Finger-Actuated Microfluidic Display for Smart Blood Typing
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Supporting Information

ABSTRACT: Accurate blood typing is required before transfusion. A number of methods have been developed to improve blood typing, but these are not user-friendly. Here, we have developed a microfluidic smart blood-typing device operated by finger actuation. The blood-typing result is displayed by means of microfluidic channels with the letter and the symbol of the corresponding blood type. To facilitate the mixing of blood and reagents, the two sample inlets are connected to a single actuation chamber. According to the agglutination aspect in the mixture, the fluids are directed to both the microslit filter channels and bypass channels, or only to the bypass channels. The dimension of the microslit filter being clogged by the red blood cell aggregates was optimized to achieve reliable blood-typing results. The flow rate ratio between two channels in the absence of agglutination was subjected to numerical analysis. With this device, blood typing was successfully performed by seven button pushes using less than 10 μL of blood within 30 s.

Human blood is classified into more than 30 types according to the presence or absence of antigens on the surface of red blood cells (RBCs). Human blood also contains the antibodies that do not react with self-antigens. Therefore, for transfusion, it is important to prevent an antigen–antibody reaction between the blood of the donor and the recipient, which can be fatal. The most common blood-typing systems, ABO and Rhesus (Rh), are the first-line pretransfusion suitability tests. Inaccurate blood typing of the donor and recipient can result in a hemolytic transfusion reaction in the recipient. Blood typing involves determination of agglutination with the anti-A, anti-B, and anti-D blood-typing reagents. Conventionally, the blood sample and each blood-typing reagent are manually loaded, and agglutination is evaluated in tubes or gel columns, or on slides or microplates. The blood type is determined according to the pattern of agglutination associated with the blood-typing reagents. However, conventional blood-typing methods depend on manual interpretation of the reactions between the reagents and the blood sample, which can lead to human-induced error. To prevent this, fully automated blood-typing systems are used in blood banks and hospitals. Although these yield reliable results, they are unsuitable for rapid blood typing of patients in urgent need of transfusion.

To address the above limitations, simpler and more accurate methods of blood typing based on microfluidic technology have been developed, wherein the blood-typing reagents and blood sample are reacted in microfluidic channels, and agglutination is assayed by a plasmonic, optical, electrical, or rheological method. However, such systems require an external equipment, rendering them bulky and complicated. As a simpler approach, pumpless microfluidic devices based on finger motion, capillary force, vacuum-driven flow, or a simple screw pump have been developed. Although these systems allow for reactions between the reagents and the blood sample, they show the agglutination pattern only. Also, the blood is typed by manually reading the agglutination pattern, resulting in human-induced error.

A paper substrate has been used for blood typing based on the principle that RBC aggregates cannot penetrate the pores in the paper. Although a paper substrate is convenient for the end user, an additional assay, for example, based on the movement of RBCs, smart phone-based images, or spectroscopy, is needed to determine the blood type. Such system displays the blood type on the surface of the filter membrane, which aggregated RBCs cannot penetrate and from which nonaggregated RBCs are washed away. In this way, the blood type can be easily determined at a glance, but the sequential loading of reagents and the incubation time prevent rapid blood typing.

Here, we present a simple and rapid blood-typing system with a finger-actuated microfluidic device that displays the blood type by means of microfluidic channels. With use of a novel working principle of the finger-actuated pump, a constant volume is dispensed regardless of differences in end user. Compared to our previous design, the finger-actuated pump utilizes space more efficiently. Multiple inlet channels are connected to the single actuation chamber, enabling...
charging with multiple fluids simultaneously, where the charge is inversely proportional to the fluidic resistance of the inlet channels. The mixing ratio of multiple fluids can be controlled by adjusting the fluidic resistance of the inlet channels. This allows the blood sample to be reacted with the anti-A, anti-B, and anti-D blood-typing reagents simultaneously by pushing a single button. To display the blood type via microfluidic channels, two flow paths are needed: a channel with a microslit filter and a bypass channel. If agglutination occurs, the mixture of blood and reagent is delivered only to the bypass channel. However, the mixture is delivered to both the microslit filter channel and the bypass channel if no agglutination occurs. On the basis of flow path, ABO and Rh blood types are displayed simultaneously, where the charge of fluids can be controlled by adjusting the fluidic resistance of the inlet channels. The blood-type display also has a control panel to prevent blood mistyping due to the aggregation of RBCs, in turn caused by a high protein concentration. With use of our system, blood can be typed rapidly without external equipment or human-induced error.

**EXPERIMENTAL SECTION**

**Device Fabrication.** The microfluidic smart blood-typing device consists of fluidic channels with a poly-(dimethylsiloxane) (PDMS) layer, pneumatic channels with a PDMS layer, a PDMS cover layer, and a thin PDMS membrane. The micromolds for fluidic channels and pneumatic channels were fabricated by photolithography. To develop the microslit filter, a 3.5-μm-thick layer of SU-8 2005 (MicroChem Corp., Westborough, MA, USA) was spin-coated onto a silicon wafer. Next, an SU-8 2050 layer with a height of 50 μm was spin-coated over the first layer. PDMS layers were obtained by curing a mixture of PDMS precursors and a curing agent (mixing ratio = 10:1) at 85 °C for 1 h. A thin PDMS membrane with a height of 25 μm was obtained by spin-coating a mixture of PDMS precursors and a curing agent (mixing ratio = 7:1) onto a bare silicon wafer at 1500 rpm for 60 s, and curing at 150 °C for 1 min. Each layer was bonded after being treated with oxygen plasma for 1 min and incubated at 65 °C for 10 min.

**Measurement of Mixture Concentration.** A solution of 1 mM erioglaucine (Sigma-Aldrich, St. Louis, MO, USA; a peak absorbance at 406 nm) was used to measure the concentration of the mixture. To produce a calibration curve, 10 μL of 0.1–1 mM erioglaucine solution was added to 200 μL of distilled water in a 96-well plate and the absorbance at 406 nm was measured. Then 1 mM erioglaucine solution was mixed with distilled water in the device. The fluid from the outlet (10 μL) was added to 200 μL of distilled water in a 96-well plate, the absorbance at 406 nm was measured, and the concentration was determined from the calibration curve.

**Blood-Typing Procedures.** Ethylenediaminetetraacetic acid-treated whole-blood samples were obtained from the Biobank of Chungnam National University Hospital (Daejeon, Korea) in compliance with safety regulations and stored at ~4 °C. For off-chip blood typing on a glass slide, each blood-typing reagent (anti-A, anti-B, and anti-D) (Shinyang Diagnostics, Seoul, Korea) was mixed with whole blood and incubated on a glass slide. The anti-A and anti-B reagents are IgG, and the anti-D reagent is a mixture of IgG and IgM. For smart blood typing, the device is preloaded with 10 μL of the blood-typing reagents and 3% bovine serum albumin (BSA) solution. The end user loads 10 μL of whole blood into the
inlet while depressing the push button. Next, the user pushes the button seven times, and the blood type is shown on the microfluidic display (Movie S1 of the Supporting Information).

RESULTS AND DISCUSSION

Design and Working Principle. The blood-typing device contains four independent fluidic channels operated by finger-actuated pumping units (Figure 1A). Each pumping unit consists of pneumatic valves and an actuation chamber. A uniform volume of fluid is charged and discharged without backflow by pushing and releasing the button (Figure 1B). Pneumatic valve 1 is operated by the pressure change in the pneumatic channel and valve 2 by the pressure change in the fluidic channel. Upon depression of the button, the increased pressure in the pneumatic channel deflects the thin PDMS membrane, closing valve 1 and compressing the actuation chamber. Next, the fluid in the actuation chamber is discharged into the outlet by opening valve 2. In contrast, when the button is released, valve 1 opens and an actuation chamber decompresses, reducing the pressure in the fluidic channel. The actuation chamber is then charged with fluid from the inlets with valve 2 closed.

Three pumping units are used to mix the anti-A, anti-D, and anti-B blood-typing reagents with whole blood to determine the presence or absence of agglutination. The other pumping unit mixes whole blood with 3% BSA to prevent mistyping caused by agglutination due to the high protein content of the blood-typing reagents. Two pumping units simultaneously draw a constant volume of whole blood and reagents and discharge them via three serpentine microfluidic channels and the control channel (Figure 1C). Each pumping unit dispenses about 0.5 μL of fluid. Because the viscosity of whole blood is higher than that of the blood-typing reagents, the inlet channel for whole blood (400 μm) was wider than that of the blood-typing reagents (300 μm) to allow the mixing ratio to be adjusted. RBC aggregates at the serpentine microfluidic channel corresponding to the blood type, and the aggregates increase in size as they pass through the reaction chamber. To enhance agglutination, the reaction chamber is higher (125 μm) and wider (1750 μm) than the serpentine channel. After the aggregates pass through the reaction chamber, the fluid path is determined according to the presence of agglutination (Figure 1D,E). In the presence of agglutination, the microslit filter is blocked by the RBC aggregates, directing the mixture into the bypass channel. In the absence of agglutination, the mixture flows into both the microslit filter channel and the bypass channel. The three microslit filter channels, but not the bypass channel, are connected to the blood-type display. To minimize the shear rate along the microslit filter channel, and to prevent the difference in flow rate compared to the bypass channel from increasing in magnitude, the width of the microslit filter channel is twice that of the bypass channel. The mixture of whole blood and 3% BSA flows into the blood-type display along the control channel. The blood-type display is composed of three microslit filter channels (dark pink) and the control channel (light pink), which are separated by the thin PDMS membrane. (Figure 1F). Aggregation in the control panel indicates protein-induced agglutination and an unreliable result.

Assessment of the Connection of Multiple Inlet Channels to a Single Actuation Chamber. In our previous work, we used one actuation chamber per inlet so multiple actuation chambers were required. The two fluids were dispensed into the outlet via a serpentine channel at a predetermined ratio. To improve the system for dispensing multiple fluids at a desirable ratio, we connected multiple inlet channels to a single actuation chamber. Fluids from multiple inlets can be simultaneously charged into the single actuation chamber and mixed in the serpentine channels (Figure 2A). To verify this concept, we designed a device with six inlets and a single actuation chamber of 2 μL volume (Figure 2B). A

Figure 2. Operation of a single-actuation-chamber device with multiple inlet channels. (A) Schematic of a single-actuation-chamber device with multiple inlet channels. Each inlet channel has the same flow resistance. (B) Enlargement of the circled area in panel A. Six inlet channels are connected to the single actuation chamber. Scale bar = 1 mm. (C) Mixture concentration according to the number of injection sites for 1 mM erioglaucine solution. One inlet is used for 1 mM erioglaucine solution, and the others are for distilled water. (D) Mixture concentration according to the location of the injection sites for the 1 mM erioglaucine solution. One inlet is used for 1 mM erioglaucine solution, and the others are for distilled water. (E) Schematic of a device with a single actuation chamber connected to two inlet channels differing in flow resistance. (F) Enlargement of the boxed area in panel E. The two inlet channels differ in width. Scale bar = 1 mm. (G) Mixture concentration according to the width ratio of the two inlet channels.
mixture of 1 mM erioglaucine and distilled water was measured by controlling the number of injection sites for 1 mM erioglaucine solution. The concentration of the mixture increased linearly with increasing number of inlets for 1 mM erioglaucine (Figure 2C). We also evaluated the effect of the angle of injection of 1 mM erioglaucine on the volume of fluid charged into the actuation chamber. Erioglaucine solution (1 mM) was injected into one inlet, and the remaining inlets were injected with distilled water. There was no significant difference in the concentration of the mixture according to the location of the inlet (Figure 2D). Therefore, a single actuation chamber can be simultaneously charged with multiple fluids irrespective of the angle of the inlet channel.

Furthermore, the fluid charging ratio from multiple fluids can be controlled by modulating the fluidic resistance of the inlet channels. To test this, the device had two inlet channels of different widths connected to a single actuation chamber 2 μL in volume (Figure 2E,F). Because the structure of the pneumatic valve in the inlet channel plays a significant role in fluidic resistance, any change in geometry of the pneumatic valve influences the fluidic resistance. Erioglaucine solution (1 mM) was injected into inlet 1 and distilled water into inlet 2. The concentration of the mixture increased according to the width ratio of channels 1 and 2 (Figure 2G). Meanwhile, the length of the inlet channel did not significantly influence the concentration of the mixture because it had no effect on the geometry of the pneumatic valve in the inlet channel (Figure S1 of the Supporting Information).

In general, the flow rate ratio of the microfluidic channels under the same pressure drop is the inverse of the hydraulic resistance, which is proportional to the viscosity of the fluid. Accordingly, because multiple fluids are charged into the actuation chamber according to the flow rate ratio under the same pressure drop, the viscosity of the fluid affects the fluid-charging ratio. Because the concentration of the mixture was 0.5 mM at a channel width ratio of 1, the viscosity of 1 mM erioglaucine solution was similar to that of water. The viscosity and fluidic resistance must be considered to determine the mixing ratio of multiple fluids of different viscosities. In the smart microfluidic blood-typing device, the inlet channel for whole blood, which is considerably more viscous than the reagents, is wider than those for the blood-typing reagents and BSA, to increase the charging rate of blood. We controlled the mixing ratio according to fluidic resistance using a device with two inlet channels, so a number of inlet channels differing in fluidic resistance could be used to control the mixing ratio of multiple fluids.

Optimizing the Design of the Microslit Filter. We optimized the design of the microslit filter as follows. First, the microfluidic blood-type display should be clear with ensured filtration efficiency; the smaller the microslit filter, the higher the filtration efficiency and the clearer the microfluidic display. Second, the number of button pushes should be minimized to enhance user-friendliness; the number of button pushes required increases with decreasing flow rate along the microslit filter channel. We first evaluated microslit filters with heights of 2, 3.5, and 5 μm and length of 200 μm. Rh+ A type blood aggregated in the anti-A and anti-D channels but not in the anti-B channel. Thus, the microslit filter of the anti-A and anti-D channels, but not that of the anti-B channel, was blocked by RBC aggregates. In all cases an “A” was displayed and an “X” appeared over the “B” (Figure 3A). However, the Rh blood-typing result was unclear using microslit filters with heights of 3.5 or 5 μm. Agglutination occurred as expected, and small RBC aggregates passed through the microslit filter in the anti-A and anti-D channels (Figure 3B). However, the aggregation caused by the anti-D reagent was weaker than that induced by the anti-A reagent, leading to more passage of small RBC aggregates through the microslit filter in the anti-D channel. The weak agglutination of the anti-D reagent is due to it being a mixture of IgG and IgM because the level of antigen D on the surface of RBCs is lower than that of antigens A and B. The blood type was displayed clearly when a microslit filter of height 2 μm was used. However, more than 10 button pushes were required, necessitating the use of large quantities of reagents and reducing the user-friendliness of the system. RBC aggregates passed through the 5 μm height of the microslit filter, hampering determination of the Rh blood type. Therefore, the optimum height of the microslit filter was 3.5 μm, which necessitated seven button pushes to obtain a reliable blood-typing result. Although some RBC aggregates appeared over the “+”, this did not hamper interpretation of the result. In short, the lower the height of the microslit filter, the better the clarity of the microfluidic display and the higher the flow resistance in the channel, resulting in a greater number of pushes being required to obtain a reliable result. The microslit filter should be designed such that the microfluidic display is clear and the number of button pushes required is minimized.

Therefore, we evaluated the number of button pushes required to obtain a reliable result according to the flow rate ratio between the microslit filter channel and the bypass channel. To obtain a reliable result, the device should be

![Figure 3. Optimizing the height of the microslit filter using Rh+ A-type blood. (A) Images of the blood-type display according to the height of the microslit filter. Using microslit filters of height 5, 3.5, and 2 μm with 5, 7, and 17 pushes, respectively, were required to obtain a reliable result. Scale bar = 1 mm. (B) Images of the microslit filters of the anti-A, anti-D, and anti-B channels. Scale bar = 200 μm.](image-url)
operated until “X”, each half of “O”, and “−” are displayed, and the higher the flow rate into the microslit filter channel, the fewer the number of button pushes. The flow rate ratio between the microslit filter channel ($Q_1$) and the bypass channel ($Q_2$) ($Q_1/(Q_2 + Q_1)$) was subjected to numerical analysis using COMSOL Multiphysics 5.3 (COMSOL, Inc., Burlington, MA, USA) according to the geometry of the microslit filter (Figure 4A). The velocity at the channel cross section is shown in Figure 4B. As expected, the fluidic resistance of the microslit filter channel was greater than that of the bypass channel so that less than 30% of the fluid was directed to the microslit filter channel (Figure 4C). Because the fluidic resistance in the rectangular channel is affected more by the height than the width of the channel, the flow rate ratio is influenced by the height of the microslit filter; the lower the flow rate ratio, the smaller the volume of mixture flowing into the microslit filter, such that a greater number of button pushes is required. Conversely, a higher flow rate ratio requires fewer button pushes. A microslit filter was 3.5 μm in height and 200 μm in length was optimum, so the number of button pushes required could be reduced by decreasing the length of the microslit filter to 100 μm. However, because of the increased flow rate, RBC aggregates did not clog the microslit filter, particularly in the anti-D channel, rendering the blood-typing result unclear (Figure S2 of the Supporting Information). The increased flow rate into the microslit filter channel resulted in a high shear rate, forcing RBC aggregates through the microslit filter. Therefore, the optimum microslit filter had a height of 3.5 μm and length of 200 μm, and seven button pushes were required to obtain a credible result; fewer than seven button pushes yielded an unreliable result. For example, for Rh+ A blood, the system should show an “X” over the “B” and half of “O”; however, this did not occur (Figure S3 of the Supporting Information). The large number of button pushes resulted in RBC aggregates passing through the microslit filter, rendering the microfluidic display unclear. This was particularly so in the anti-D channel.

Figure 4. Numerical analysis of the flow rate ratio between the microslit filter channel and the bypass channel. (A) Schematic of the numerical analysis model with a single microslit filter and bypass channel. Inset, enlarged three-dimensional schematic of a microslit filter. (B) Numerical analysis of the velocity profile of the channel cross section with respect to the height and length of the microslit filter. (C) Flow rate ratio between channels according to the geometry of the microslit filter.

Figure 5. Results of finger-actuated microfluidic smart blood typing. (A) Expected results of the eight blood types. (B) Photograph of the blood-typing device, indicating Rh+ A. Scale bar = 5 mm. (C) Images of the four blood-type displays. (D) Demonstration for the blood typing of Rh− blood samples. Bovine serum albumin (BSA) solution (3%) was used in place of the anti-D reagent. Scale bar = 1 mm.
Results of Smart Blood Typing. The results of finger-actuated microfluidic smart blood typing are shown in Figure SA. The “A,” “B,” “+,” and control panel are presented via the control channel, while the “X,” two halves of “O,” and “−” are displayed via the microslit filter channels. The blood type is shown on the display after pushing the button seven times (Figure SB). We performed the typing Rh+ A, B, AB, and O blood using our system (Figure SC). We could not obtain Rh− blood samples, so Rh− blood typing was demonstrated by injecting 3% BSA in place of anti-D reagent, on the assumption that the anti-D reagent does not induce agglutination of RBCs (Figure SD). Agglutination in the control panel indicates aggregation of RBCs due to the protein content of the blood-typing reagents. Therefore, the result of the blood typing should not be accepted if agglutination occurs in the control panel. BSA solution (3%), the viscosity of which is similar to that of the blood-typing reagent (Figure 1C), was used for quality control of agglutination.

The system displayed “A,” “B,” and “O” clearly in some cases, with debris over the “+” due to weak binding between the anti-D antibody and RBCs. The microslit filter was designed to minimize the flow rate difference between the microslit filter channel and the bypass channel without distorting the displayed result. The clarity of the Rh blood-type display is associated with the flow rate into the microslit filter channel, which is in turn related to the geometry of the microslit filter. Reducing the shear rate would prevent the passage of RBC aggregates through the microslit filter, but a large number of button pushes would be required. To prevent disturbance of the microfluidic display without increasing the number of button pushes required, a smaller microslit filter with lower flow resistance can be used. Because blood viscosity increases over time, the mixing ratio of the blood and reagents differs depending on the storage time of blood. Blood of high viscosity has a low drawn ratio, resulting in an unclear microfluidic channel-based blood-type display. However, the system performed well even using an old blood sample (Figure S4 of the Supporting Information).

Our smart blood-typing system has several issues that must be overcome before it can be used in the clinical laboratory. First, clinical trials involving analysis of various blood samples are required because the hematocrit, protein content, and viscosity of blood differ according to gender, age, and disease status. Second, although PDMS facilitates rapid prototyping, its hydrophobicity results in the generation of bubbles in fluid. To overcome this, the channels of the smart blood-typing system were filled with distilled water; ideally, this step should be eliminated to improve the user-friendliness of the system. Therefore, clinical trials of smart blood-typing systems manufactured from a hydrophilic material and involving various blood samples are warranted.

**CONCLUSIONS**

In summary, blood typing was performed using a finger-actuated microfluidic device. The results are displayed by means of microfluidic channels according to the agglutination aspect. The actuation chamber has multiple inlet channels to allow facile mixing of the blood-typing reagents with whole blood. The geometry of the microslit filter was optimized and the flow rate ratio between the microslit filter channel and the bypass channel was analyzed numerically; furthermore, the system does not require manual interpretation of the results. The blood type of an unknown sample can be determined within 30 s using <10 μL of blood. Our novel blood-typing system prevents blood mistyping due to errors in analysis of the agglutination patterns, or to agglutination caused by the protein content of the blood-typing reagents. Furthermore, the working principle of the device can be expanded to minor human blood-type systems (e.g., the Kell, Kidd, MNS, and Lewis systems). Our system facilitates rapid blood typing and could, together with our recently developed finger-actuated blood cross-matching test, prevent hemolytic transfusion reactions.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b02129.

Effect of the mixture concentration on the length of each inlet channel, typing of Rh+ AB blood sample with the 100 μm length and 3.5 μm height of the microslit filter, typing of Rh+ A blood sample according to the number of button pushes, and typing of the 22-day-old Rh+ AB blood sample (PDF)

Operation of the device (MP4)

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Notes

The authors declare no competing financial interest.

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