rounds seated in the inner-middle channel and middle-outer channel, the WBCs stay in the inside channel, while the RBCs are gathered from middle and plasma from outer. Specially, a 40μm-high step (shown in Fig. 1 (B)) is designed between the inner and middle channels, which can improve purity of WBCs collection. By virtue of the step, the difference of pressure between the inner and middle channels is bigger than that of the isobathic chip. Increased amount of RBCs would be driven by inertia to the pillar arrays and pass through the gaps to reach the middle channel. The principle of the improvement is elaborated below aided by simulation.

According to equation of continuity of incompressible fluid, by using Fluent to verify the device design, the model of steady laminar flow with material constant set as blood plasma is employed. Different inflow boundary conditions are carried out for comparison of the isobathic and splitlevel chip. At the inlet, the velocity and the pressure are the same, 0.00207m/s and 1.02×10^5 Pa, respectively. As the result of the simulation shown in Tab. 1, the flow of inner channel is 50.5%

Tab.1 The simulation results of velocity inlet and pressure inlet boundary condition

	Isobathic chip		Splitlevel chip	
	Velocity inlet/ Pressure inlet		Velocity inlet/ Pressure inlet	
	Flux(10^{-9}kg/s)	Percentage	Flux(10^{-9}kg/s)	Percentage
Inner	29.88/20.1	14.9/14.8	38.75/39.05	19.4/19.4
Middle	69.46/47.04	34.6/34.6	98.64/100	49.3/49.3
Outer	101.35/68.77	50.5/50.6	62.48/63.59	31.3/31.4
Total	200.7/135.92	100.0	199.9/202.65	100.0

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in isobathic chip, but that of splitlevel chip is decreased to 31.3%. In contrast, the flow of middle and outer channel increased from 49.5% (isobathic chip) to 68.7% (splitlevel chip). Under the same pressure, the flow of the outer and middle channels in the splitlevel chip sharply increases, whereas the flow of the inner channel remains almost constant, which is good for WBC gathering. Fig. 2(A) and (B) show the pressure distribution at the medium height under boundary conditions of velocity inlet. In terms of pressure analysis, it illustrates that the pressure in every channel along radial direction decreases, especially at the gap, which plays a key role in blood separation.

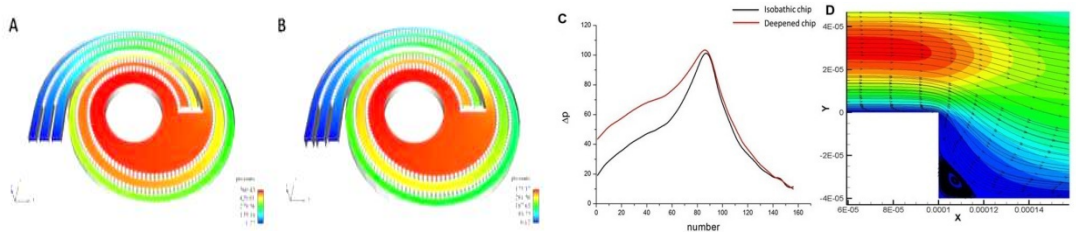

Figure 2: The simulation results. (A) The simulation result of pressure in isobathic chip at the section of 30μm; (B) the simulation result of pressure in splitlevel chip at the section of 30μm; (C) the difference of pressure between the first gap array in two kinds of chip; (D) the cross section of stream line at the step of splitlevel chip .

In velocity inlet model, the average pressure of isobathic chip is 550Pa, whereas that of the splitlevel chip is 367Pa, which decrease the flow resistance. The comparison between the different pressures located at the gap region of the two kinds of separation chip show in Fig. 3(C). It means that the pressure located at gap region of the splitlevel chip outweighs the isobathic chip, especially in the first loop. The simulation results of the differential pressure essentially explain that the more flow through gaps in the latter design than the former. As seen in Eq. 1, Δp , differential pressure between inlet and outlet, includes two pressure differences

$$\Delta p \approx \Delta p_1 + \Delta p_2 = (\frac{3\alpha\mu}{2h^3}L + \beta\gamma)Q_1 \quad (1)$$

Δp_1 , which is in line with Δp in poiseuille flow, is pressure drawdown due to frictional resistance; Δp_2 is pressure losses through the gap and proportional to the flux of gaps, which illustrated as $\Delta p_2 = \beta Q_2$. β is a form factor. In terms of two-dimensional poiseuille flow, Δp can be expressed as $\Delta p = \frac{3\mu Q}{2h^3}$. μ is viscosity coefficient, while Q is flow. h is the depth of the channel. For our case, α is a constant value in isobathic and splitlevel chip. L stands for the length of channel. However, the Δp_1 is expressed as $\Delta p_1 = \frac{3\alpha\mu Q_1}{2h^3}L$. Reynolds number in our chip is calculated as $Re = \frac{\rho u l}{\mu} =$ 0.078, which affords viscous force significantly compared with inertia force. Q_1 is the total flux of middle and outer channel, while Q_2 is the flux of the sample passing through gaps. The relationship between Q_1 and Q_2 is declared as $Q_2 = \gamma Q_1$. γ varies with the position of the gap. Based on deducing above, Eq. 1 is rewrited as

$$\Delta p \approx \Delta p_1 + \Delta p_2 = (\frac{3\alpha\mu}{2h^3}L + \beta\gamma)Q_1 \quad (2)$$

Providing that $(\Delta p)_1 = (\Delta p)_2$, $h_{isobathic} < h_{Splitlevel}$, the total flux of middle and outer channel in two kinds of chip has a relationship as $(Q_1)_{isobathic} < (Q_1)_{Splitlevel}$. Based on the analysis above, it is clear that $(Q_2)_{isobathic}$ is less than $(Q_2)_{Splitlevel}$. This consolidates that the deepened modification of design gives the latter chip a competitive advantage in separating efficiency. The results of simulation and theory analysis are in accordance with the testing results.

FABRICATION

Fabrication of the microfilter is schematically summarized in major process steps in Fig. 3. The substrate is silicon. DRIE process is used to etch the microchannels with different depths. The glass wafer is anodically bond with the silicon wafer to encapsulate the microchannels. A PDMS layer is attached on the backside of the chip in order to realize the chip packaging and plumbing. SEM (scanning electron microscope) pictures and an optical picture of the packaged chip are shown in Fig. 4.

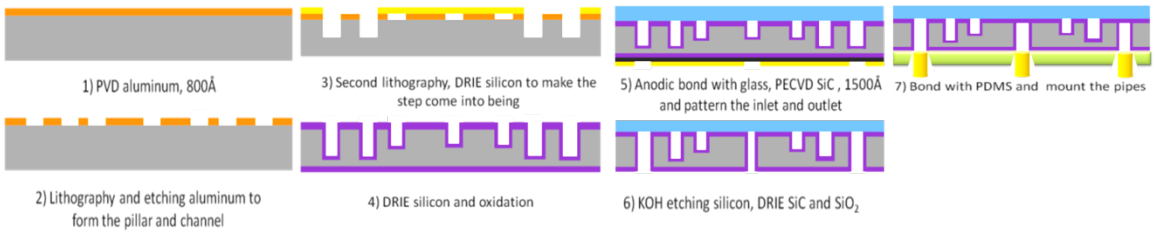

Figure 3: The schematic of micro fabrication process for separation microfluidics chip

RESULTS AND DISCUSSION

The separation chip was characterized by injecting microbeads solution and diluted human blood. The flow rate was 8.1μl/min, controlled by a syringe pump. The performances of the filter were evaluated qualitatively through an optical microscope during separation, shown in Fig. 5(A) and (B) respectively. Fig. 5(A) illustrates that the polystyrene (PS) microbeads with diameter of 1.5μm selectively pass through the gaps among the first row of micropillars, while a mass of PS beads reached the middle channel in Fig. 5(B). The phenomenon proved the rationality of the design. Avoiding the

risk of channel blocking, the whole human blood diluted by 20 times with 0.9% NaCl physical saline before the experiment. To make the surface of channel hydrophilic, 0.1% Pluronic F-127 solution was guided to flush the separation channel in more than fifteen minutes at the speed of 1ml/min. After 20 times diluted by solution of NaCl (0.9%), the testing sample of blood contained 2.63×10^{11}/L RBCs and 2.48×10^{8}/L WBCs. After separation, about 8.12×10^{11}/L RBCs gathered in inner and middle outlets of the isobathic chip. The concentration of WBCs in inner channel was 7.34×10^{8}/L. However, 95μl plasma flowed to the outer channel (shown in Fig. 5(C)). Comparatively, in the splitlevel chip, the values of WBCs in inner channel, RBCs in middle channel and plasma in outer channel were 1.24×10^{9}/L, 6.31×10^{11}/L and 110μl, respectively (shown in Fig. 5(D)). The differences of separation results in those two kinds of chips were shown in Tab.2. In the chip with the splitlevel channels, the number of RBCs gathered from inner outlet was decreased to 8.87% of value from the isobathic one. Furthermore, the collected plasma volume was 44%, compared to 39% in isobathic one.

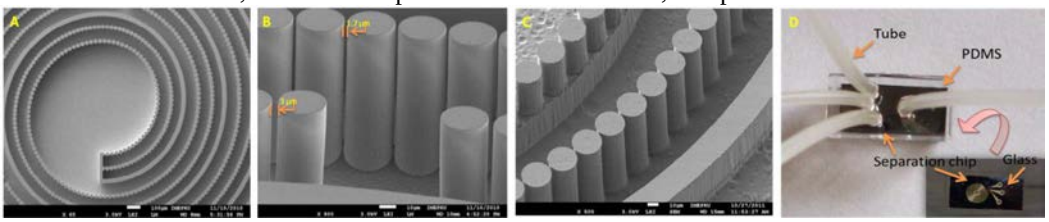

Figure 4: SEM of the chip. (A) Top view of the chip; (B) micropillars with micro-gap; (C) the step of splitlevel chip; (D) separation chip after package.

Tab. 2 The results difference in two kinds of chips

	WBCs in Inner channel	RBCs Middle	Plasma in Outer channel
Isobathic spiral channel chip	7.34×10^{8}/L	5.02×10^{11}/L	95μl
Splitlevel spiral channel chip	1.24×10^{9}/L	6.31×10^{11}/L	110μl

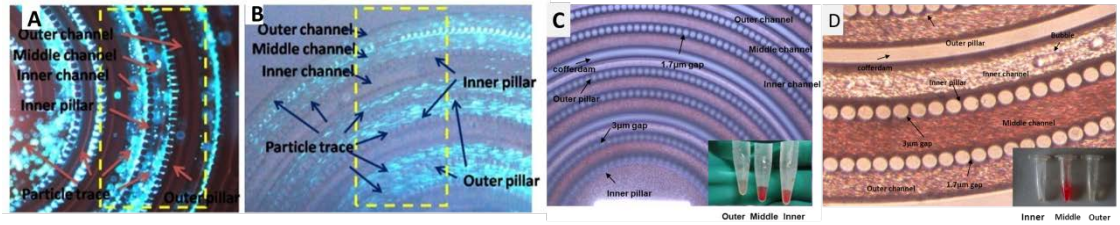

Figure 5: The testing results. (A)The result of 1.5μm PS beads experiment in isobathic chip; (B) the result of 1.5μm PS beads experiment in splitlevel chip. (C) the result of the whole blood experiment in isobathic chip; (D) the result of whole blood experiment in splitlevel chip;

CONCLUSION

In this paper, a silicon-based microfluidic spiral filters, constituted by double pillar arrays, crossflow and splitlevel-channel has been investigated. RBCs, WBCs and plasma were successfully separated from the blood in this device. The design of the splitlevel channels has efficiently increased the purity of WBCs and separated value of plasma.

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