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ARTICLE

Rapid and inexpensive blood typing on thermoplastic chips†

Jun-You Chen,‡ Yi-Ting Huang,‡ Hsin-Hao Chou, Cheng-Po Wang and Chien-Fu Chen*

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A portable and cost-effective colorimetric diagnostic device was fabricated for rapid ABO and Rh blood typing. Using microfluidic construction on a thermoplastic chip, blood antibodies were preloaded into a reaction channel and exposed to blood samples to initiate a haemagglutination reaction. Downstream high-aspect ratio filters, composed of 2 μm high microslits, block agglutinated red blood cells (RBCs) to turn the reaction channel red, indicating the presence of the corresponding blood antigen. Users manually actuate the blood sample using a simple screw pump that drives the solution through serpentine reaction channels and chaotic micromixers for maximum interaction of the preloaded antibodies with the blood sample antigens. Mismatched RBCs and antibodies elute from the channel into an outlet reservoir based on the rheological properties of RBCs with no colorimetric change. As a result, unambiguous blood typing tests can be distinguished by the naked eye in as little as 1 min. Blood disorders, such as thalassemia, can also be distinguished using the device. The required blood volume for the test is just 1 μL, which can be obtained by the less invasive finger pricking method. The low reagent consumption, manual driving force, low-cost of parts, high yield, and robust fabrication process make this device sensitive, accurate, and simple enough to use without specialized training in resource constrained settings.

Introduction

Accurate identification of blood groups is critical for blood banks and medical centers to provide safe blood transfusion and transplantation for donors and recipients.[1, 2] Mistyped blood groups can cause a hemolytic transfusion reaction with potentially fatal consequences. Blood groups are also used as valuable generic bioinformatic markers for clinical applications, such as disease therapeutics and prognoses.[3-5] For these reasons, rapid, accurate, and inexpensive blood typing tests are necessary for quality healthcare services.

Conventional blood typing tests that are performed in medical centers include column agglutination and solid phase tests.[6-8] 328 different antigens on the erythrocyte surface have been identified and classified into 30 different blood type groups.[9] Among these, ABO blood groups and rhesus (Rh) typing are the most frequently classified categories. The mechanism of antiglobulin based blood typing tests is the observation of RBC agglutination when specific antibodies in the serum conjugate with the corresponding antigens on the RBC surfaces. This antibody-antigen interaction brings RBCs closer together, thus causing visual accumulation. Although these haemagglutination reaction tests are reliable and accurate, they also require well-trained personnel and sophisticated equipment to obtain results. For example, the column agglutination method requires preparation of the blood sample and multiple centrifugation steps to clarify blood typing results. These necessities limit usage in resource constrained environments, such as battlefields or developing countries. In addition, typical agglutination tests require a blood sample of more than 1 mL, which cannot be obtained using low invasive methods, such as finger pricking. A rapid, inexpensive, and painless blood sampling tool would be a valuable addition to medical diagnostics.

In response to these issues, several cell typing platforms have been developed based on the concept of point-of-care diagnosis in combination with low blood sample requirements.[10-14] Among the choices of substrate materials, paper-based assays are attractive due to their wicking behavior, flexibility, scalability of manufacturing, and convenience of colorimetric readouts.[10, 11] Filtration of agglutinated RBCs allows unreacted RBCs to be transported through a paper substrate, enabling the haemagglutination results to be observed by the naked eye. Blood typing can also be realized on paper *via* chromatographic elution,[10] or by direct text report,[11] requiring a blood sample volume of only 3 μL. However, these techniques require copious amounts of buffer solution to rinse the reaction zone in order to obtain a clear result. In addition, the immune interaction between RBCs and antibodies on paper substrates depends on limited diffusion and finite incubation times, which may increase the amount of time it takes to obtain a result for weakly interacting blood groups such as A_3 (subgroups of A), B_3 (subgroups of B), and patients with hematologic malignancy or thalassemia.[2]

In addition to paper substrates, polymers can provide another cost-effective approach for blood typing using fluidic control elements.[12-14] Sufficient interaction between RBCs and antibodies can be realized by combining chaotic micromixers and incubation chambers. Agglutinated RBCs can then be collected using different sized sieves with results displayed in a readout corresponding to

Graduate Institute of Biomedical Engineering, National Chung Hsing University, Taichung 402, Taiwan.
Email: stevechen@nchu.edu.tw
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‡ The authors contributed equally to this work.

where the RBCs have been blocked.[12] However, bulky external pumping systems and power source requirements for these tests prevent processing in resource limited environments. In addition, tubing and fluidic interfaces complicate the operation and induce more dead volume into the system. Various fluidic methods have been developed for cell typing on polymer chips based on the detection of RBC agglutination *via* rheological measurements[13] or dielectrophoretic characterization of polarized RBCs.[14] Despite the elegance and precise control of these methods, analysis must be further simplified with fewer blood samples, equipment, and operation requirements in order to realize truly rapid and inexpensive blood typing tests.

In this study, we propose a portable colorimetric detection platform, which employs a screw pump, serpentine reaction channels, chaotic micromixers,[15-17] and low-aspect-ratio filters for rapid blood typing on a polymer chip. In order to eliminate active fluid-transport systems and obtain visualized results for quick on-site diagnosis, commercially available stainless steel screws served as the pressure source to actuate the flow of blood and buffer solution towards interaction with downstream antibodies. Filters block the agglutinated RBCs and turn the reaction channel on the device the color red, corresponding to the indicated ABO and Rh blood types. Our design requires only 1 µL of blood, which can be obtained using a simple finger prick. The RBC agglutination result can be visualized on the device in 1 min for both normal blood samples and those exhibiting thalassemia, a disorder which causes the patient's RBCs to have a smaller mean corpuscular volume (MCV)[18] and a lower hematocrit. This makes it a rapid and effective blood typing method that is also simple and low-cost enough to implement in a variety of circumstances and conditions.

Experimental

Chemicals

Methanol, ethanol, 2-propanol, cyclohexane, phosphate buffered saline (PBS), and tris-borate-EDTA were purchased from Sigma-Aldrich (St. Louis, MO). Monoclonal Anti-A, Anti-B, and Anti-D antibodies were received from Sanquin (Amsterdam, Netherlands). Ultrapure water (18.2 mΩ • cm) was obtained using a Milli-Q system (Millipore, Milford, MA).

Instrumentation and characterization

The dimensions of the flow channels and filters were inspected using a scanning electron microscope (SEM; JSM-6700F, JEOL, Tokyo, Japan). X-ray photoelectron spectroscopy analysis of the modified channel surface was characterized utilizing a ULVAC-PHI 5000 VersaProbe ESCA microprobe. Back pressure resistance of the screw pump was verified using an analytical liquid chromatography (LC) pump (AD20, Shimazhu, Kyoto, Japan). Water contact angle measurements were performed using a goniometer (First Ten Angstroms, Portsmouth, VA). Blood agglutination results for the blood typing test were observed both by the naked eye and using a Leica DMI3000 B inverted microscope (Leica Microsystems, Mannheim, Germany) to collect images.

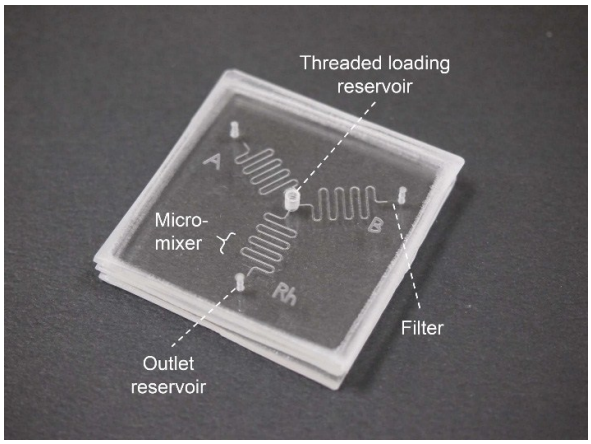


Fig. 1. The blood typing chip. Using a seamless contact between the screw and the threaded interface at the loading reservoir, the blood sample can be manually actuated by rotating the screw to create pressure that causes the sample to flow into the three downstream reaction channels. The RBCs' surface antigens rapidly interact with preloaded specific antibodies through the arrangement of the serpentine reaction channels and chaotic micromixers. A matched blood type causes a haemagglutination reaction, which blocks flow at the 2 µm high filters located at the channel outlets, turning the reaction channel from transparent into red.

Fabrication of the blood typing chip

The blood typing chip was composed of several components, including a loading reservoir with a screw interface for screw installation, a chaotic micromixer for rapid cell-antibody interaction, outlet reservoirs, and low-aspect-ratio filters for blocking agglutinated blood. A schematic illustration and images of the fabrication process is shown in Fig. S1. The device was fabricated from two COP chips (Zeonor 1020R, Zeon, Tokyo, Japan), which were 3 cm × 3 cm × 4 mm and 3 cm × 3 cm × 2 mm in size. The thicker top cover chip was drilled to create a threaded screw interface at the loading reservoir port, as well as the outlet reservoirs (details below). The bottom layer chip was patterned with the serpentine reaction channels, chaotic micromixers, and low-aspect-aspect ratio filters (details below). The two chips were sealed into one device after the bottom chip was swelled with cyclohexane during the selective solvent swelling process, followed by directly pressing the two substrates together using a uniform pressure of 3.45 MPa for 1 min at room temperature.

Fabrication of the top cover COP chip

The top cover COP polymer chip was sequentially cleaned with methanol, 2-propanol and ultrapure water, then blown dry with N_2. After deaerating in a 50 ^{0}C vacuum oven for 8 h, three outlet reservoirs were drilled through the chip, 650 µm in diameter. A 1.50 mm diameter drill bit was used to drill a hole in the center of the device, which was subsequently threaded using a commercially available 2 mm diameter stainless steel screw (Fig. S1a).

Fabrication of the bottom COP chip

Microchannels and chaotic micromixers were first milled onto a bare COP chip using a 125 µm diameter end mill that was controlled using a computer numerical control (CNC) milling machine (EGX 400, Roland, Irvine, CA). The channel depth was 100 µm. The micromixer component was made by drilling 200 µm deep holes every 500 µm at the outlet reservoirs and half-way up the length of each flow channel (Fig. S1b). The COP chip was then sonicated for 30 min in ultrapure water to remove debris, followed by sequential rinsing using methanol, 2-propanol, and ultrapure water. The cleaned COP chip was dried with N_2 and deaerated at 50 ^{0}C in a vacuum oven for 8 h. Filters for the agglutinated RBCs were fabricated at the ends of the flow channels and aligned with the outlet reservoirs of the matching top cover chip (Fig. 1). Blue dicing tape was milled to create a 125 µm × 2 mm open window. This stencil was then attached to the chip surface. The open window was aligned to connect the flow channels and outlet reservoirs. The COP chip and stencil complex was exposed to UV/ozone (UVO; Novascan, Ames, IA) for 30 min. After removing the tape, the COP chip was placed in a vapor-phase cyclohexane container for 6 min (Fig. S1c) followed by bonding to the top cover COP chip using just pressure alone (3.45 MPa) for 1 min at room temperature (Fig. S1d).

Pressure resistance measurement

A microfluidic chip composed of a single channel featuring a screw pump and a high-pressure needle interface[19] at either end was prepared to measure the pressure resistance of the screw pump. First, the stainless steel screw was twisted to the bottom of the threaded hole. Next, the hypodermic needle segment was installed at the reservoir as a fluidic transportation interface. The pressure resistance of the screw pumping interface was monitored by pumping liquid from an analytical LC pump through a capillary tube connected to the chip *via* the needle interface. Eight different drill bits of diameters ranging in 50 µm intervals, from 1.35-1.70 mm, were used to prepare the loading reservoir with screw pump interfaces for the pressure tests. The burst pressure was defined as the maximum pressure that can be read on the LC pump console while continuously pumping ultrapure water to the pump at 10 µL/min.

Optimal loading volume tests

The same chip for the pressure resistance test was prepared using the aforementioned processes. 5 different volumes of ultrapure water, from 4-8 µL in 1 µL intervals, were pipetted into the loading reservoir. The stainless steel screw was then inserted and twisted to the bottom. The pumping volume of each test was estimated by measuring the difference in the weight of paper after absorbing solution at the outlet reservoir. The waste volume, including the water within the flow channel and the dead volume, was calculated as the total loading volume minus the pumping volume. The measurement process and the remaining water within the flow channel was also confirmed using a microscope. Before each loading volume test, the flow channel and the interfaces were dried with N_2 and allowed to sit at room temperature for 4 h.

Blood typing diagnosis

The blood typing chip devices were prepared using the aforementioned processes. 0.5 µL of anti-A, anti-B, and anti-D antibody reagents were separately pipetted into the outlet reservoirs. The antibody reagents were then allowed to flow half way up the reactive flow channel by gently applying vacuum pressure using a withdrawing syringe connected to the loading reservoir *via* plastic tubing. The liquid outlets of the chip were then covered with blue dicing tape (Nitto Denko, Osaka, Japan) and the chip was sealed in a plastic bag with a sterilized screw and stored at 4 ^{0}C in the refrigerator before the test.

30 blood samples were obtained from male and female volunteers, ages 20-40. All experiments were performed in compliance with relevant laws and institutional guidelines. All procedures were approved by the CGM hospital. Informed consent was obtained from these patients prior to sample collection. 1 µL of blood obtained *via* finger prick performed using a lancing device was pipetted into the threaded loading reservoir followed by the addition of 5 µL PBS buffer solution. The screw was inserted and twisted to pump the blood sample down the reaction channels and micromixers to encounter and react with the antibodies until the screw came into contact with the bottom of the loading reservoir. The agglutination of the blood sample blocked at the filters was observable by the naked eye and recorded using a camera.

Results and discussion

The blood typing platform was fabricated from cyclo-olefin polymer (COP). This substrate was chosen for its unique material properties including high biological compatibility, high transparency in the visible region of the spectrum, low water absorption, as well as low oxygen and moisture permeability.[20-22] The chip features several components, which are shown in Fig. 1. The sample loading reservoir is located at the center of the chip and was made with a threaded side-wall interface, which allows a screw pump to be installed for manual pressure actuation. Three serpentine reaction channels and corresponding chaotic micromixers and low aspect-ratio filters extend from the central loading reservoir. At the end of each reaction channel is an outlet reservoir where unreacted blood samples accumulate.

Two COP chips were used to fabricate different components of the device. The chips were then pressed and sealed together to complete the microfluidic features. The threaded loading reservoir and the three outlet reservoirs were constructed from the top cover chip. The serpentine reaction channels and chaotic micromixers were milled out of the bottom chip. The low-aspect ratio filters were also fabricated from the bottom COP chip using a selective solvent swelling method.[23] Antibody reagents were separately preloaded into the outlet reservoirs and subsequently flowed half way up the reactive flow channels by gently applying vacuum pressure using a syringe connected to the loading reservoir *via* plastic tubing to avoid cross contamination and false positive results during the diagnostic test. The consumption of reagents for the anti-A, anti-B, and anti-D antibodies was just 0.5 µL each. The antibodies can be pre-loaded

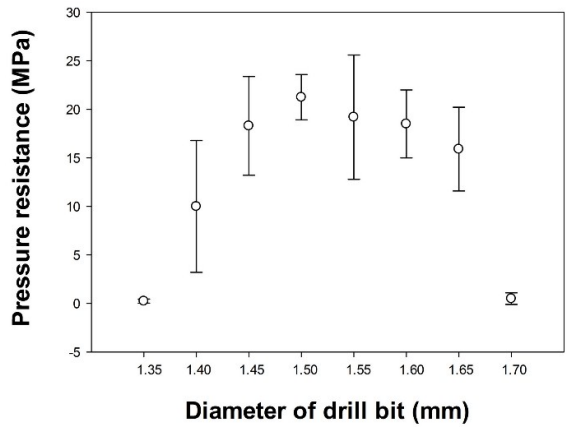


Fig. 2. A pressure resistance test was used to confirm what size pre-drilled hole should be used to fabricate the threaded interface at the loading reservoir. The maximum mean pressure resistance with relatively low variation (21.25 ± 2.33 MPa) was obtained using a 1.50 mm diameter drill bit to pre-drill the hole before tapping (n = 7).

into the fluidic channel and stored at 4 ^{0}C for up to 6 months prior to application of the test and accurate blood typing results will still be obtained. A, B, and Rh symbols were milled onto the top cover chip to indicate the locations of the antibodies and the corresponding blood type results.

Loading reservoir with threaded screw interface

A screw was used in the device to actuate the flow of blood in order to avoid complicated and costly pumping systems used in other blood typing techniques, which cannot be easily be integrated and realized for practical applications. In addition, the screw-pump actuator eliminates the need for tubing and fluidic interfaces to further simplify the system, lowering the cost, and limiting the dead volume. A seamless contact between the screw and the threaded sample loading reservoir was critical for achieving low reagent consumption and minimal dead volume in the COP chip. Epoxy glue was not used in the interface fabrication process to avoid device contamination or stability issues. In addition, cross contamination can be avoided by applying uniform pressure from the screw into the three serpentine reaction channels to eliminate backflow into other channels.

A stainless steel screw with a 2 mm diameter was chosen for the actuator port based on its suitable actuation volume (µL), as well as its corrosion resistance, cost effectiveness, and biocompatibility.[24, 25] In order to create a mating threaded sidewall at the loading reservoir, the screw was used to tap the thread directly rather than using a conventional thread-tapping tool. When the thread was formed using a conventional tapping tool, voids existed between the screw and the thread, which caused leakage during solution transport. In contrast, using the screw itself to tap the thread created a seamless contact between the screw and the threaded reservoir. However, to achieve this seamless contact, without crack or void, it was necessary to drill a pilot hole before tapping. If the pre-drilled hole was too small, the induced stress during tapping caused cracking. Alternately, if the pre-drilled hole was too large, voids were subsequently observed.

To confirm the seamless nature of the loading port, a single channel microfluidic chip was fabricated to test for pressure resistance. This test setup incorporated a loading reservoir at one end of the channel and a high-pressure needle interface[19] at the outlet reservoir on the other end. First, the stainless steel screw was twisted to the bottom of the loading reservoir. Next, the hypodermic needle was installed at the outlet reservoir and connected to an analytical LC pump to measure the back-pressure. Eight different drill bits featuring diameters, from 1.35-1.70 mm, were used to pre-drill holes for the threaded interfaces of the loading reservoir. A low variation, maximum mean pressure resistance of 21.25 ± 2.33 MPa was observed when the 1.50 mm diameter drill bit was used to pre-drill the hole for tapping (Fig. 2). When the drill bit diameter was greater than 1.7 mm and less than 1.35 mm, the pressure resistance of the threaded screw interface was below 0.5 MPa. The highest pressure resistance measurement for these leakage tests indicated the best seal between the screw and the threaded interface. Therefore, the 1.5 mm diameter drill bit was used to pre-drill holes for all subsequent tests.

We next investigated the optimal sample loading volume in order to maximize the blood/antibody interaction while also minimizing the amount of solution waste to keep blood sampling requirements as small as possible. The total sample volume was conceptually divided into the pumping volume and the waste volume. The pumping volume was the amount of solution that flowed from the loading reservoir and collected into the outlet reservoir. This fraction represents the amount of sample that fully interacts with the preloaded antibodies. The pumping volume was estimated by absorbing the solution collected in the outlet reservoir using a piece of paper and measuring the difference in the paper's mass before and after solution absorption. The waste volume was defined as the amount of sample that remained within the flow channel and never collected into the outlet reservoir, representing the portion of the sample that does not fully interact with the antibodies. The waste volume was estimated by subtracting the measured pumping volume from the total sample loading volume. To investigate the optimal loading volume, 5 different amounts of ultrapure water (4-8 µL) were loaded into the device. The pumping and waste volumes were subsequently measured as described (Table S1). The waste volume remained the same (1.2 µL) when the loading volume was ≤ 6 µL, however the amount of waste continued to increase for loading volumes of > 6 µL. The pumping volume plateaued at 4.8 µL when the loading volume was ≥ 6 µL. These results indicate that 6 µL is the optimal loading volume for the present device, maximizing the amount of sample that interacts with the reaction channel antibodies while also minimizing the amount of solution waste. The loading volume could be increased in future iterations of the device by increasing the thickness of the top cover chip to fabricate a larger loading reservoir.

Aggregated blood filter formation

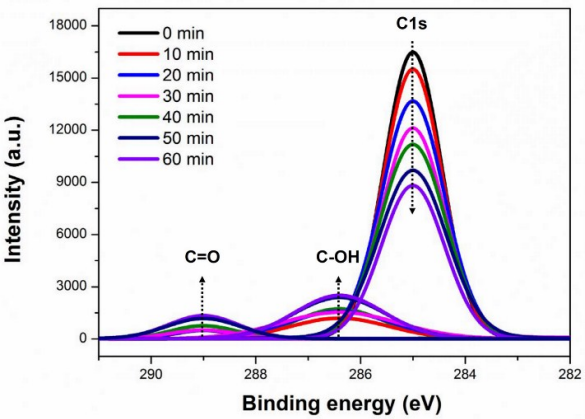


Fig. 3. XPS spectra of a UV/ozone oxidized COP chip surface. The C1s (main peak component C–C/C–H, 285 eV) continuously converted into C=O (289 eV) and C-OH (286.4 eV) with increasing UV/ozone exposure time.

Human RBCs have a biconcave-discoidal shape with a diameter of 6-8 µm. Under physiological conditions, the center of the RBC is slightly narrower in width (~1 µm) compared to the surrounding edge (~3 µm).[26, 27] RBCs are flexible and will bend into a parachute or slipper shape when they are at the center or off-center of the microfluidic channel due to the reduction of flow resistance caused by the fluidic field or device geometry.[28, 29] Based on the rheological properties of RBCs, a fluidic channel with a height of 2 µm (lower than the thickness of the RBCs outer edge) will block agglutinated RBCs, while non-conglomerated RBCs will flex and pass through the filter gap. To further eliminate the possibility of false positive results due to clogging of the filter and to enhance the visibility of the result readout, a wide and low-aspect-ratio fluidic channel was required to enable non-agglutinated RBCs to pass horizontally through the filter.

The selective solvent swelling method[23] was used to construct low-aspect-ratio filters in a cost-effective manner. Selective solvent swelling allowed shallow open channels to be fabricated in the bottom COP chip, which were subsequently enclosed using the top cover chip. The concept of selective solvent swelling is based on the selective modification of a specific area of a polymeric surface, which causes it to resist absorbing non-polar solvents. The unmodified surface will absorb solvent that has a similar Hildebrand solubility parameter, causing it to swell and grow, effectively confining the modified, non-swelling region of the polymer to create channels and other features. Swelling height can be controlled by the duration of solvent uptake. In addition, because a solvent is used to swell the polymer, the channel can be subsequently enclosed by directly pressing the top cover polymer chip to the solvent swollen bottom chip without heating or any other treatment.

In this study, UV light and ozone was used to create high-density ozone and excited oxygen atoms for the modification of the COP chip surface. UV and ozone was used due to its efficient oxidative ability compared to oxygen plasma.[30, 31] Using electron spectroscopy for chemical analysis (ESCA), we observed that the C1s (main peak component C–C/C–H, 285 eV) on the COP surface progressively converted to C=O (289 eV) and C-OH (286.4 eV) with increasing UV/ozone exposure time (Fig. 3). The surface modification results were also confirmed by water contact angle measurements (Fig. S2).

In order to achieve both effective solvent resistivity and minimum fabrication time, COP chips with different UV/ozone exposure times were examined, from 10-60 min, followed by 10 min solvent exposure. As a result, 30 min UV/ozone exposure time was adopted because no further chip morphology change was observed for longer exposure times. To further simplify the process, blue dicing tape was milled to create an open window for UV/ozone exposure. The milled tape was attached to the COP chip surface to act as a masking layer against the UV/ozone modification. This fabrication technique allowed us to eliminate complicated photolithography processes, which enables the device to be fabricated in a normal lab environment without cleanroom equipment. After UV/ozone modification and removal of the masking tape, the chip surface was exposed to cyclohexane in the gas phase for 6 min, causing the unexposed region of the polymer to swell. The chip was subsequently covered and pressed with the top cover COP chip. The resulting structure formed a low-aspect ratio filter, ~200 µm in width and 2 µm high (Fig. 4).

Blood typing diagnosis

A schematic illustration of the ABO and Rh blood typing test is shown in Scheme 1. A finger prick was performed using a lancing device to

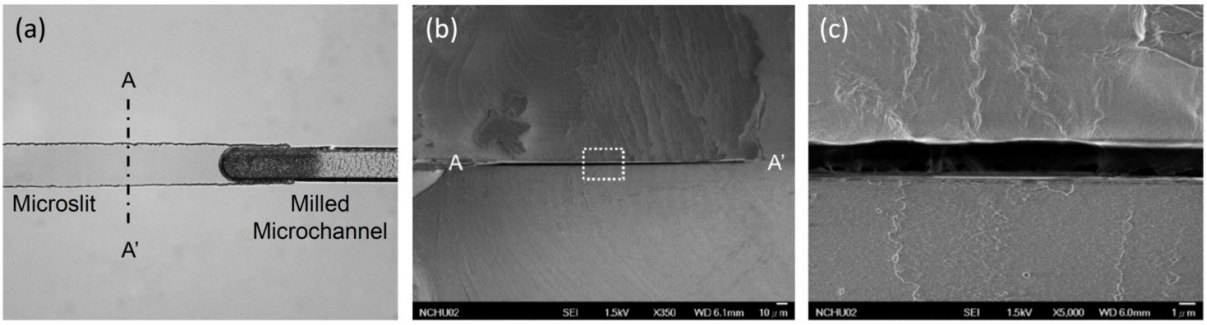
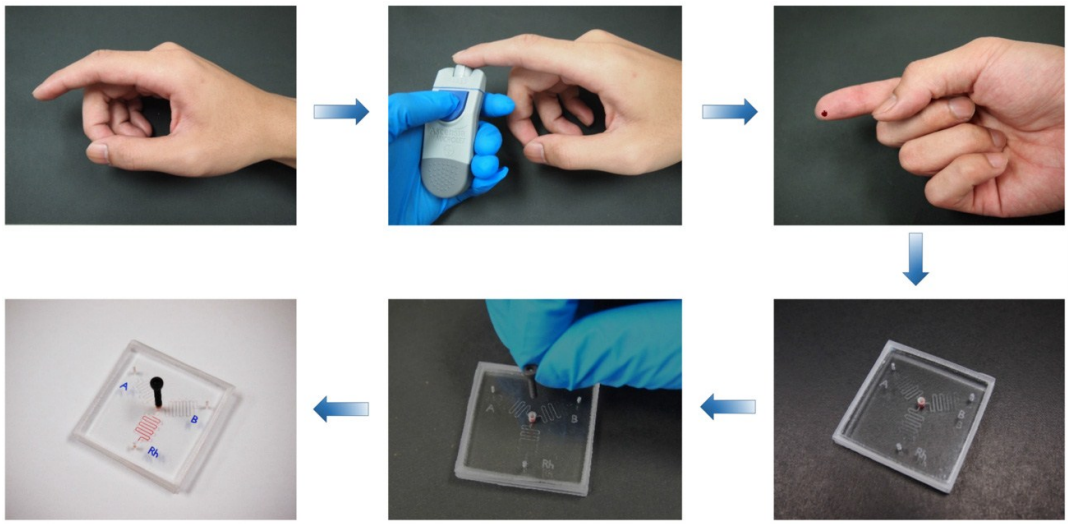


Fig. 4. Images of the fabricated low-aspect-ratio microfilter that were taken on a (a) optical microscope and (b), (c) SEM. A-A' cross-section was obtained using a freeze-fractured method to break the COP chip in liquid nitrogen. The SEM images show a low-aspect ratio filter, 2 µm in height, which is formed after 30 min UV/ozone modification followed by 6 min of cyclohexane exposure.



Scheme 1. A schematic process illustrating the on-site ABO and Rh blood typing test. A finger prick was first used to obtain a small amount of blood from the donor. The blood was then transferred to the loading reservoir. After addition of the PBS buffer to the same reservoir, the screw was installed and rotated using one's fingers or a screwdriver to actuate the blood stream into the three separate reaction channels, consisting of Anti-A, Anti-B, and Anti-D blood type antibodies. If the blood sample contains the corresponding antigen, then an agglutination reaction occurs. Agglutinated RBCs block the filters near the flow outlet and as a result the reaction channel turns red. This visualized result is clearly indicated and can be referenced against the A, B and Rh symbols patterned on the top of the chip to indicate which blood type is present in the sample.

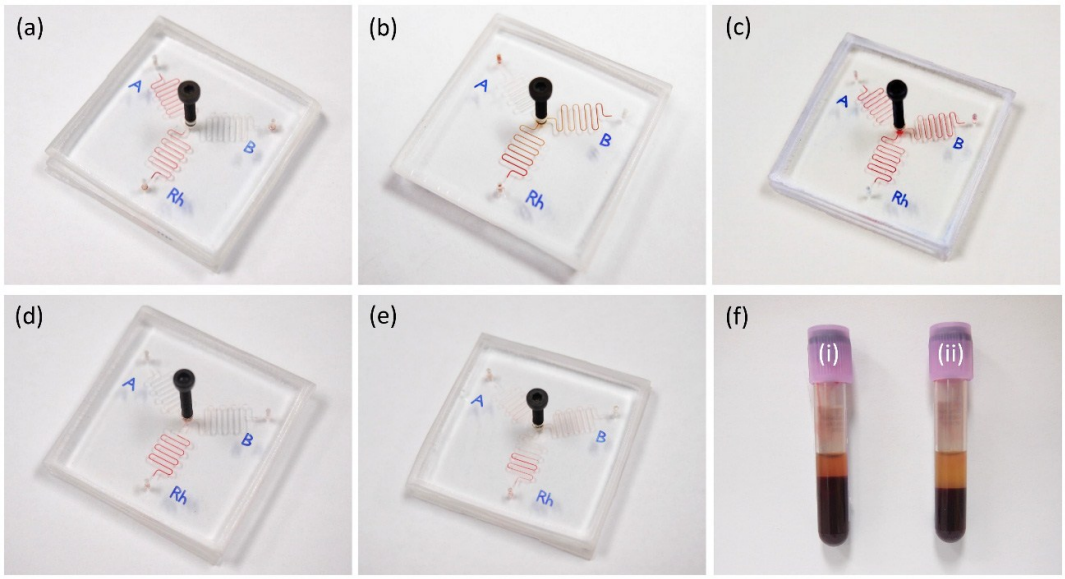


Fig. 5. Representative chip test results are shown for (a) A Rh^+, (b) B Rh^+, (c) AB Rh^+ and (d) (e) O Rh^+ blood types. These visualized results are clearly indicated by the red lines and the corresponding A, B, and Rh symbols on the top of the chip which label the antibody locations. (e)(f) Thalassemia samples with smaller RBCs and lower hematocrit were also successfully verified, displayed as a clear but shorter agglutinated RBC line compared to the (d) healthy blood sample. (f) An image of a (i) normal blood sample (age 23; male) and (ii) thalassemia blood sample (age 37; male) after extraction and storage in the 4 ^{0}C refrigerator for 1 hour.

obtain a small amount of blood (typically < 20 µL) from the donor. The blood was then transferred to the chip's loading reservoir *via* a metering needle. PBS solution was added followed by installation of the screw which was rotated using one's fingers or a screwdriver to actuate the sample flow into the three separate channels consisting of the Anti-A, Anti-B, and Anti-D antibodies. RBCs that reacted with the matching antibody in the specific reaction channel agglutinated and blocked the filters, turning the reaction channel red near the flow outlet. These visualized results are clearly indicated using the A, B, and Rh symbols previously patterned on the top of the chip. Representative blood typing test results of A Rh^+, B Rh^+, AB Rh^+, and O Rh^+ blood types are shown in Fig. 5.

To make the test suitable for point-of-care and pediatric disease diagnostics, it was critical that the required volume of blood could be taken using a finger prick to cause minimal wound and relative painlessness.[32-34] As the optimal loading volume was determined to be 6 µL, 1 µL of blood and 5 µL PBS buffer was adopted to provide sufficient solution to flow into the 3 separate reaction channels and interact with the pre-stored antibodies. Any resulting haemagglutination reaction resulted in the appearance of red channels as the agglutinated RBCs accumulated and blocked their respective filters, providing unambiguous results that can be easily read. The pressure difference between the interactive (matched blood type) and non-interactive (mismatched blood type) channels cause the PBS buffer solution to elute the non-aggregated RBCs out into the outlet reservoir. The non-interactive channel thus appears clear colored. As a result, the visual signal between red and clear channels can be distinguished by the naked eye in just 1 min. However, pipettes or other accurate volume dispensing tools are not necessary to obtain accurate blood typing results. If circumstances make it so accurate measurement of the blood sample volume is not possible, a blood volume greater than 1 µL can also be used for the test as long as one additional PBS or water washing step is applied to elute the non-aggregated RBCs.

In order to verify the longevity of samples for the blood typing tests, a blood sample was obtained by finger prick and transferred to a glass slide to observe the rate of natural agglutination. Once a minute, a sample of this blood was transferred to the diagnostic chip for blood typing. For the healthy donors (n=3), agglutination on the glass slide began after ~3 min. At this time, the blood could still be transferred to the blood typing test and obtain accurate results. 5 min after the blood was transferred to the glass slide, more RBCs agglutinate and the blood can no longer be pumped through the device. If an anticoagulant is used, the blood sample can sit at room temperature for ~19 min and still be pumped into the flow channel to obtain accurate blood typing results.

Micromixers are one of the device's key elements, which enable rapid and accurate diagnosis. Based on our experiments, if the chip is constructed without the mixer features, it is difficult to obtain accurate and rapid blood typing due to insufficient interaction between the RBCs and antibodies. Without mixers, the antibodies in the serum cannot fully conjugate with the corresponding antigens on the RBC surfaces during their short period of interaction, resulting in blurred and unclear RBC agglutination when the experimental time is limited to 1 min. In order to obtain clearer blood typing results without the micromixers, it is necessary to instead decrease the rate of solution flow by rotating the actuating screw more slowly, sacrificing the speed of the test. In addition, without the micromixers, the majority of the antibody reagent is purged out of flow channel as the blood stream enters. Therefore, an insufficient amount of antibodies are present for RBC agglutination, which can lead to false negative results.

In order to verify the feasibility of the proposed platform, 30 blood samples were tested that had been obtained from healthy male and female volunteers, ages 20-40. The results showed 100% sensitivity and specificity compared to test results verified by an accepted clinical assay of blood typing at a medical center (Table S2). Among these tests, thalassemia samples from one male and one female featuring smaller RBCs and lower hematocrit, were verified by their blood types. Thalassemia causes a type of anemia due to deficient globin-chain production during erythropoiesis. Blood transfusion is sometimes requested for patients with thalassemia to eliminate the complications of anemia and compensatory bone marrow expansion for normal childhood development and extended survival rates.[35, 36] Patients with thalassemia typically possess smaller MCV of red blood cell size and lower mean corpuscular hemoglobin per red blood cell.[18] A visual comparison between normal and thalassemia blood samples can be seen in Fig. 5f. Due to the smaller MCV and lower hematocrit, thalassemia blood samples possess a greater challenge for point-of-care (POC) tests, which require small volumes and minimal sample variation. In this study, a clear but shorter agglutinated RBC line was observed for samples featuring thalassemia (Fig. 5e). The unambiguous and highly accurate diagnostic results were attributed to the pressure-based blood typing design and highly efficient cell-antibody interaction, which allows even thalassemia samples to be identified. Sample pretreatment steps and external equipment, such as centrifuges or optical sensors, were unnecessary for reading results, making blood type diagnosis straightforward and easy. Users without any specialized training can perform it.

A comprehensive comparison between the slide method and the proposed polymeric platform for ABO and Rh blood typing tests is listed in Table 1. The conventional slide tests for ABO and Rh blood typing has been adopted for comparison with other POC platforms.[10, 12, 13] According to the literature, the slide tests requires the blood sample to be divided into three parts for Anti-A, Anti-B, and Anti-D antibody interaction on a labeled glass slide. The volume of blood sample necessary for one test is ~20 µL. A comparable amount of antibody solution is also needed for the test. The extracted blood sample may need an additional dilution step using sodium chloride solution in order to observe a clear result. If the slide is only shaken gently to induce agglutination without using a stir bar, 15 min is required to obtain a visual result. On the other hand, a stir bar can be used to enhance the interaction between the antibodies and the antigens on the RBCs' surfaces, resulting in a shorter turnaround

Table 1. A comprehensive comparison between the slide method and the proposed polymeric platform for ABO and Rh blood typing tests.

	Slide method	Proposed polymeric chip
Blood volume required	~20 µL	1 µL
Antibody serum volume required	~7 µL	0.5 µL
Manual processes	More	Less
Reaction time	3 ~ 15 min	1 min
Material waste	Glass slide	Polymer
Biological waste	Exposed	Sealed

time. However, using a stir bar may damage RBCs and affect the result. Although the slide test is simple and straightforward, the multiple manual processes, larger amount of blood sample and antibody requirement, and longer reaction time limit its application for POC tests. Moreover, it should be noted that discarded glass slides and exposed blood waste present more serious safety conisderations, particularly for facilities in developing countries. Based on the abovementioned factors, the proposed polymeric platform for ABO and Rh blood typing possesses cheaper, faster, and safer advantages compared to the slide method.

Conclusions

Accurate identification of blood groups is critical to provide safe blood transfusions. In this study, a portable and cost-effective colorimetric detection platform was fabricated for simple ABO and Rh blood typing. The thermoplastic device employed a screw pump, serpentine reaction channels, chaotic micromixers, and low-aspect-ratio filters to rapidly determine the sample blood type without need for bulky analytical equipment or technical personnel. The required blood volume for the test was 1 μL, which allows the sample to be obtained using a finger prick method that not only simplifies the sampling and storage process, but also causes minimal wound or pain for the patient. A screw pump was used to provide a manual driving force for blood flow with low reagent consumption and minimal dead volume. Blood samples and antibodies fully interact *via* serpentine reaction channels and chaotic micromixers. Agglutinated RBCs block the entrance of low-aspect-ratio filters, which creates the appearance of red lines that show the unambiguous blood typing results in as little as 1 min. High sensitivity and specificity was obtained using the blood typing chip compared to test results determined using a standard column agglutination method. In this work, rapid prototyping was achieved using machine milling to construct the diagnostic chip. However, other more cost-effective methods, such as injection molding or hot embossing, could be easily adopted for practical mass production of the blood-typing device. It is expected that this platform could be further expanded to other aggregation-based infectious disease diagnoses for affordable, sensitive, specific, user-friendly, rapid and equipment-free (ASSURED) diagnostic applications.

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