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Microfluidic chips for forward blood typing performed with a multichannel waveguide-mode sensor

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ABSTRACT

We designed a microfluidic chip for use with an evanescent field optical sensor to conduct simultaneous measurements for ABO and Rh(D) forward blood typing. The microfluidic chip was designed to include multiplexed mixers for blood-reagent mixing and chambers for optical measurements. To achieve simple and rapid mixing in the microfluidic channels, holes containing freeze-dried reagents were employed as mixers. In addition, capillary stop valve-based multiplexing of the measurement chambers was used to effectively fill the chambers with samples without empty spaces or leaks. The five-channel microfluidic chip was designed and tested, including the mixing holes and measurement chambers. A multi-channel waveguide-mode sensor was used for optical measurement. Forward blood typing tests using human whole blood samples and anti-A, anti-B, anti-D, and Rh control reagents were conducted on the microfluidic chip. Simultaneous measurements were successfully performed using 40 μL of diluted blood samples, and ABO and Rh(D) blood types could be determined within 5 min.

1. Introduction

Blood typing is important in clinical practice; in particular, ABO and Rh(D) blood typing are commonly performed prior to blood transfusions to prevent hemolysis caused by type mismatched. Manual test methods, including those using a slide or a tube, and fully automated test instruments are widely used at present [1]. In recent years novel methods have been proposed to improve the sensitivity, usability, or cost of conventional blood typing. These methods include optical sensor-based [2–5], microfluidic device-based [6–8], and paper-based [9–11] techniques. Methods that are suit for on-site use [5,6,9–11] are candidates for point-of-care blood typing. A method needs to fully satisfy criteria, including sensitivity, usability, and low cost, to be used for point-of-care blood typing. However, such a method has not been developed.

Recently, we developed a method to rapidly and sensitively detect hemagglutination [12]. The method, herein called the *restrictive channel method*, uses a microfluidic channel paired with an evanescent field optical sensor. The channel functions as a spatial restraint against agglutinated red blood cells (RBCs), resulting in signal differences between agglutinated and non-agglutinated samples and thus allowing hemagglutination detection. In the previous report, we used a single-channel microfluidic chip and manual sample-reagent mixing before injection into the channel. Although hemagglutination detection has been demonstrated, multiplexing and automation of the measurements, which are essential for point-of-care blood typing, have not yet been established. The development of such a system holds promise for improving conventional blood typing methods and merits further investigation.

In this paper, we present a microfluidic chip for simultaneous ABO and Rh(D) forward blood typing tests based on the restrictive channel method. To enable simultaneous and semi-automated measurements on microfluidic chips, a "mixer" for sample-reagent mixing and a "measurement chamber" on the microfluidic chips were designed. A waveguide-mode sensor, i.e., an evanescent field optical sensor equipped with a slab optical waveguide, was used with the microfluidic chips. Five measurement channels were included on the microfluidic chips to allow testing with anti-A, anti-B, anti-D, Rh control reagents, and a reference blank. ABO and Rh(D) blood typing using our measurement system were demonstrated and are described here. A droplet of diluted blood (40 μL) was sufficient for distinguishing blood types.

2. Material and methods

2.1. Materials

Reagents were used as received with no further purification. Human whole blood containing an anticoagulant (ethylenediaminetetraacetic

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acid dipotassium salt; EDTA 2K) was purchased from Tennessee Blood Services Corporation (Memphis, TN, USA). Tubes containing fresh blood samples were shipped by air and used within 2 weeks from the day of collection. Hemolysis of samples was checked before use and only non-hemolyzed samples were used. Blood types were confirmed by the supplier in advance. Blood-typing reagents for the ABO system (monoclonal anti-A and anti-B, Neo Kokusai) were purchased from Sysmex Corporation (Kobe, Japan). Blood-typing reagents for the Rh(D) antigen (Ortho BioClone anti-D and Rh control) were purchased from Ortho Clinical Diagnostics, Inc. (Raritan, NJ, USA) Phosphate-buffered saline (PBS) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Polydimethylsiloxane (PDMS) pre-polymer and its curing reagent (SILPOT 184) were purchased from Dow Corning Toray Co., Ltd. (Tokyo, Japan). Refractive index matching liquid for fused silica (Fused Silica Matching Liquids 50,350) was purchased from Cargille Laboratories Inc. (Cedar Grove, NJ, USA). Waveguide-mode sensor chips were obtained from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan).

2.2. Waveguide-mode sensor

A multi-channel waveguide-mode sensor allowing up to 8 channels for simultaneous measurements was used. A schematic diagram of the measurement system is shown in Fig. 1(A). A collimated white light was irradiated onto a waveguide-mode sensor chip at an incident angle of 70.7°, and the reflected light was measured by a spectrometer. The waveguide-mode sensor chip consisted of a surface silicon oxide waveguide layer, an embedded silicon layer, and a silica substrate [13]; the thicknesses of the layers were approximately 360 nm, 45 nm, and 0.725 mm, respectively. The chip exhibited spectral dip minima at a wavelength of approximately 540 nm during blood sample measurements. The chip was sensitive to RBC detection, as RBCs exhibit a peak of absorbance at 540 nm [5]. The sensor measured

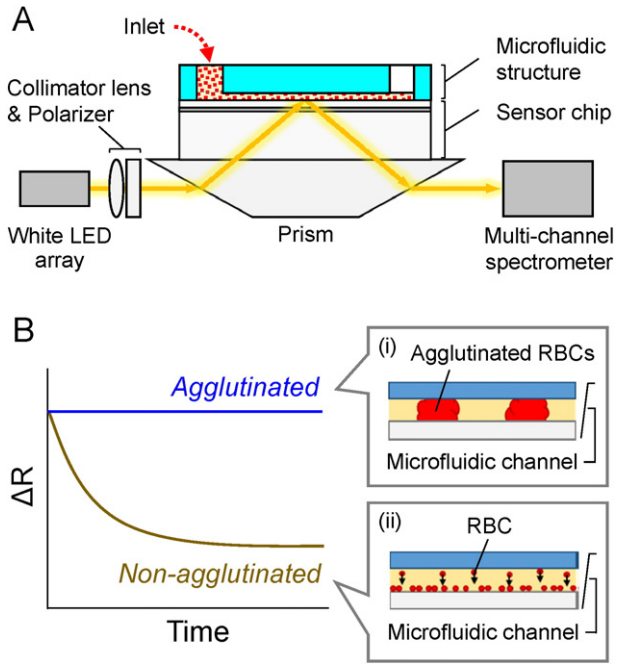


Fig. 1. Schematic diagrams of hemagglutination detection using a microfluidic channel and a waveguide-mode sensor. (A) Optical setup of a multi-channel waveguide-mode sensor. Multi-optical paths are arrayed in the direction perpendicular to the paper surface. (B) Typical curves representing changes in bottom reflectance at the spectral dip minima (ΔR) against time measured using the waveguide-mode sensor. Insets show the cross-sections of microfluidic channels filled with (i) agglutinated and (ii) non-agglutinated blood samples.

$27 \times 12 \times 7.5\ cm^3$ in external dimensions, a size amenable to portability (see Supplementary Fig. S1).

2.3. Microfluidic structure preparation

The microfluidic structures were fabricated by replica molding of PDMS using a silicon template. A 4 in. silicon wafer was processed by a maskless lithography system (DL-1000; NanoSystem Solutions, Inc., Okinawa, Japan) and a reactive ion etching system (RIE-101iPH; SAMCO Inc., Kyoto, Japan) to form the template. The PDMS pre-polymer was mixed with the curing agent at a weight ratio of 10:1. The mixture was poured onto the silicon template and degassed in a vacuum for 60 min. The PDMS volume was adjusted to reach a thickness of approximately 3 mm. The template was then placed on a hot plate at 80 °C for 90 min. After removal from the template, the cured PDMS microfluidic structures were punched to make inlets and outlets using a biopsy punch with a diameter of 1.5 mm (BPP-15F; Kai Industries Co., Ltd., Seki, Japan). The bonding between PDMS-glass surfaces or PDMS-PDMS surfaces was performed using a plasma-bonding technique. The bonding surfaces were treated with a plasma cleaner (PDC-32G; Harrick Plasma, Ithaca, NY, USA) for 20 s. The treated surfaces were placed in contact immediately, and the assembly was placed on a hot plate at 80 °C for 1 min. The assembled microfluidic chips were placed in a dry chamber for 2 days before use to decrease hydrophilicity resulting from the plasma treatment, since hydrophilic surfaces in the microfluidic channels inhibit gapless sample filling.

2.4. Blood testing

Forward blood typing tests were conducted using microfluidic chips and a waveguide-mode sensor to detect hemagglutination. Human whole blood was diluted with PBS to 10% of the RBC volume. The microfluidic chips were placed on a prism of the waveguide-mode sensor and the matching liquid filled the intermediate space. Tubing containing the diluted blood was connected to the inlet; the other side of the tubing was connected to a syringe pump. The blood samples were introduced into the microfluidic chips using a syringe and mixed with blood typing reagents. RBCs of blood samples that mixed with the corresponding blood typing reagents formed aggregates. After stopping the flow, reflected light spectra from the samples were measured by the waveguide-mode sensors, and changes in reflectance at the spectral dip minima (ΔR) were analyzed.

A schematic diagram of typical measurement data for the agglutinated and non-agglutinated blood samples is shown in Fig. 1(B). For the agglutinated sample, aggregates of RBCs stayed motionless since their gravitational sedimentation was restrained by the microfluidic channel, and thus ΔR was nearly zero. However, for the non-agglutinated sample, individual RBCs settled on the bottom surface of the microfluidic channel. Since the waveguide-mode sensor is a surface-sensing technique, ΔR decreased over time owing to the settled individual RBCs. Hemagglutination was detected by the presence or absence of a decrease in ΔR [12].

3. Results and discussion

3.1. Design and examination of the mixer

Microfluidic channels have low Reynolds numbers and are not conducive to the mixing of liquids; accordingly, to facilitate mixing in microfluidic channels, various micromixer devices have been developed [14–16]. Although these mixers are effective, it is not easy to make them compact; the lengths of the mixers range from millimeters to tens of millimeters, and reservoirs for liquid reagents are needed. Our aim was to design a simple and compact microfluidic system for use on the sensor chips. Therefore, we explored various mixing techniques as well as the use of freeze-dried reagents [17] encapsulated in “mixing

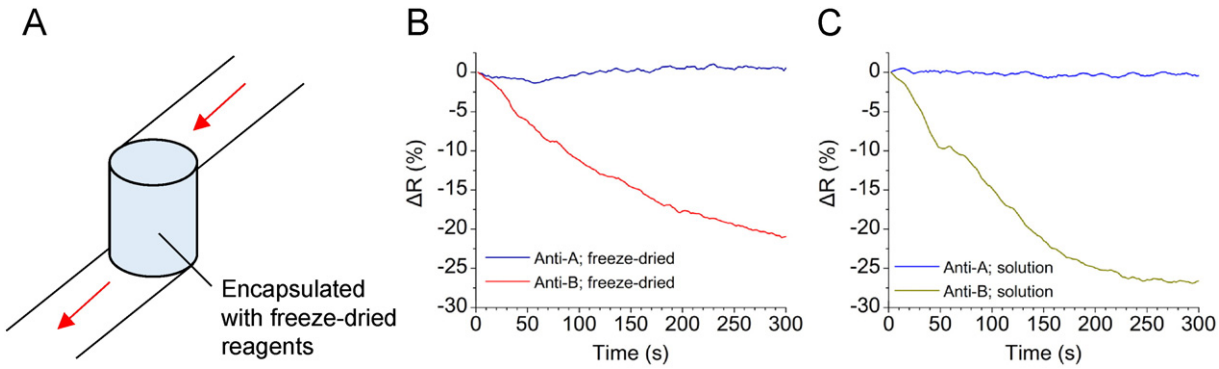


Fig. 2. Investigation of mixing holes to facilitate the mixing of samples and reagents. (A) Schematic diagram of a mixing hole. (B) Plots of changes in reflectance at the spectral dip minima (ΔR) against time measured using blood samples mixed at the mixing hole with freeze-dried reagents. The sample used in these measurements was type A blood. The data were sampled every 2 s. (C) Plots of ΔR against time measured using blood samples mixed with liquid reagents. The sample used in these measurements was the same blood as that used in (B).

holes." A schematic diagram of the mixing hole is shown in Fig. 2(A). The mixing holes are through-holes between the upper and lower levels of the microfluidic chip. When samples pass through the mixing holes containing the freeze-dried reagents, the reagents are expected to dissolve into the sample. For forward blood typing, aggregates of RBCs form in the mixing holes when blood samples are mixed with the corresponding blood-typing reagents, and aggregates are then introduced into measurement chambers. An important feature of the system is that the mixing holes can produce aggregates of RBCs that are larger than the height of the measuring chamber, since the height of the mixing holes is larger than that of the chambers. As shown in Fig. 1(B), the restrictive channel method detects hemagglutination based on differences in ΔR that result from the difference in size between aggregates and individual RBCs. Larger aggregates effectively suppress decreases in ΔR of the agglutinated samples. Thus, a distinct difference between ΔR of agglutinated and non-agglutinated samples is expected owing to the use of the mixing holes.

The mixing holes containing freeze-dried reagents were tested using a microfluidic channel and waveguide-mode sensor. The blood-typing reagents were freeze-dried using FDU-1200 (AS ONE Corporation, Osaka, Japan). Droplets of the reagents were put on a resin case and frozen by incubation at −80 °C for 3 h, then freeze-dried at approximately 30 Pa for 3 h. The freeze-dried reagents retained their ability to agglutinate RBCs (see Supplementary Fig. S2). The microfluidic channel used in this test was a single straight channel with a width of 1.5 mm and a height of 0.05 mm, which was identical to that used in a previous study [12]. Through-holes with a diameter of 1.5 mm were made at the ends of the channel using a biopsy punch and one hole was used as the mixing hole. Owing to deformation of the PDMS structures during the punching process, the holes were not cylinders, but narrowed in the middle. Approximately 0.04 mg of freeze-dried reagent was put into the mixing hole. To connect the tubing, the upper PDMS structure was bonded to the mixing hole with a 10-second plasma treatment and 5 min of hot plate heating at 36 °C. Milder bonding conditions were employed compared to normal bonding conditions (described in Section 2.3) to prevent damage to the encapsulated reagents. Diluted type A blood was introduced into the channel and passed through the mixing hole, and reflection spectra were measured with the waveguide-mode sensor. Fig. 2(B) summarizes the ΔR values obtained for the freeze-dried anti-A and anti-B reagents. Clear differences in ΔR between the agglutinated ("Anti-A; freeze-dried") and non-agglutinated ("Anti-B; freeze-dried") samples were observed, similar to the typical curves shown in Fig. 1(B). For comparison, blood-typing reagents without freeze drying were tested. For these measurements, droplets of the reagents and the blood sample were gently mixed in advance using a pipette, and the mixture was then introduced into the channel. The results are shown in Fig. 2(C). A similar signal was obtained for the measurements using the liquid reagents as compared to freeze-dried reagents. These results confirmed that the mixing holes containing the freeze-dried reagents effectively mixed the blood samples and reagents.

3.2. Design and examination of the measurement chambers

To conduct sample injections and blood typing in parallel, measurement chambers equipped with capillary stop valves [18] at their termini were examined. A schematic diagram of the chambers is shown in Fig. 3(A). According to our design, samples are injected into the chambers in sequence, with samples stopping at the valves due to capillary force, until all chambers are filled. Multichannel systems are typically based on bifurcation-based multiplexing [6,19]. However, capillary valve-based multiplexing can provide robust microfluidic systems that allow fluid resistance variation, whereas bifurcation-based multiplexing requires the precise control of fluid resistance for sample filling. In addition, in the case of capillary valve-based multiplexing, the microfluidic

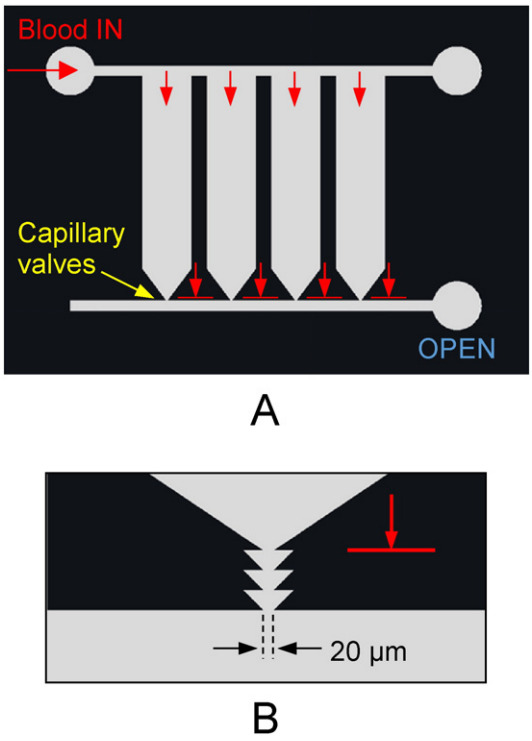


Fig. 3. Design of measurement chambers. (A) Schematic diagram of the measurement chambers. (B) Schematic diagram of capillary stop valve equipped with extra valves.

system is easily expandable to chamber numbers other than 2^n. We therefore investigated a capillary valve-based multiplexing system.

The effects of the width of the capillary valve opening on function were examined using microfluidic chips containing three test chambers. Capillary valves with various opening widths were equipped at the ends of the chambers. The diluted blood samples were introduced into the chambers at various flow rates controlled by a syringe pump. Stopping was determined as "successful" if the samples were stopped at all three chambers. The width, length, and height of the chambers were 1.5 mm, 7 mm, and 0.05 mm, respectively. Test results are summarized in Table 1. The results indicate that an opening width not exceeding 20 μm is appropriate to stop the blood samples. A narrower opening was considered to be unfavorable for smooth fluid operation. Thus, we employed 20 μm as the opening width and added three additional valves at the terminus of each chamber, as shown in Fig. 3(B). These valves effectively enhanced the stopping efficiency. In chambers equipped with this valve structure, only a few instances of sample overflow were observed for more than a hundred sample injections.

3.3. Design of the five-channel microfluidic chip

A microfluidic chip for the simultaneous blood typing test was designed and tested based on the same objectives as those for the mixing holes and measurement chambers. A schematic diagram of the five-channel-integrated microfluidic chip is shown in Fig. 4(A). The microfluidic chip consisted of an upper microfluidic structure, a lower microfluidic structure, and a waveguide-mode sensor chip. The upper level contained an inlet and sample-dividing channel and the lower level contained five measurement chambers, with mixing holes situated between the levels. Blood samples injected from the inlet were directed to five channels, passed through the mixing holes, and then introduced into the measurement chambers. The freeze-dried anti-A, anti-B, anti-D, and Rh control reagents were located in separate mixing holes at a weight of approximately 0.04 mg per hole; the remaining mixing hole was kept as a reference blank. The diameter and height of the mixing holes were 1.5 mm and 3 mm, respectively. The shape of the chambers was chosen to cover the spot size of incident light of the waveguide-mode sensor. The height of the chamber was set at 50 μm, which is preferable for the restrictive channel method [12]. The three-strand capillary stop valves with an opening width of 20 μm were located at the ends of the chambers. The microfluidic chips were assembled using plasma bonding under normal conditions (Section 2.3) and mild conditions (Section 3.1).

Blood injection into the microfluidic chip was tested, without encapsulation of freeze-dried reagents, and the result is shown in Fig. 4(B). Undiluted human whole blood was introduced into the chip by a syringe pump with a flow rate of 15 μL/min. Blood was successfully divided into five channels, passed through the mixing holes, and introduced into the measurement chambers without empty spaces or leaks.

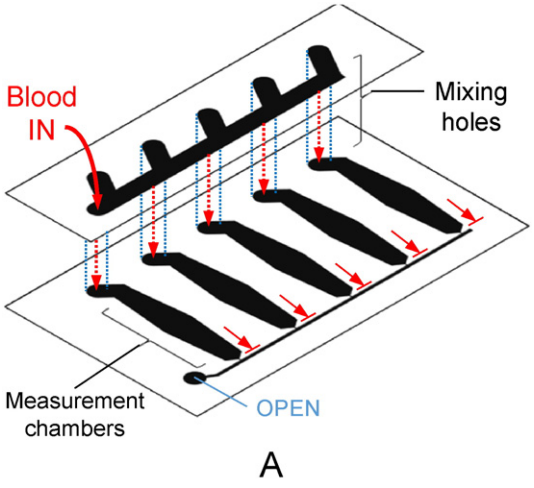
3.4. Blood testing using the five-channel microfluidic chip

Fig. 5(A) summarizes the blood testing data obtained using our five-channel microfluidic chip and type A+ blood and type A+ blood with respect to time elapsed after blood samples reached the first

Table 1
Summary of the capillary stop valve test results.

Flow rate (μL/min)	Opening width (μm)				
	20	40	60	80	100
1	○	×	×	×	×
5	×	○	×	×	×
10	○	×	×	×	×

○: successful; ×: unsuccessful.



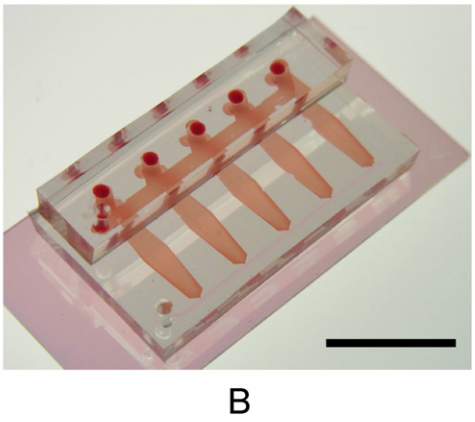


Fig. 4. Five-channel microfluidic chip for simultaneous blood typing measurements. (A) Schematic diagram of the microfluidic chip design. (B) Photograph of the microfluidic chip filled with human whole blood. Scale bar is 10 mm.

measurement chamber. Blood samples (40 μL) were introduced into the tubing connected to the chip inlet and injected into the chip with a flow rate of 15 μL/min. Approximately 30 s elapsed from the time when samples passed the inlet to the time when they reached the first chamber. All of the chambers were filled in approximately 70 s (a series of pictures illustrating the sample injection are shown in Supplementary Fig. S3), and then the syringe pump was stopped. As shown in Fig. 5(A), a larger decrease in reflectance over time was observed for the non-agglutinated samples ("Anti-B" and "Rh control") than the agglutinated samples ("Anti-A" and "Anti-D"). The reflectance at the plateau for the "Blank" was considered the reflectance level of ideal non-agglutinated samples. Fig. 5(B) shows the ΔR values calculated from the measurements shown in Fig. 5(A). In this measurement, the decrease in ΔR of the non-agglutinated samples was larger than that of the agglutinated samples, and thus hemagglutination was distinguishable. A picture of the microfluidic chip after the test is shown in Fig. 5(C). Agglutination of RBCs was visually observed in a comparison of the chamber containing anti-A reagent and that containing anti-B reagent. By contrast, the difference between the chamber containing anti-D and Rh control was unclear based on visual observations. However, ΔR measured by the sensor showed a difference caused by the hemagglutination reaction against the anti-D reagent, indicating that the restrictive channel method can detect smaller aggregates of RBCs than visual inspection.

As revealed in Fig. 5(A), the determination of hemagglutination in all measurement chambers was completed within 270 s; thus, blood type was determined within 5 min, including sample delivery from the inlet and mixing.

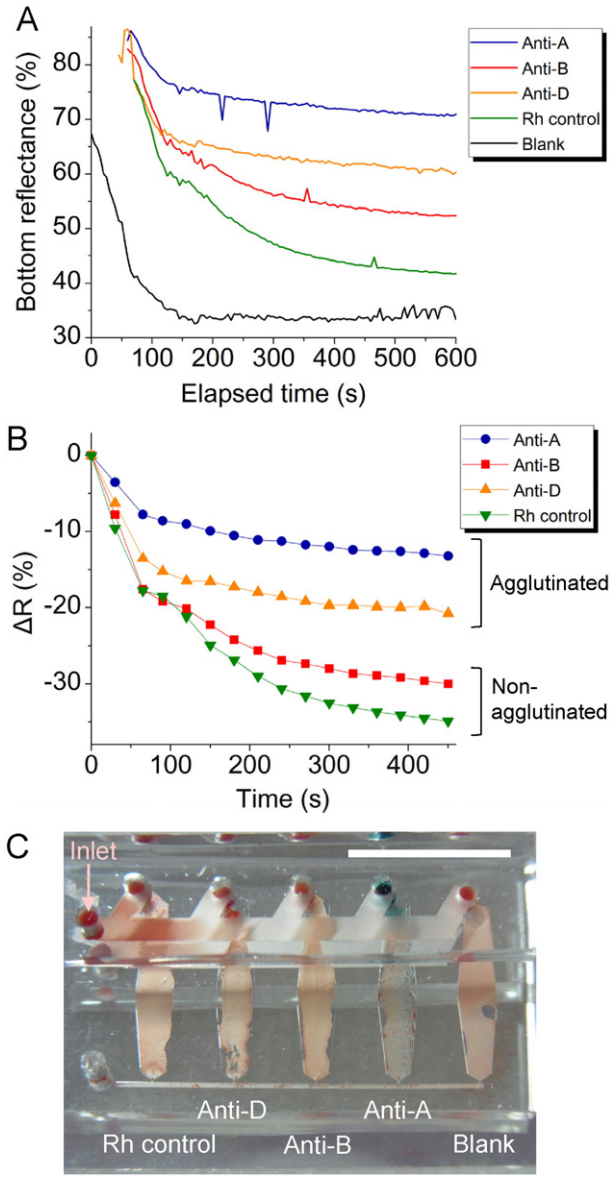


Fig. 5. Blood testing using the five-channel microfluidic chip. (A) Plots of reflectance at the spectral dip minima ("Bottom reflectance") against time. The origin of the abscissa ("Elapsed time") is the time point when blood samples reached the first measurement chamber. The data were sampled every 5 s. (B) Plots of changes in the bottom reflectance (ΔR) against time. The origin of the abscissa ("Time") is the time point when the reflectance measurements of each chamber began, i.e., the start points for the plots in Fig. 5(A) are shifted to 0 s. (C) Photograph of the microfluidic chip after blood testing. Reagents encapsulated in each of the mixing holes are indicated. Scale bar is 10 mm. Although the blood sample overflowed from the chambers, this occurred after the procedure when the tubing was removed from the chip. The stop valves worked efficiently during the procedure, as shown in Supplementary Fig. S3.

Table 2
Summary of repeated measurements (N = 4) for type A+ blood samples.

Reagent	Condition of RBCs	ΔR at 180 s (%)	
		Average	SE
Anti-A	Agglutinated	−12.5	3.3
Anti-B	Non-agglutinated	−23.2	0.5
Anti-D	Agglutinated	−22.5	2.4
Rh control	Non-agglutinated	−30.5	1.5

SE: standard error.

3.5. Mixing performance of the five-channel microfluidic chip

Repeated measurements were conducted using the same blood samples as those used to generate Fig. 5, and the ΔR values at 180 s are summarized in Table 2. A similar trend as that shown in Fig. 5(B) was obtained, i.e., the decrease in ΔR of the non-agglutinated samples was larger than that of the agglutinated samples. A difference between agglutinated and non-agglutinated samples was observed. However, ΔR of the agglutinated samples showed a decrease, which was not observed for the single-channel measurement shown in Fig. 2(B). The |ΔR| of the agglutinated sample shown in Fig. 2(B) was less than 1.5%. These results imply that mixing in the five-channel chip was less effective than that in the single channel, although the design of the mixing hole was identical. We hypothesized that the difference in mixing efficiency reflects a difference in the volume of the flowed blood sample. Since the flow of the sample stopped at the end of the chamber, the volume of the sample that flowed in each channel of the five-channel chip was smaller than that of the single channel test. Therefore, mixing in the five-channel chip was insufficient and less effective than that in the single channel test. To perform complete mixing in the five-channel chip, an increased sample volume should be passed through the measurement chamber. This can be implemented by increasing the channel volume, i.e., via the addition of a reservoir between the measurement chamber and stop valve. Such a microfluidic chip should perform mixing as effectively as the single channel test.

Mixing performance can also be improved by revising the choice of reagents. We used commercially available blood-typing reagents, which were not intended to be freeze dried. Thus, some components included in the reagents could disturb the ability to dissolve in blood samples. Moreover, improving the structure of the mixing holes may be effective. A simple columnar structure was used for the mixing holes in this study. By introducing a functionally-improved structure, such as that used in micromixers to produce a vortex, the passive mixing of reagents could be enhanced. Active mixing using external fields, including magnetic and electric fields, is also applicable. These approaches can improve the performance of mixing, resulting in improved stability and accuracy of hemagglutination detection.

3.6. Comparison among methods

Our microfluidic chip was capable of simultaneous measurements for ABO and Rh(D) forward blood typing. To accommodate multichannel measurements using multiple reagents on microfluidic channels, the system can become complicated, requiring a number of pumps, mechanical or electrical valves, and other microfluidic devices. The complexity of the system arises from the need for a number of liquids, including samples and reagents. In contrast, our microfluidics chip uses reagents in the form of freeze-dried powders and a single liquid sample; accordingly, the fluidic system is simple and requires only a single pump. Although we used a syringe pump in this study, a diaphragm-type micro-pump would also be applicable and would allow for a more compact fluidic system. By improving the usability of the inlet, a portable and fully automated measurement system could be developed. Although our microfluidic chip was designed for point-of-care blood typing, it can also be utilized for other assays by changing the encapsulated reagents and applying surface modifications on the measurement chambers, if needed. Such microfluidic chips are applicable to the automation of other immunoassays and can decrease the volumes of samples and reagents.

With respect to point-of-care blood typing, the leading candidates among current blood typing methods are paper-based techniques [9,11]. The measurement time for the developed microfluidic chip was less than 5 min, which is compatible or faster than that of paper-based systems (5–10 min). Our system is suitable for automation, and thus the ease of operation is potentially compatible to that of the paper-based assays. The cost of the test would be advantageous for the

paper-based tests; however, our system provides additional benefits, such as its speed and automation. At the present stage, the sensitivity and accuracy of the microfluidic chip have not been fully evaluated owing to the limited number of available samples. Such tests will be conducted in future work and these tests will include the improvements related to mixing performance described in Section 3.5.

From the viewpoint of practical operations, sample preparation is an important step; our microfluidic chip requires the dilution of blood, whereas some paper-based systems can be implemented with undiluted blood. Thus, it is necessary to consider when and how collected whole blood is diluted. Since the hematocrit of blood samples is usually unknown, it is difficult to prepare diluted samples containing a specific percentage of RBCs. However, the appropriate dilution can be obtained by using a tool for blood collection and a "6-fold dilution." In a previous study, the restrictive channel method was applicable for diluted blood samples containing RBC volumes ranging from 5% to 10% [12]. The microfluidic chip developed in this study operates in the same way; thus, a 6-fold dilution of the collected blood can account for hematocrit variation from 30% to 60%. The tool can be used to collect a specific amount of patient blood and mix it with a specific amount of PBS. An anticoagulant may be mixed along with PBS. Then, the 6-fold diluted blood is introduced into the microfluidic chip. The development of such a tool as well as a sensing instrument should be a priority for practical applications.

4. Conclusion

A multi-channel microfluidic chip for simultaneous hemagglutination measurements was developed. To conduct parallel sample filling and sample-reagent mixing using a simple fluidic system, an appropriate mixer and measurement chamber were designed. A mixing hole encapsulated with freeze-dried reagents was employed as a mixer, which allowed sample-reagent mixing by passing the samples through the holes. The measurement chambers were equipped with capillary stop valves at their ends to facilitate parallel sample filling. The capillary valves, including four sequential valves with an opening width of 20 μm, showed almost 100% stopping efficiency for dozens of measurements. Based on these results, a five-channel microfluidic chip was designed and tested. The chip was tested using freeze-dried anti-A, anti-B, anti-D, and Rh control reagents, which were encapsulated in the mixing holes to conduct ABO and Rh(D) forward blood typing. Diluted blood samples (40 μL) were injected into the chip and simultaneous blood typing measurements were conducted in each of the chambers. Hemagglutination was detected using the restrictive channel method, and ABO and Rh(D) blood types were determined within 5 min. Further improvements of the microfluidic chip can be implemented to increase the stability and accuracy of hemagglutination detection, including the introduction of a reservoir to increase mixing performance.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.sbsr.2016.01.012.

References

[1] W. Malomgré, B. Neumeister, Anal. Bioanal. Chem. 393 (2009) 1443–1451.
[2] J.G. Quinn, R. O'Kennedy, M. Smyth, J. Moulds, T. Frame, J. Immunol. Methods 206 (1997) 87–96.
[3] S. Narayanan, L. Galloway, A. Nonoyama, G.F. Leparc, L.H. Garcia-Rubio, R.L. Potter, Transfusion 42 (2002) 619–626.
[4] J.S. Robb, D.J. Roy, P. Ghazal, J. Allan, J. Petrik, Transfus. Med. 16 (2006) 119–129.
[5] H. Ashiba, M. Fujimaki, K. Awazu, M. Fu, Y. Ohki, T. Tanaka, M. Makishima, Sens. Bio-Sens. Res. 3 (2015) 59–64.
[6] D.S. Kim, S.H. Lee, C.H. Ahn, J.Y. Lee, T.H. Kwon, Lab Chip 6 (2006) 794–802.
[7] T.R. Kline, M.K. Runyon, M. Pothiawala, R.F. Ismagilov, Anal. Chem. 80 (2008) 6190–6197.
[8] S. Makulska, S. Jakiela, P. Garstecki, Lab Chip 13 (2013) 2796–2801.
[9] M.S. Khan, G. Thouas, W. Shen, G. Whyte, G. Garnier, Anal. Chem. 82 (2010) 4158–4164.
[10] M. Li, W.L. Then, L. Li, W. Shen, Anal. Bioanal. Chem. 406 (2014) 669–677.
[11] J. Noiphung, K. Talalak, I. Hongwarittorrn, N. Pupinyo, P. Thirabowonkitphithan, W. Laiwattanapaisal, Biosens. Bioelectron. 67 (2015) 485–489.
[12] H. Ashiba, M. Fujimaki, K. Awazu, M. Fu, Y. Ohki, T. Tanaka, M. Makishima, Jpn. J. Appl. Phys. 55 (2016) 027002.
[13] M. Fujimaki, C. Rockstuhl, X. Wang, K. Awazu, J. Tominaga, Y. Koganezawa, Y. Ohki, T. Komatsubara, Opt. Express 16 (2008) 6408–6416.
[14] R.H. Liu, M.A. Stremler, K.V. Sharp, M.G. Olsen, J.G. Santiago, R.J. Adrian, H. Aref, D.J. Beebe, J. Microelectromech. Syst. 9 (2000) 190–197.
[15] A.D. Stroock, S.K.W. Dertinger, A. Ajdari, I. Mezić, H.A. Stone, G.M. Whitesides, Science 295 (2002) 647–651.
[16] N.-T. Nguyen, Z. Wu, J. Micromech. Microeng. 15 (2005) R1–R16.
[17] K. Morimoto, S. Upadhyay, T. Higashiyama, N. Ohgami, H. Kusakabe, J. Fukuda, H. Suzuki, Sensors Actuators B Chem. 124 (2007) 477–485.
[18] R. Takagi, J. Fukuda, K. Nagata, Y. Yawata, N. Nomura, H. Suzuki, Analyst 138 (2013) 1000–1003.
[19] K. Sato, M. Yamanaka, H. Takahashi, M. Tokeshi, H. Kimura, T. Kitamori, Electrophoresis 23 (2002) 734–739.