Finger-actuated microfluidic device for the blood cross-matching test†

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A blood cross-matching test should be carried out to prevent a hemolytic transfusion reaction as the final verification step. To simplify complicated procedures of a conventional blood cross-matching test requiring bulky systems and skilled people, we present a finger-actuated microfluidic device for the blood cross-matching test. Although finger actuation is a simple action that anyone can easily accomplish, there would be a variation in the individual finger actuation that may induce the user-dependent errors of the device. Therefore, the working principle of the finger-actuated microfluidic device is newly designed to reduce the user-dependent errors by indirectly controlling the pressure of fluidic channels. The constant volume was repeatedly dispensed by pushing and releasing a pressure chamber regardless of the different pushed depths of the pressure chamber, the pushing time interval, and the end-users. The dispensed volume was linearly increased according to the number of pushing times applied to the pressure chamber and determined by adjusting the diameter of an actuation chamber. In addition, multiple fluids can be dispensed with a desirable ratio by pushing and releasing the pressure chamber. Finally, a finger-actuated microfluidic device for the blood cross-matching test was developed, which can simultaneously actuate four fluidic channels. After loading 50 μL of whole blood samples from a donor and a recipient into two inlets of the device, the blood plasma from each individual was separated through the two plasma separation membranes. The blood cross-matching test results can be achieved by cross-reacting the donor’s blood plasma with the recipient’s whole blood as well as the donor’s whole blood with the recipient’s blood plasma by pushing and releasing only a single pressure chamber within 10 min.

Introduction

The blood cross-matching test is an essential and the final verification process in blood transfusion to prevent a hemolytic transfusion reaction which is caused by the antigen–antibody reaction due to the incompatibility of the ABO blood type and the unexpected antibodies.1,2 Thus, the transfusion suitability between donor and recipient should be finally confirmed through the blood cross-matching test. To identify whether or not the donor’s blood is compatible with that of the recipient, red blood cells (RBCs) and blood plasma are cross-reacted between the donor and the recipient.2 In particular, the reaction between the donor’s RBCs and the recipient’s blood plasma is the major test, while the reaction between the recipient’s RBCs and the donor’s blood plasma is the minor test. The transfusion suitability is determined by identifying the agglutination that occurs in the major or minor test. As a gold standard method for the blood cross-matching test, the blood plasma is first separated using a centrifuge and the results are obtained by a gel column agglutination method or incubation in tubes after the cross-reactions.4,5 However, because of the bulky system of the centrifuge and its expensive price, it is difficult to achieve a simple and rapid blood cross-matching test which is required for patients who need urgent transfusion. Additionally, the separated blood plasma must be manually moved into the tubes or gel column for the cross-reactions between RBCs, which makes the operation principle complicated.

Meanwhile, various microfluidic devices have been developed for simple and rapid point-of-care testing (POCT) by miniaturization to compensate for the drawbacks of bulky laboratory systems.6–8 Although the simplicity is getting better in miniaturized microfluidic devices, they still require an expensive and user-unfriendly external pumping system.9 For these reasons, a number of easy-to-use microfluidic systems for POCT have been introduced, including paper-based analytical devices,10 self-powered microfluidic devices using the degassing process for poly(dimethylsiloxane) (PDMS),11–13 and capillary force-derived microfluidic devices.14,15 On the other hand, the blood cross-matching test has also been
demonstrated in pumpless microfluidic systems. A smart pipette tip was used to separate the recipient’s blood plasma which was reacted with the donor’s RBCs in another plate for the major test. Micronics, Inc. used a filtration method to separate the recipient’s blood plasma which was reacted with the donor’s RBCs for the major test using the finger actuation-induced pressure change.

Likewise, finger actuation, one of the simplest actions that people can do, is an appropriate method to drive the flow in a microfluidic device for POCT. By applying the pressure to the microfluidic channels with a finger actuation, the flow can be simply achieved. In this sense, in addition to the blood cross-matching test, finger actuation has widely been applied to microfluidic devices for glucose assay, dispensing multiple reagents, blood typing, and antibiotic susceptibility tests. Various applications of finger-actuated microfluidic devices have been dealt with, but the flow behavior induced by finger actuation can be greatly affected depending on how much pressure is exerted by the end-user. In other words, it is difficult to provide accurate, standardized guidelines due to the different personal characteristics of finger actuation. Moreover, since fluidic channels are directly connected to the pressure chamber, the sample solution mistakenly introduced into the pressure chamber may affect the repeated actuation of the pressure chamber.

To overcome these limitations of previous finger-actuated microfluidic devices, the working principle of a finger-actuated microfluidic device is newly proposed to reduce user-dependent errors by indirect control of the pressure in a fluidic channel. Two pneumatic valves and one actuation chamber can control the pressure in a fluidic channel and the direction of flow. In particular, one pneumatic valve and an actuation chamber are actuated by pushing and releasing the pressure chamber while the other pneumatic valve is actuated according to the pressure in a fluidic channel. The constant volume can be repeatedly dispensed by finger actuation regardless of the end-users, the pushed depth of a pressure chamber, and the pushing time interval. Additionally, multiple reagents from different fluidic channels can be dispensed with different predesigned ratios. Finally, the blood cross-matching test is demonstrated in a finger-actuated microfluidic device. After separation of blood plasma from the donor and recipient’s whole blood through the two plasma separation membranes, the reactions between the donor’s RBCs and the recipient’s plasma as well as between the recipient’s RBCs and the donor’s plasma are performed. The results of the blood cross-matching test, whether or not agglutination occurs, are shown.

Experimental

Fabrication of a finger-actuated microfluidic device

A finger-actuated microfluidic device consists of three PDMS layers (thickness = 3 mm), one thin PDMS membrane layer (thickness = 25 μm), and two plasma separation membranes (see Fig. S1, ESI†). The mold for the PDMS layers was fabricated by photolithography. To prevent the structure of pneumatic valves being bonded to the PDMS membrane, SU-8 2005 (MicroChem Corp., MA, USA) was first spin-coated onto a silicon wafer to obtain a 5 μm thickness. Subsequently, SU-8 2100 was spin-coated to obtain a 95 μm thickness. PDMS precursors and a curing agent were mixed well in a ratio of 10:1 and poured onto the SU-8 mold. The PDMS layer was peeled off from the SU-8 mold after baking at 150 °C for 10 min. The PDMS membrane was obtained by spin-coating PDMS (PDMS: curing agent = 10:1) onto a bare silicon wafer at 1500 rpm for 60 s and curing at 150 °C for 10 min. The PDMS layer and the PDMS membrane were bonded to each other after treatment with oxygen plasma for 1 min and incubated at 65 °C for 10 min. The top PDMS layer was first bonded to the middle PDMS layer, including actuation channels. The assembly was then bonded with the PDMS membrane on a bare silicon wafer. After peeling off the assembly from the bare silicon wafer, it was finally bonded with the bottom PDMS layer, including fluidic channels. For the blood cross-matching test, the plasma separation membrane (PES HD1.8B-A; Cobetter Filtration, Hangzhou, China) was partially pressed with a press machine (SWP-HP180-120S; Sam Woo, Siheung, Korea) to facilitate the integration into the device (see Fig. S2, ESI†). Finally, it was integrated into the assembly using double-sided tape before being bonded to the bottom PDMS layer. The finger-actuated microfluidic device is ready for use after the negative pressure in the pneumatic channel is generated by pushing the pressure chamber and releasing it after blocking the air outlet with an acrylic block (see Fig. S3, ESI†). Then, after loading the fluid into the inlet, the fluid can be dispensed into the outlet by pushing and releasing the pressure chamber.

Determination of the volume and concentration of the dispensed sample solution

To determine the volume of the dispensed solution, 1 mM erioglaucine was used as a sample solution. 1 to 20 μL of 1 mM erioglaucine that has a peak absorbance at 406 nm were pipetted into 200 μL of distilled water. The calibration curve according to the volume of 1 mM erioglaucine was obtained by measuring the absorbance of the mixture at 406 nm. The absorbance of the dispensed sample solution through a finger-actuated microfluidic device at 406 nm was obtained after mixing the solution with 200 μL of distilled water. Finally, the dispensed sample volume was calculated using the calibration curve.

To determine the concentration of the dispensed solution, 10 μL of 0.1 to 1 mM erioglaucine were pipetted into 200 μL of distilled water. The calibration curve according to the concentration of 10 μL erioglaucine was obtained by measuring the absorbance of the mixture at 406 nm. The finger-actuated microfluidic device was operated until 10 μL of the mixture was dispensed. The dispensed solution was mixed with 200 μL of distilled water and the absorbance at 406 nm was
measured. The concentration of the mixture was calculated using the calibration curve.

**Procedures for the blood cross-matching test**

For the off-chip blood cross-matching test, the donor and recipient’s plasma was separated by 24 h sedimentation. 2 μL of the donor’s RBCs at the lower part was mixed with 10 μL of the recipient’s plasma at the upper part for the major test on a glass slide. For the minor test, 2 μL of the recipient’s RBCs at the lower part was mixed with 10 μL of the donor’s plasma at the upper part. Then, the results of the blood cross-matching test were confirmed using a stereoscope (SZX16; Olympus, Japan) whether agglutination occurs or not.

For the on-chip blood cross-matching test, the blood plasma of the donor and the recipient was first separated through the two plasma separation membranes by loading 50 μL of whole blood into each inlet. After 5 min, the separated blood plasma and RBCs were cross-reacted by pushing and releasing the pressure chamber five times. Then, whether agglutination has occurred or not was observed with a stereoscope after 5 min of incubation. The EDTA-treated whole blood samples were obtained from the biobank of the Chungnam National University Hospital in compliance with safety regulations. Before each experiment, the whole blood samples were stored at approximately 4 °C. To demonstrate the four possible results of the blood cross-matching test, an appropriate combination of blood types was used as the donor and recipient’s blood (see Table S1, ESI†). For the results where agglutination occurred in the major and minor tests, the blood type A, Rh⁺ was used as the donor’s blood and the blood type B, Rh⁺ was used as the recipient’s. For the results where agglutination occurred in the major and not in the minor tests, the blood type AB, Rh⁺ was used as the donor’s blood and the blood type O, Rh⁺ was used as the recipient’s. For the results where agglutination occurred in the minor and not in the major tests, the blood type O, Rh⁺ was used as the donor’s blood and the blood type A, Rh⁺ was used as the recipient’s. For the results where agglutination did not occur in the major and minor tests, the blood type A, Rh⁺ was used as the donor and recipient’s blood.

**Results and discussion**

**Design and working principle**

The finger-actuated microfluidic device for the blood cross-matching test is designed to separate the donor and recipient’s blood plasma and to cross-react them with whole blood from each individual (Fig. 1A). It consists of two inlets, one for the donor’s whole blood and the other for the recipient’s whole blood. The plasma separated through the plasma separation membrane and the whole blood exceeding the capacity of the plasma separation membrane are cross-reacted by pushing and releasing the pressure chamber that simultaneously actuates four finger-actuated microfluidic pumping units. Afterwards, the mixture of the donor’s whole blood and the recipient’s plasma and that of the recipient’s whole blood and the donor’s plasma are dispensed into each outlet. During the incubation of the mixture in the outlet, the mixture is refluxed into the fluidic reaction chamber. Finally, the results of the major and minor tests can be visualized at the reaction chamber.

The working principle of the finger-actuated microfluidic device is shown in Fig. 1B and C. The finger-actuated microfluidic pumping unit consists of pneumatic and fluidic channels that are partitioned by the PDMS membrane. The pressure change in the pneumatic channel actuates valve 1 and the actuation chamber by pushing and releasing the pressure chamber. On the other hand, the pneumatic valve 2 is not actuated by the pressure change in the pneumatic channel, but in the fluidic channel. When the pressure chamber is pushed, the increased pressure in the pneumatic channel closes valve
1 and compresses the actuation chamber sequentially, resulting in the discharge of the fluid in the actuation chamber by opening valve 2. In contrast, when the pressure chamber is released, the reduced pressure in the pneumatic channel opens valve 1 and decompresses the actuation chamber. Then, valve 2 is closed due to the decreased pressure in the fluidic channel, and the fluid is charged into the actuation chamber. Finally, the flow into the outlet can be achieved without backflow by repeatedly pushing and releasing the pressure chamber.

**Evaluation of the finger-actuated microfluidic device regarding the characteristics of the finger actuation**

For more precise dispensing of the fluid using a finger-actuated microfluidic device, it is better to have less influence on the dispensed volume from the personal characteristics of the finger actuation. In this sense, the dispensed volume was evaluated according to the various characteristics of the finger actuation in the finger-actuated microfluidic device wherein the diameter of the actuation chamber was 4000 μm (Fig. 2A). The compression and decompression of the actuation chamber is controlled by the deflection of the PDMS membrane, and the maximum deflection of the PDMS membrane cannot exceed the height of the fluidic and pneumatic channels which are fabricated aiming for 100 μm. On the one hand, the dispensed volume is determined by the compressed volume of the actuation chamber. This means that there exists an upper limit for the dispensed volume. The volume change of the pressure chamber induced by finger actuation determines the compressed volume of the actuation chamber. Therefore, the constant volume of the dispensed fluid can be obtained by pushing and releasing the pressure chamber if the compressed volume of the pressure chamber is equal to or larger than the volume of the actuation chamber.

To confirm that constant dispensed volume is achieved, we have measured the volume of the dispensed fluid according to the pushed depth of the pressure chamber with different initial pushed depths of the pressure chamber (Fig. 2B). The pushed depth of the pressure chamber was controlled using the end-tip of the vernier calipers at intervals of 0.5 mm and 1 mm for the initial pushed depth. The constant volume was dispensed when the pushed depth of pressure chamber was larger than the initial pushed depth of the pressure chamber while the device did not work when the pushed depth of the pressure chamber was equal to or less than the initial pushed depth. In other words, if the pressure generated by pushing the pressure chamber was enough to fully compress the actuation chamber, the constant volume corresponding to the volume of the actuation chamber can be dispensed.

We analyzed whether the pressure generated by the fingertip was sufficient to fully compress the actuation chamber and how large the volume of the actuation chambers can be fully compressed by the pressure generated with the fingertip. In general, the force which can be applied by the human fingertip ranges between 21–60 N. There is an upper limit in the compression of the actuation chambers so the finger-actuated microfluidic devices should be designed such that the actuation chambers can be fully compressed with minimum force. With the minimum force of 21 N, a pressure of 268 kPa is applied over the pressure chamber considering the area of the pressure chamber of 78.5 mm². The maximum deflection of the pressure chamber \( (w_{\text{max}}) \) can be calculated with the following equation under the assumption that the pressure induced by finger actuation is uniformly loaded over the pressure chamber:26

\[
 w_{\text{max}} = \left( 1 + 5.72 \left( \frac{h}{r} \right)^2 \right) \left( \frac{3}{16} \left( 1 - \nu^2 \right) \frac{pd^4}{Eh^3} \right)
\]

where \( h \) is a thickness of the cover plate over the pressure chamber, \( r \) is a radius of the pressure chamber, \( \nu \) is Poisson’s ratio, \( E \) is the Young’s modulus of the cover plate material, and \( p \) is an applied pressure. The equation can only be used for thick circular plates \( (h/r > 0.1) \). The \( h \) is 3 mm, \( r \) is 5 mm, \( \nu \) is 0.5, and \( E \) is 2.59 MPa, so the maximum deflection of the pressure chamber was calculated to be 1.03 mm.27 Then, the volume change of the pressure chamber \( (\Delta V) \) by finger actuation can be calculated using the following spherical cap equation:28

\[
 \Delta V = \frac{\pi w_{\text{max}}}{6} \left( 3r^2 + w_{\text{max}}^2 \right)
\]

Finally, the minimum volume change of the pressure chamber by human finger actuation is about 41 μL which
means that the actuation chamber having a volume of 41 μL can be operated. Also, the pressure change in the pneumatic channels (ΔP) when the pressure chamber is pushed can be represented by using the ideal gas law as follows:

\[ \Delta P = \frac{\Delta V - V_{\text{act}}}{V_p + V_c - \Delta V + V_{\text{act}}} P_i \]

where \( V_p \) is the volume of the pressure chamber, \( V_c \) is the volume of the pneumatic channels, \( V_{\text{act}} \) is the volume of the actuation chambers which should not be over \( \Delta V \), and \( P_i \) is the initial pressure. Considering that \( V_p \) is 235.5 μL, \( \Delta V \) is 41 μL, and \( V_{\text{act}} \) can be ranged from 0 to 41 μL, the \( V_c \) of the finger-actuated microfluidic device should be designed not to significantly reduce the pressure change in the pneumatic channels. In other words, quite long pneumatic channels that have a large volume comparable with \( V_p \) and \( \Delta V \) can reduce the pressure change in the pneumatic channels which can result in malfunction of the valves and the actuation chambers.

In addition, if the volume of the actuation chamber is small enough to be decompressed in a short time, it is expected to reduce user-dependent errors regarding the pushing time interval. The dispensed volume according to the pushing time interval was evaluated by pushing the pressure chamber four times (Fig. 2C). The dispensed volume of the device wherein the diameter of the actuation chamber is 4000 μm was not significantly different even if the pushing time interval was as short as 1 s. It was calculated that the 41 μL volume of the actuation chamber can be compressed with the minimum force of the finger actuation. However, the smaller the volume of the actuation chamber is, the less the effect of the pushing time interval is, since the actuation chamber is compressed more quickly by finger actuation. Therefore, the volume of the actuation chamber is recommended to be less than 41 μL. Lastly, to verify that the user-dependent errors could be reduced, we measured the dispensed volume by pushing the pressure chamber four times for ten people (5 men and 5 women) (Fig. 2D). As a result, a volume of 8.7 μL was dispensed on average and the coefficient of variation (CV) was 3.17%, indicating that there was no significant difference between the performances of the device actuated by different persons.

**Analysis of the dispensed volume**

Since the expected amount of fluid dispensed may vary due to backflow, it is important to understand how much backflow occurs during operation of the finger-actuated microfluidic device. The backflow may occur if valve 2 does not work well when the pressure chamber is released. We analyzed the backflow by measuring the volume change in the outlet before and after releasing the pressure chamber (Fig. 3A). There was no significant difference in the volume before and after releasing the pressure chamber for the various diameters of the actuation chambers. Thus, it can be understood that valve 2 works well by the pressure change in the fluidic channel induced by releasing the pressure chamber. The pressure change in the fluidic channels due to the release of the pressure chamber is different according to the diameter of the actuation chambers, but it seems that the amount of the pressure change would be sufficient to close valve 2. Because there was little backflow during actuation of the device, the volume dispensed by actuation of the device can be calculated as follows:

\[ V_{\text{act}} = \pi \left[ \frac{d^2}{4} h_1 + \left( \frac{d}{2} - t \right) h_2 \right] \]

where \( d \) is the diameter of the actuation chamber, \( h_1 \) and \( h_2 \) are the height of the pneumatic and fluidic channels, and \( t \) is the thickness of the PDMS membrane. The volume of an actuation chamber is dispensed by actuating the device and the dispensed volume can be easily adjusted by controlling the number of pushing times that the pressure chamber is pushed and the diameter of the actuation chamber at the designing step. As shown in Fig. 3C, the dispensed volume linearly increased with respect to the number of pushing times. The dispensed volume was measured by actuating the device that is pre-filled with a fluid. This linearity was confirmed for

![Fig. 3](image-url)

**Fig. 3** Analysis of the dispensed volume according to device actuation. (A) It was estimated for various diameters of the actuation chambers whether or not backflow occurs during the release of the pressure chambers. Error bars represent the standard deviation from the mean (\( n = 4 \)). (B) The cross-sectional schematic of the actuation chamber when the pressure chamber is pushed and released. (C) Graph showing the dispensed volume of the finger-actuated microfluidic device with different diameters of the actuation chamber with respect to the number of pushing times applied to the pressure chamber. Error bars represent the standard deviation from the mean (\( n = 3 \)). (D) The calculated volume of the actuation chamber was compared to the slope of the linear fitting in panel C.
various diameters of the actuation chamber, which were 3000, 4000, 5000, and 6000 μm, whose slope was 1.16, 2.07, 3.21, and 4.45, corresponding to the volume dispensed by actuation of the device.

The experimentally measured volume dispensed by an actuation of the device was compared to the calculated volume of the actuation chambers (Fig. 3D). The $h_1$ of the SU-8 mold where the diameter of the actuation chamber was 3000, 4000, 5000, and 6000 μm was fabricated to be 92.15, 90.13, 94.36, and 90.03 μm, respectively. The $h_2$ of the SU-8 mold was fabricated to be 90.27, 86.83, 89.06, and 90.18 μm, respectively. Based on the above equation, the volume of the actuation chamber whose diameter was 3000, 4000, 5000, and 6000 μm was calculated to be 1.27, 2.19, 3.56, and 5.05 μL, respectively. The experimentally measured values decreased by 8.7%, 5.5%, 9.8%, and 11.88%, on average by 8.97% compared to the calculated volume of the actuation chamber. Even though there was little backflow during actuation of the device, there was little difference between the experimentally measured and calculated values. It is supposed that the PDMS shrunk during curing so the actual dimensions of the actuation chamber would be decreased compared to the SU-8 mold. However, as mentioned in the previous section, a significant difference was not shown in the dispensed volume according to the various characteristics of the personal finger actuation, and there was also no significant difference in the dispensed volume according to the device made at another time (see Fig. S4, ESI†). It can be understood that the device prototyping with PDMS showed good repeatability.

Furthermore, for the wide availability of the finger-actuated microfluidic devices, there should be little difference between the designed volume of the actuation chamber and the dispensed volume. In this manner, mass manufacturing would improve the limitation regarding the shrinkage of the PDMS because the materials suitable for mass manufacturing would not show shrinkage during curing. Then, the volume corresponding to the volume of the actuation chamber can be dispensed by actuation of the device. On the other hand, for the mass manufacturing of devices, the design of each functional part that exhibits the elastic properties of PDMS would be considered to be compatible with other materials suitable for mass manufacturing.

We estimated the dispensed volume by actuation of the device after an actuation chamber is pre-filled with a fluid, but when the device is actually being used, the device will be operated with an empty actuation chamber as mentioned in the experimental section. However, when a fresh device is actuated for the first time, a volume smaller than the volume of the actuation chamber is dispensed into the outlet (see Fig. S5, ESI†). This means that the actuation chamber is not fully filled with a fluid after the first actuation of the device. The actuation chamber can be fully filled with a fluid after actuation of the device at least twice, so that the volume corresponding to an actuation chamber can be dispensed into the outlet. It is understood that this is due to the hydrophobic surface properties of PDMS. This should be improved to obtain a precise desired volume through actuation of the device and we expect hydrophilic surface treatment or the use of a hydrophilic material to resolve this limitation.

The dispensable volume is determined depending on the diameter of the actuation chamber at the designing step. Although the adjustability of the dispensed volume in the fabricated device is difficult to be achieved by precise control of the pressure chamber, the predesigned volume can be repeatedly dispensed and the desired volume will be obtained by adjusting the number of pushing times applied to the pressure chamber. The dispensable volume from the actuation of the fresh device can be calculated by adding the dispensed volume for the first time and the multiplication value of the actuation chamber’s volume and the number of actuations after the first dispensing of the fluid. We believe that the working principle of our finger-actuated microfluidic device is suitable for finger actuation which does not require elaborate control of the motion.

Dispensing of dual fluids with different predesigned ratios

The fluidic channels of our finger-actuated microfluidic device are not directly connected to the pressure chamber, so it can be freely designed not concerning the position of the pressure chamber. Furthermore, multiple fluids can be dispensed by connecting each pneumatic channel into the single pressure chamber. According to the aforementioned design principle, many fluidic channels can be simultaneously actuated with finger actuation. We have confirmed the dispensing of fluids with finger actuation by designing the device as shown in Fig. 4A. 3000, 4000, 5000, and 6000 μm diameter of actuation chambers were combined to achieve various dispensing ratios of dual fluids. 1 mM erioglaucine was used as the sample solution and distilled water was used as the diluent. With respect to the diameter ratio of the actuation chamber, 1 mM erioglaucine was diluted with a predesigned concentration (Fig. 4B and C). Because of the different volume of each actuation chamber, it was simultaneously dispensed as much as the volume of the smaller actuation chamber. The remaining volume of the larger actuation chamber was dispensed alone. The simultaneously dispensed fluids were mixed through the curved channels, but the fluid dispensed alone did not mix with the other fluid. However, the mixture and fluid dispensed alone were supposed to be mixed by diffusion at the outlet chamber. Even if the diffusion does not occur completely, the sample solution and diluent are dispensed with a predesigned ratio so that the diluted sample solution with a desirable concentration can be achieved after pipetting or stirring.

Results of the blood cross-matching test

Finally, a finger-actuated microfluidic device was developed for the blood cross-matching test as shown in Fig. 5A. To separate blood plasma from whole blood of the donor and the recipient, a blood plasma separation membrane was integrated into the finger-actuated microfluidic device (Fig. 5B).
Four microfluidic channels are required corresponding to the donor's whole blood and blood plasma and the recipient's whole blood and blood plasma. Thus, the device was designed to actuate four fluidic channels with a single pressure chamber, two for the blood plasma and two for the whole blood that actuates a total volume of 6.19 μL of the actuation chambers. The diameter of the actuation chambers was designed to be 4000 μm and 2000 μm aiming to dispense 2.48 μL of blood plasma and 0.61 μL of whole blood. Since the volume of the actuation chamber for blood plasma is larger than that for whole blood, the remaining volume of the actuation chamber for blood plasma after the simultaneous compression of the actuation chambers will be dispensed alone into the outlet. Thus, an incubation time of 5 min is required to provide enough time for the reaction at the outlet chamber. For 5 min, the cross-reacted mixtures at the outlet chamber slowly flow back into the chambers for the major and minor tests due to the pressure of the mixtures.

Fig. 5C shows the four possible results of the blood cross-matching test. It is worth noting that the agglutination aspect was the same as the results of the off-chip blood cross-matching test although the blood plasma was cross-reacted with whole blood instead of plasma-free RBCs. According to the agglutination in the major and minor tests, the transfusion suitability is interpreted as follows. For the results where agglutination did not occur in the major and minor tests, the donor’s blood may be transfused to the recipient. Transfusion should not be done for the other cases. However, for the result where agglutination occurred in a minor test and not in a major test, a little amount of donor’s blood can be transfused by monitoring whether or not the hemolytic transfusion reaction occurs if the transfusion is urgently required. The most basic blood cross-matching test without the addition of reagents such as response enhancers and enzyme solution was demonstrated in a finger-actuated microfluidic device. Actually, response enhancers, such as polyethylene glycol, low-ionic strength solution and bovine albumin, and enzyme solutions, such as bromelin, were used to achieve more sophisticated blood cross-matching results. Although our device is not designed to handle response enhancers or enzyme solutions, additional inlets for these solutions can be supplemented for more accurate blood cross-matching tests within the allowed volume of the actuation chamber.

We simply performed the blood cross-matching test without ancillary equipment, but a stereoscope was used to analyze the results of the blood cross-matching test. However, a low magnification of the stereoscope was used for the analysis so that the stereoscope can be replaced by a magnifying glass or a miniaturized and portable microscope like a foldscope. Furthermore, although we used anti-coagulant-treated whole blood to demonstrate the four possible results of the blood cross-matching test, the whole blood obtained directly from the fingerstick of donors and recipients can be used in the real-world emergency state requiring transfusion.
Conclusions

We demonstrated a blood cross-matching test in a finger-actuated microfluidic device. The blood cross-matching test was simply performed with only a few finger actuation steps that do not require any bulky, expensive systems and well-trained people. The blood plasma was simply separated from the whole blood through the plasma separation membrane which was integrated in the device, and the blood plasma and the whole blood of the donor and the recipient were easily cross-reacted with a predefined volume ratio. It is expected that the finger-actuated microfluidic device can provide rapid results of the blood cross-matching test within 10 min which is useful for patients who need urgent transfusion. Also, various functions of bulky and expensive systems are expected to be replaced by finger actuation which is one of the simplest actions humans can do. Compared to other finger-actuated devices, it is meaningful that the user-dependent errors depending on the various characteristics of the personal finger actuation can be reduced. All components required for fluid dispensing are indirectly actuated by pushing and releasing the pressure chamber. Therefore, the various characteristics of the personal finger actuation were corrected more uniformly. Because of this working principle of the finger-actuated microfluidic device, the constant volume was repeatedly dispensed with a CV of 3.17% according to end-users. Besides the blood cross-matching test, we expect that many kinds of POCT can be performed with the finger-actuated microfluidic device with the integration of pre-treatment and analysis functions.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

This research was supported by the National Research Foundation of Korea (NRF) (NRF-2016R1A2B3015986, NRF-2017M3A7B4039936, and NRF-2016-Global Ph.D. Fellowship Program) funded by the Ministry of Science and ICT. The biospecimens and data used for this study were provided by the Biobank of Chungnam National University Hospital, a member of the Korea Biobank Network.

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