Characterization of thermoplastic microfiltration chip for the separation of blood plasma from human blood

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In this study, we developed a fully thermoplastic microfiltration chip for the separation of blood plasma from human blood. Spiral microchannels were manufactured on a PMMA substrate using a micromilling machine, and a commercial polycarbonate membrane was bonded between two thermoplastic substrates. To achieve an excellent bonding between the commercial membrane and the thermoplastic substrates, we used a two-step injection and curing procedure of UV adhesive into a ring-shaped structure around the microchannel to efficiently prevent leakage during blood filtration. We performed multiple filtration experiments using human blood to compare the influence of three factors on separation efficiency: hematocrit level (40%, 23.2%, and 10.9%), membrane pore size (5 μm , 2 μm , and 1 μm), and flow rate (0.02 ml/min, 0.06 ml/min, 0.1 ml/min). To prevent hemolysis, the pressure within the microchannel was kept below 0.5 bars throughout all filtration experiments. The experimental results clearly demonstrated the following: (1) The proposed microfiltration chip is able to separate white blood cells and red blood cells from whole human blood with a separation efficiency that exceeds 95%; (2) no leakage occurred during any of the experiments, thereby demonstrating the effectiveness of bonding a commercial membrane with a thermoplastic substrate using UV adhesive in a ring-shaped structure; (3) separation efficiency can be increased by using a membrane with smaller pore size, by using diluted blood with lower hematocrit, or by injecting blood into the microfiltration chip at a lower flow rate. *Published by AIP Publishing.* [<http://dx.doi.org/10.1063/1.4964388>]

I. INTRODUCTION

Microfluidic technologies have developed rapidly since micro-gas chromatography was first reported in 1979.¹ Compared to large-scale instruments, microfluidic devices require smaller samples and reagent volumes and provide better separation efficiency due to (1) a high surface-to-volume ratio, (2) shorter analysis time, and (3) higher sensitivity. Microfluidic devices can even be integrated with other components toward a lab-on-a-chip (LOC). Blood analysis^{2–13} involves separating blood plasma from whole human blood to prevent blood cells from affecting final assay results. The conventional approach for plasma separation involves fitting commercial centrifuge tubes with a filter to separate the blood plasma from the whole human blood; however, the centrifugal forces induced by high speed rotation are difficult to be realized on microfluidic platforms. This has led to the development of other separation methods that can be realized or integrated with a microfluidic platform.

Microfiltration is the most common technique used to separate plasma from blood, and microfluidic-based filtration approaches include constructing obstructions within the microchannels,^{2–4} packing beads to create a microfilter,^{5,6} and integrating filters within microfluidic

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chips.^{7–11} Examples of the different microfiltration approaches are described over subsequent paragraphs.

A. Microfiltration based on microchannel obstruction

Kang *et al.* previously developed submicron-scale vertical pillars along microchannels to enable the continuous separation of blood plasma from whole blood.² The principle behind that filtration technique was based on the size exclusion of cells, whereby only plasma was allowed to pass between the vertical pillars. The effectiveness of Kang *et al.*'s approach in separating blood plasma from whole blood was demonstrated experimentally; it was found to have a filtration efficiency of 99.9% and a plasma collection rate of $0.67 \mu\text{l min}^{-1}$. In another study, Li *et al.* developed a Poly(methyl methacrylate) (PMMA) microfluidic chip to separate red blood cells (RBCs) from whole rat blood.³ Specifically, those researchers used dry etching to create microchannels and micro-dams on silicon wafers, followed by hot embossing to fabricate polymeric microfluidic chips. With this device, when rat blood flows through the main microchannels, perfusion flow through the side channels washes red blood cells away, as red blood cells are small enough to enter the gaps between the micro-dams and the cover plate.

B. Microfiltration based on microfilters

Shim *et al.* previously used two sizes of silica beads to create microfilters at inlet reservoirs.^{5,6} In that study, when silica beads were well packed within the inlet reservoir, capillary forces drew whole human blood through gaps between the silica beads, which succeeded in separating plasma from blood cells.

C. Microfiltration based on integrating filters within microfluidic chips

Other researchers have used microfabrication technology to create pores with well-defined geometries on silicon-based substrates, and these have been used as membranes in a variety of filtration applications.^{7,8} For example, Lee *et al.* developed a $\text{Si}_3\text{N}_4/\text{SiO}_2/\text{Si}_3\text{N}_4$ triple layer membrane sieve for blood plasma separation.⁷ The thickness of this membrane was $0.4 \mu\text{m}$ and the diameter of the pores was $1 \mu\text{m}$. The membrane was fabricated using a semiconductor fabrication process. Using the device designed by Lee *et al.*, plasma separation is achieved by driving whole human blood through the membrane by capillary forces. An experiment investigating the efficacy of this device demonstrated its effectiveness by separating $1 \mu\text{l}$ of blood plasma from $5 \mu\text{l}$ of whole human blood.

Commercial membranes can be integrated with microfluidic platforms and to separate blood plasma from whole blood. The benefits of using commercial membranes have easy accessibility, low-cost, and many choices for different applications. For example, Thorslund *et al.* designed a hybrid microfluidic chip with two Polydimethylsiloxane (PDMS) microchannels sandwiching a polymeric membrane. That device used commercial membranes made of polyethersulfone (PES), polypropylene and polyvinylidene fluoride, cellulose acetate, polycarbonate, and polyvinylpyrrolidone (PVP)/polyethersulfone.⁹ Multiple experiments have been conducted on hybrid microfluidic chips to understand how different membranes affect blood plasma extraction. In one study, Wang *et al.* developed a disposable four-layer microfluidic chip to separate blood plasma and viruses from whole human blood.¹⁰ The microchannels were fabricated on a PMMA substrate via laser cutting, and then a polycarbonate membrane (with pore sizes ranging from 0.4 to $3 \mu\text{m}$) was bonded to the PMMA substrate using double-sided adhesive. Using a filter based chip with a pore size of $2\text{-}\mu\text{m}$, those researchers succeeded in separating the Human Immunodeficiency Virus (HIV) from HIV-spiked whole blood. Moreover, they achieved high recovery efficiencies of $89.9\% \pm 5.0\%$, $80.5\% \pm 4.3\%$, and $78.2\% \pm 3.8\%$, for viral loads of 1000, 10 000, and 100 000 copies/ml, respectively. Meanwhile, $81.7\% \pm 6.7\%$ of red blood cells and $89.5\% \pm 2.4\%$ of white blood cells (WBCs) were retained on the $2 \mu\text{m}$ pore-sized filter microchips. In another study, Aota *et al.* reported a multilayer microfluidic chip for plasma separation. The microchannels in that chip were fabricated on glass substrates

and bonded to a Cyclopore membrane using a stainless steel holder.¹¹ This device takes advantage of axial migration to separate plasma from whole blood and reportedly prevents hemolysis and cell clogging. In experiments, the device was able to separate 65% of plasma in 200 μl of whole blood. In a final example, Homsy *et al.* developed a hybrid microfluidic chip for the separation of plasma from whole blood.¹² The microfluidic chip comprised three parts: (1) microchannels fabricated by soft-embossing of a thiolene-based UV-curable adhesive, (2) a coverplate of milled polycarbonate, and (3) a commercially available blood filter membrane. In experiments, the microfluidic chip designed by Homsy *et al.* was able to extract 12 μl of plasma from 100 μl of whole blood in less than 10 min.

Researchers have reported that leakage is a critical issue when integrating commercial membranes within thermoplastic microfluidic chips. Thorslund *et al.*⁹ reported leakage at the interface between the polyvinylpyrrolidone (PVP)/polyethersulfone (PES) membrane and the PDMS substrates. Homsy *et al.*¹² adopted the method of Thorslund *et al.*⁹ but changed the substrate material from PDMS to PC and NOA 81 in an attempt to prevent leakage between the two heterogeneous materials. In the current study, we used a two-step injection and curing procedure of UV adhesive to fabricate a fully thermoplastic microfiltration chip for separating blood plasma from human blood, and this chip had ring-shaped structural features on the both poly(methyl methacrylate) (PMMA) substrates around the microchannels and a commercial polycarbonate membrane was sandwiched between the top and bottom substrates. All of the micro features were fabricated on PMMA substrates using a micromilling machine. We then injected UV adhesive in the ring-shaped structures around the microchannels to tightly assemble the membrane and thermoplastic substrates to prevent any leakage during the filtration process. Figure 1 shows the idea of the microfiltration chip, in which a blood sample is injected into the chip via the top microchannel. Blood then flows through the membrane, and filtered blood plasma is collected from the bottom microchannel. Throughout filtration experiments, we ensured that pressure in the top microchannel did not exceed 0.5 bars in order to prevent hemolysis. Multiple experiments were conducted to elucidate the influence of three factors on separation efficiency: blood injection flow rate, pore size of the membrane, and hematocrit (i.e., volume percentage of red blood cells in blood) level.

II. DESIGN, FABRICATION, AND ASSEMBLY OF MICROFILTRATION CHIP

A. Chip design and fabrication

Figure 2(a) presents the structure of the fully thermoplastic microfiltration chip, which includes two PMMA substrates (as top and bottom covers), two PMMA substrates with spiral microchannels and ring cavity structures, and a polycarbonate membrane. To connect the tubing, the two PDMS connectors were bonded to the PMMA substrates. The top spiral microchannel provides an inlet for the introduction of blood, whereas the bottom spiral microchannel provides an outlet for the collection of filtered blood plasma. The reasons behind designing a spiral microchannel for this filtration chip include: (1) avoiding the bubble stuck in the path of blood flowing, which was a common phenomenon in the chamber type microfluidic chip and (2) avoiding any sharp corner, which would cause difficulty in micromilling machining and circular eddy to deteriorate the filtration performance.

FIG. 1. Working principles underlying our microfiltration chip for the separation of blood plasma from human blood. Raw blood is introduced into the top microchannel and then passes through a membrane. Filtered blood is collected from the bottom microchannel.

FIG. 2. (a) Five-layer structure of this fully thermoplastic microfiltration chip: two PDMS connectors attached to two PMMA substrates, two PMMA substrates with microchannels and ring cavity structures, and a polycarbonate membrane; (b) dimensions of the microfluidic chip.

Figure 2(b) presents a top-view of the microfluidic chip, which has dimensions of $37.5 \text{ mm} \times 50 \text{ mm} \times 1 \text{ mm}$. The width and depth of microchannels are 1.2 mm and 0.5 mm , respectively; and the width and depth of the ring cavity structure are 3.5 mm and 0.5 mm , respectively. The structure of the ring cavity in this microfiltration chip was used to ensure a good assembly between the membrane and PMMA substrates without leaving any gaps that could cause leakage. Micro features were fabricated on the PMMA substrate using a micromilling machine that possessed five major components: a spindle (E3000c, Nakanishi, Japan), a laser non-contact tool setting system (NC4, Renishaw, United Kingdom), a numerical controller (M515i, LNC Technology Co. Ltd., Taiwan), a compressed air/oil coolant system, and a milling bit holder for tool exchange. Throughout the cutting process, air was supplied to the cutting surface of the PMMA substrates from a nozzle. The micromilling bit comprised a 2-flute end mill with a diameter of $200 \mu\text{m}$ (Taiwan Microdrill Co. Ltd., Taiwan). Cutting parameters included a feed rate of 600 mm/min , a spindle speed of $12\,000 \text{ rpm}$, a step over of $100 \mu\text{m}$, and a cutting depth of $100 \mu\text{m}$, which resulted in a surface roughness R_a (arithmetic average roughness) of $0.015 \mu\text{m}$.¹⁴

B. Chip assembly

In this study, we used polycarbonate membranes with three different pore sizes: $1 \mu\text{m}$, $2 \mu\text{m}$, and $5 \mu\text{m}$ (PDC-32G, Harrick Plasma Corp., NY, USA). Figure 3 presents scanning electron microscopy (SEM) images of the membranes. Specifically, Figure 3(a) shows a membrane with a pore size of $1 \mu\text{m}$, Figure 3(b) shows a membrane with a pore size of $2 \mu\text{m}$, and Figure 3(c) shows a membrane with a pore size of $5 \mu\text{m}$. After microchannels and ring-shaped structures had been fabricated on PMMA substrates, two bonding methods (solvent bonding and adhesive bonding) were employed to assemble the microfiltration chip, as shown in Figure 4. Specifically, Figures 4(a)–4(c) illustrate the steps involved in bonding the PDMS connector to the PMMA substrates using the solvent bonding method.¹⁵ In brief, a plasma machine [PDC-32G, Harrick Plasma, USA] was used to break the original polymer chains and create hydroxyl groups on the PMMA and PDMS surfaces (Figure 4(a)). Modified PDMS was then soaked in 1% (v/v) aqueous solutions of aminosilane (ATPES) to create amine functionalities, while the modified PMMA was soaked in 1% (v/v) aqueous solutions of epoxysilane (GTPES) to create epoxy functionalities (Figure 4(b)). The two substrates were then brought together to create an amine-epoxy bond (Figure 4(c)).

FIG. 3. Polycarbonate membranes used in the proposed microfiltration chip: (a) membrane with a pore size of $1\ \mu\text{m}$, (b) membrane with a pore size of $2\ \mu\text{m}$, and (c) membrane with a pore size of $5\ \mu\text{m}$.

After assembling the PDMS connector and PMMA substrates, adhesive bonding was used to bond the polycarbonate membrane and the PMMA substrates,¹⁶ as shown in Figures 4(d)–4(f). For this, a very thin layer of UV adhesive was distributed across the PMMA substrates, and the polycarbonate membrane was then sandwiched between and bonded to the two PMMA substrates (Figure 4(d)). To prevent leakage, UV adhesive was injected into the ring cavity using a manual pipette so that the gap between the membrane and substrates was completely sealed (Figure 4(e)). Finally, the entire thermoplastic chip was exposed to UV irradiation to promote solidification of UV adhesive prior to experiments (Figure 4(f)).

III. EXPERIMENT METHODS

A. Determining maximum volume of injected blood without causing hemolysis

Figure 1 illustrates the working principle underlying the proposed microfiltration chip. As the pores of the membrane are blocked by filtered components, pressure inside the top microchannel would increase significantly. If pressure in the top microchannel was to exceed 0.5 bars, hemolysis could occur, and this would contaminate filtered blood plasma in the bottom microchannel.^{12,17} Thus, we determined the maximum injection volume of blood at a pressure threshold of 0.5 bars in order to prevent hemolysis. In those experiments, pressure inside the top microchannel was monitored while either whole blood or diluted blood was introduced into the microfiltration chip at a flow rate of 0.06 ml/min. To measure pressure inside the top microchannel, we designed a fluidic system. A schematic diagram of this system is presented in Figure 5(a) and the real system is shown in Figure 5(b). In brief, the fluidic system includes (1) a syringe pump to generate pressure, (2) a pressure sensor (PS100-10 Bar, Yalab, Taiwan) which is connected to a computer to measure and record experiment data, and (3) an aluminum chip holder to ensure that the connection between the microfluidic chip and the tubing remains

FIG. 4. Bonding process used in the fabrication of microfiltration chips: (a)–(c) bonding between PDMS connectors and PMMA substrates; and (d)–(f) bonding between polycarbonate membranes and PMMA substrates.

tight during the experiments. A three-way connector was used to obtain accurate pressure measurements within the top microchannel.

Hematocrit is the volume percentage (vol. %) of red blood cells in blood, which can alter the viscosity and flow behavior of liquids. In this study, we employed blood samples with three different hematocrit values: 40%, 23.2%, and 10.9%. Equation (1) was used to estimate the

FIG. 5. (a) Schematic of the fluidic system used to measure pressure within the top microchannel. The system includes a pressure sensor, a three-way connector, a syringe pump, a chip holder, and a synchronized camera; (b) photograph of fluid system.

hematocrit level when diluting blood with PBS (Phosphate buffered saline, Sigma-Aldrich) solutions

$$\text{Hct} (\%) = \frac{\text{Hct} (\%)_{\text{Blood}} \times V_{\text{Blood}}}{V_{\text{Blood}} + V_{\text{PBS}}} = \frac{V_{\text{RBCs}}}{V_{\text{Total}}}, \quad (1)$$

where Hct (%) is hematocrit of diluted blood, $\text{Hct} (\%)_{\text{Blood}}$ is hematocrit of whole blood, V_{Blood} is the volume of whole blood, V_{PBS} is the volume of PBS, V_{RBCs} is the volume of the red blood cells, and V_{Total} is the total volume after dilution. The blood samples used in experiments were drawn from the author, a young, healthy, 24 year old male. After the blood was drawn, it was mixed with PBS to dilutions of 40%, 23.2%, and 10.9%.

B. Separation efficiency

To quantify the performance of the microfiltration chip, Equation (2) was used to estimate separation efficiency (ϕ)

$$\text{Separation Efficiency} (\phi) = \frac{C_S - C_P}{C_S} \times 100, \quad (2)$$

where C_S is the numbers of cells before filtration (numbers/ μl) and C_P is the numbers of cells after filtration (numbers/ μl). For example, a separation efficiency of 70% means that 70% of the injected cellular components were blocked by the membrane. Thus, higher separation efficiency leads to superior microfiltration performance. We used a hematology analyzer (XT-1800I, Sysmex) to determine the concentration of cells before and after filtration, and the cellular components focused in this study were white blood cells (WBCs), red blood cells (RBCs), and platelets. Figure 6 illustrates the procedure followed for filtration experiments. Specifically, in step 1, each type of cell in the blood sample was identified using a hematology

FIG. 6. Experimental procedure: (a) Dilution of blood with PBS to prepare samples with various hematocrit levels (40%, 23.2%, and 10.9%); concentration of white blood cells, red blood cells, and platelets were measured using a blood analyzer; (b) injection of the blood sample into the microfiltration chip using a syringe pump at a constant flow rate, filtered blood plasma was collected in a centrifuge tube at the outlet; (c) collection of blood plasma to determine separation efficiency and the concentration of each cellular component (measured using a blood analyzer).

analyzer (Figure 6(a)). In step 2, a syringe pump was used to introduce whole or diluted blood into microfiltration chips at a constant flow rate, and filtered blood plasma was collected in a centrifuge tube (Figure 6(b)). In step 3, filtered blood plasma was measured using a hematology analyzer to determine the concentration of each cell type (Figure 6(c)), the results of which were used to calculate separation efficiency using Equation (2).

C. Factors associated with separation efficiency

Three factors (hematocrit level, flow rate, and membrane pore size) were used to characterize the performance of the proposed microfiltration chip. Specifically, we conducted experiments using blood samples with hematocrit values of 40%, 23.2%, and 10.9%; and we compared the efficacy of applying flow rates of 0.02 ml/min, 0.06 ml/min, and 0.1 ml/min and pore sizes of 5 μm , 2 μm , and 1 μm . In experiments investigating the influence of membrane pore size and hematocrit on separation efficiency, the flow rate was maintained at 0.06 ml/min. In experiments investigating the influence of flow rate on separation efficiency, hematocrit was maintained at 40% and the membrane pore size was 1 μm .

IV. RESULTS AND DISCUSSION

A. Maximum volume of blood that can be injected without inducing hemolysis

Figure 7 presents pressure values measured inside the top microchannel as blood with various hematocrit levels passed through the microfiltration chip at a flow velocity of 0.06 ml/min. Our aim of this experiment was to determine the maximum volume of blood that could be injected into the chip without inducing hemolysis. From Figure 7, it shows that the corresponding maximum injection volumes were as follows: hematocrit of 40% (7:26 min and 371.6 μl), hematocrit of 23.9% (12:22 min and 618.3 μl), and hematocrit of 10.9% (15:36 min and 780 μl). Therefore, diluted human blood (e.g., 10.9% hematocrit) reached the 0.5 bar pressure line more slowly than did less diluted samples. Conversely, pumping blood with a hematocrit of 40% at a flow rate of 0.06 ml/min over a period of 7:26 min resulted in pressure values near 0.5 bars, which is close to the limit beyond which hemolysis is likely to occur. We used the results of these tests to formulate maximum injection volumes for subsequent experiments, which were determined as follows: hematocrit of 40% (350 μl), hematocrit of 23.9% (600 μl), and hematocrit of 10.9% (750 μl).

B. Discussion on filtration experiments

Figure 8 presents the experimental results comparing separation efficiency and membrane pore size as a function of hematocrit. Figure 8(a) presents the results for white blood cells, Figure 8(b) presents the results for red blood cells, and Figure 8(c) presents the results for platelets, and the results are listed in Table I. Figure 9 and Table II present the results of

FIG. 7. This figure can be used to determine the corresponding maximum injection volume in terms of different hematocrit levels: hematocrit of 40% (7:26 min and 371.6 μl), hematocrit of 23.9% (12:22 min and 618.3 μl), and hematocrit of 10.9% (15:36 min and 780 μl).

FIG. 8. Graph depicting separation efficiency as a function of membrane pore size and hematocrit level: (a) White blood cells showed the highest separation efficiency (97.5%), which was achieved using a smaller membrane pore size and higher blood dilution; (b) The highest separation efficiency for red blood cells was 96.7%; this was also achieved using a smaller membrane pore size and higher blood dilution; (c) The highest separation efficiency for platelets was achieved with a smaller membrane pore size and higher blood dilution; however, the trend in separation efficiency was less obvious for platelets than for red blood cells and white blood cells, due to their smaller diameter of 2–3 μm .

TABLE I. Experimental results illustrating separation efficiency in terms of membrane size and hematocrit level.

Separation efficiency											
WBCs				RBCs				PLT			
	5 μm	2 μm	1 μm		5 μm	2 μm	1 μm		5 μm	2 μm	1 μm
Hct 40%	1.4	38.9	82.9	Hct 40%	0	1.97	93.9	Hct 40%	15.7	35.9	38.7
Hct 23.2%	9.9	42.3	85.1	Hct 23.2%	0	3.83	94.2	Hct 23.2%	29.4	37.3	32.8
Hct 10.9%	10.7	43.4	97.5	Hct 10.9%	0	5.79	96.7	Hct 10.9%	22.3	31.5	50

FIG. 9. Separation efficiency in terms of flow rate. Higher separation efficiency was achieved at a lower flow rate, regardless of cellular components.

experiments investigating separation efficiency of each cellular component under flow rates of 0.02 ml/min, 0.06 ml/min, and 0.1 ml/min.

As shown in Figures 8(a) and 8(b), separation efficiency clearly increased as membrane pore size and/or hematocrit level decreased. Indeed, the highest separation efficiency was achieved when the membrane pore size was 1 μm and the hematocrit level was 10.9%. Specifically, separation efficiency for white blood cells was 97.54% and separation efficiency for red blood cells was 96.69%. In these experiments, the diameter of white blood cells always exceeded 10 μm , while the diameter of red blood cells was 6–8 μm .¹⁸ Both types of blood cells possess elasticity characteristics which allow them to flow through a membrane with a pore size of 5 μm . However, when the pore size was reduced to 1 μm , deformed cells were unable to pass through membrane pores, which caused them to separate from the blood plasma. Figure 8(c) presents the results comparing the separation efficiency and membrane pore size for platelets. Based on the results, it is difficult to draw any conclusion which can describe the influence of the membrane pore size on the separation efficiency. Moreover, the results shown in Figure 9 clearly illustrate how separation efficiency is related to the flow rate and the size of cellular components. For example, white blood cells have the largest diameter and therefore the highest separation efficiency. Conversely, platelets have the smallest diameter and therefore the lowest separation efficiency. In addition, at a higher flow rate, greater pressure was accumulated in the top microchannel, which caused greater cell deformation and allowed more cells to pass through the membrane, thereby reducing separation efficiency. At a flow rate of 0.02 ml/min, the highest separation efficiency values were as follows: white blood cells (96.86%), red blood cells (92.51%), and platelets (30.12%).

V. CONCLUSIONS

A fully thermoplastic microfiltration chip was developed for the separation of blood plasma from whole human blood. The proposed multilayer structure was fabricated on thermoplastic substrates and integrated with a commercial polycarbonate membrane. Bonding between the membrane and substrates was achieved by applying two-step injection and curing of UV

TABLE II. Experimental results illustrating separation efficiency in terms of flow rate.

	Separation efficiency		
	0.1 ml/min	0.06 ml/min	0.02 ml/min
WBC (%)	88.7	93.9	96.9
RBC (%)	76.9	81.2	92.5
PLT (%)	20.9	23.7	30.1

adhesive into the ring-shaped structure and the major aim was to prevent leakage during the filtration process. Compared to other microfluidic chips,^{2–13,19–21} the onsite blood filtration could be realized with this device by inserting the tip of the plastic syringe into this device. The bonding technique, two-step injection and curing of UV adhesive, would ensure an efficient filtration process and no leakage would happen during the filtration process.

In this type of microfiltration chip (driven by hydraulic pressure), hemolysis can be avoided by maintaining pressure at less than 0.5 bars within the top microchannel. Prior to filtration experiments, preliminary testing was conducted to determine the maximum volume of blood that could be injected without inducing hemolysis, and the results were used to help determine the maximum injection volumes for subsequent experiments, which were as follows: hematocrit of 40% (350 μ l), hematocrit of 23.9% (600 μ l), and hematocrit of 10.9% (750 μ l). In addition, three factors (at three levels) were considered in filtration experiments: hematocrit (40%, 23.2%, and 10.9%), membrane pore size (5 μ m, 2 μ m, and 1 μ m), and flow rate (0.02 ml/min, 0.06 ml/min, and 0.1 ml/min). Our experimental results clearly demonstrated the following: (1) This fully thermoplastic microfiltration chip is able to separate WBCs and RBCs from blood with a separation efficiency exceeding 95%; (2) No leakage occurred during any of the experiments, thereby confirming the effectiveness of the proposed bonding method; (3) Higher separation efficiency can be achieved using a membrane with a smaller pore size, increasing the dilution of blood, and/or by injecting blood at a lower flow rate.

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