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# Microchip technology applications for blood group analysis

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## ABSTRACT

Blood group analysis techniques are some of the most in demand immunological applications in clinical transfusion praxis and organ transplantation. In order to aid the advance towards higher throughput and increased sensitivity, analytical solutions dealing with a minimal amount of blood samples and the miniaturization of diagnostic equipment using microchip technologies have been evolving into an optimal solution. Here we review fabrication technologies for various types of microstructure on microchips, related operating procedures, and characterization approaches. Our focus is on examples of microchip technology and instrumentation used for blood group analysis ranging from classical serological methods of glycoprotein detection and solid phase assays, to nucleic acid amplification techniques. Molecular typing using microchip-based techniques is emerging as a supplement to standard serological methods. Microchip technology will play its key role to support blood group analysis at the molecular scale by using microliters of blood samples for extremely sensitive, quantitative, and high throughput analyses.

**Keywords:** microchip technology, microfluidics, microarray technology, blood group typing

**Abbreviations:** POC, Point-of-Care; ISBT, the International Society of Blood Transfusion; RBCs, red blood cells; HDN, hemolytic disease of the newborn; MMT, microplate monolayer technique; NGS, next-generation sequencing; SERS, surface-enhanced Raman scattering; QCM, quartz crystal microbalance; μPAD, microfluidic paper-based analytical device; PDMS, polydimethylsiloxane; PEO, polyethylene oxide; PMMA, polymethyl methacrylate; PEGMA, polyethylene glycol methacrylate; MCM, microplate coagglutination method; MAM, microplate agglutination method; SPAM, solid-phase adherence method; SPRCA, solid-phase red cell adherence; MIT, molecular imprinting technology; MIPs, molecularly imprinted polymers; SPR, surface plasmon resonance; SPRi, surface plasmon resonance imaging; UV-vis, ultraviolet-visible; GNPs, gold nanoprisms; SNPs, single nucleotide polymorphisms; NAT, nucleic acid amplification techniques; PCR, polymerase chain reaction; LR-PCR, long-range polymerase chain reaction; Rh, Rhesus Macacus

## INTRODUCTION

Analytical applications in medicine have been showing increasing demand in miniaturization of instrumentation and growing need for rapid, quantitative and sensitive analyses of a large number of samples, which are available only in microliter and/or microgram quantities. Especially, the requests

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are continuously arising for the clinical testing of blood groups and detection of various biomarkers at the single-cell level in fields relating to blood transfusion, organ transplantation, tumor diagnostics, as well as infectious and non-infectious diseases. Therefore, classical assays of molecular biology and immunology are now being scaled down to the category of micrometer level devices and more sensitive detection technologies. In this review we will describe some of these new microchip technologies for blood group analysis.

Karl Landsteiner reported a series of tests to identify the ABO blood group system and classified blood into three groups according to its agglutination properties[1]. According to the International Society of Blood Transfusion (ISBT), there are now 38 formally registered blood group systems[2]. Some systems are more important in daily practice than others, such as ABO, Rh, Kell, Kidd, Duffy, and MNS. The Rhesus system is the second most important blood group system after ABO. The ABO blood group system in human bevings was recapitulated for clinicians and scientists in a comprehensive and easy way[3]. Generally, only blood with the same ABO and Rh type as the recipient is transfused from the donor. Therefore it is essential and routine to determine ABO and Rh blood type prior to transfusion.

ABO and Rh typing is also performed as a prenatal screening method for pregnant women in order to identify the risk of hemolytic disease of the newborn (HDN). Major antigens of the Rh system are named D, C, E, c, and e. Rh-negative individuals produce anti-D antibodies when exposed to Rh-positive cells by either transfusion or pregnancy. These anti-D antibodies can cross the placenta to the fetus to cause hemolytic disease of the newborn. In addition, transfusion reactions will take place when Rh-positive blood is applied. ABO grouping is mostly required in transfusion, organ and tissue transplantation, cellular or molecular therapies, and paternity testing. ABO compatibility between donor and recipient is crucial because anti-A and anti-B antibodies react with antigens *in vivo* and lead to life-threatening acute hemolysis.

Many examples of diagnostic devices are used in current clinical practices at the Point-of-Care (POC), including some for blood group analysis. There is an obvious trend towards the implementation of new generation POC medical devices based on microchip technologies, which are now offering highly automated analytical procedures and instrumentation portability. The development of microchips includes multidisciplinary approaches based on engineering, physics, chemistry, and biology knowledge on a microscale. Below, state-of-the-art approaches to the fabrication of functional microstructures on chips, patterning methods, operating procedures and characterization techniques will be presented.

## TECHNOLOGIES FOR BLOOD GROUPING AND TYPING

In order to understand the potential and advantages of microchip technologies in blood grouping and typing, it is important to first describe the principles of blood grouping and typing. Basic principles for blood group analysis fall into three main categories: (1) serological blood typing; (2) solid-phase adherence assays; and (3) molecular typing. These technologies are based on the analysis of different phenotypes and samples´ genotypes. For each category, new methods and techniques were developed to meet the evolving clinical demands and achieve accurate results.

Serological blood typing is a conventional and widely used strategy in current clinical practice. For the ABO and Rh blood group system, conventional blood typing is a series of tests performed to detect surface antigens on red blood cells (RBCs) by the agglutination test and the following compatibility test to determine relevant antibodies against these antigens.

In ABO blood typing, the RBCs of the recipient mix with anti-A and anti-B sera from the donor or standard samples. This process is known as forward grouping. When the recipient´s serum mixes with standard type A and B cells, the process is reverse grouping. The routine practice in clinic is to test A, B, and D (Rh) antigens and several other antigens in some special cases when necessary. Antibody screening tests based on a similar principle are used to discover unexpected antibodies. ABO blood group typing is confirmed by reverse grouping that detects expected isoagglutinins. Prior to transfusion, a crossmatch test between donor and recipient is performed as a final check for incompatibility.

Serological blood typing tests are used to distinguish genetically determined blood groups by determining the phenotype of circulating cells (forward typing) and reactivity of serum antibodies (reverse typing). Frequently used methods include the slide method, tube test, microplate technology, column/gel centrifugation, etc[4]. Evaluation of the gel system for ABO blood grouping and D typing showed it performed as well as tube assay method for detecting A, B, and D, but for detecting B isohemagglutinins, the tube assay has slightly better accuracy[5].

The second category includes solid-phase adherence assays. Aside from using normal agglutination reac-

tions, these have the advantage of using monolayers of antigen-bearing red blood cells (RBCs) or antibodies to minimize the quantity of reagents or improve test sensitivity. Examples such as microplate monolayer technique (MMT) will be described in more detail in the following paragraphs.

These two categories of classical typing techniques outlined above are based on specific reactions of antibody-antigens on the erythrocyte surface. However, certain limitations exist, for example, the availability of rare antiserum, blood typing of recently-transfused patients, etc. Molecular blood typing methods residing in the third category are suitable for addressing these issues. In this way, the genetic background of the blood group polymorphism is discovered and depicted to reveal the necessary phenotypes.

Among molecular typing technologies, there are different methods such as the determination of alleles by DNA hybridization or sequencing. For example, target enrichment next-generation sequencing (NGS) is able to assess genetic variations and can be used to develop an extended blood group genotyping assay system. Several high-throughput multiplex blood group molecular typing platforms have also been developed to predict and determine blood group antigen phenotypes[6].

Though serological typing is reliable and practical for routine use, it has its own limitations and drawbacks. Molecular typing emerges as an alternative or supplemental way in many diagnostic cases. One of its advantages is precisely its ability to identify clinically significant antigens missed by serological typing. In order to obtain more comprehensive and dependable results, it is better to use these two principles in conjunction with each other if conditions permit, especially for important and/or rare cases.

## MICROCHIP TECHNOLOGY FOR BLOOD TYPING

Microchip technology is a combination of technologies including the fabrication of various microstructures on a chip, the operation among integrated elements, and relevant characterization approaches. For this, it is very useful for the construction of a simple but robust assay and readout system. Microchip technology has shown its big potential in blood group analysis, with lots of successful cases having been demonstrated in experimental studies and industrial applications.

According to different functional structures, microchip technology can be classified into: (1) microfluidic chips; (2) microplate-based platforms; (3) microarray-based chips; (4) microtiter trays; (5) combinations thereof. Each structure has its own advantages in facilitating the test procedures and achieving accountable results.

From the perspective of fabrication techniques, there are many approaches for patterning the substrates, such as: (1) photolithography; (2) wet etching; (3) soft lithography; (4) wax printing; (5) molecular imprinting. With today´s advanced development of microfabrication technologies, different functional microstructures can be easily manufactured to meet the diverse and highly specialized need for research laboratories, hospitals, and industries. The implementation of this microchip technology reached sufficiently manageable operating procedure simplicity and equipment accessibility, necessary for mass production.

To quantify and characterize the typing results, a variety of detection techniques are used. Some of the relevant techniques for blood group analysis are: (1) visual observation; (2) dielectrophoretic response; (3) surface-enhanced Raman scattering (SERS); (4) surface plasmon resonance (SPR); (5) quartz crystal microbalance (QCM) technology. In a typical microchip application, signals are detected and further analyzed through equipment compatible with the microchip.

As shown in ***Table 1***, the three underlying aspects of microchip technology are: functional microstructures, fabrication techniques, and specific characterization approaches. In practice, the techniques above are combined according to the desired application in order to minimize the device down to scale. In the following sections, we will give some examples from the above for a better understanding of the various applications of microchip technology for blood group analysis.

## ADVANTAGES AND DISADVANTAGES OF MICROCHIP TECHNOLOGY

Microchip technology has many advantages for scientific research and industrial applications in physics, chemistry, and biology, especially in molecular biology and immunology. As described below, it demonstrates several advantages for blood grouping and typing, such as being suited to a small sample size, providing low energy consumption, and having a high throughput.

The first advantage in using microchip technology lies in its ability to work off a very small sample quantity, usually in the scale of microliters or even less. This makes it indispensable for minimal samples with limited supplies. Accordingly, fewer reagents are required in the following procedures. These reagents could be missing, very rare or expensive in some cases.

Another advantage is microchip technology´s ability to save energy, which brings together convenience and simplicity in the construction and operation

***Table 1*** **A general view of microchip technologies from three main aspects**

| Aspects | Functional microstructures | Fabrication techniques | Characterization approaches |
|---|---|---|---|
| 1 | Microfluidic chip | Photolithography | Visual observation |
| 2 | Microplate-based platform | Wet etching | Dielectrophoretic response |
| 3 | Microarray-based chip | Soft lithography | Surface-enhanced Raman scattering (SERS) |
| 4 | Microtiter trays | Wax printing | Surface plasmon resonance (SPR) |
| 5 | Their combinations | Molecular imprinting | Quartz crystal microbalance (QCM) technology |

of different medical devices. With the aid of capillary force, for example, it is possible to construct self-driven microfluidic devices without the need for external power supply, which are able to perform fast and sensitive immunoassays using minute volumes of analytes. By embedding and removing cylindrical microfilaments in a microfluidic chamber, several compartments can be easily formed[7]. This technology provides low-cost solutions for constructing well-controlled microenvironments for biochemical and cellular assays with the benefit that it works without the use of pneumatic valves or external equipment.

With integral microarrays, the third advantage of using microchip technology is the capability to analyze thousands of samples simultaneously, therefore replacing multiple individual assays[8]. By combining these microarrays with image processing technology, rapid and accurate determination of ABO blood types can be easily conducted with an automated blood analyzer[9].

Like other technologies, microchip technology has some disadvantages. In some cases, the fabrication procedures are complicated and need special equipment, which are not easily accessible for users without professional laboratories. The cost of fabrication of a single microchip just for preliminary experiments can also be high. However, with the mass production of commercial devices, the cost of using microchips is quite lower than before. These aspects limit the development of its applications. With the improvement of research facilities, the availability of prototypes however is becoming easier.

In this review, we focus on the vast applications of microchip technology in blood grouping and typing, from classical serological methods and solid phase assays to emerging nucleic acid amplification techniques, including studies realized by manual and automated instruments.

## FUNCTIONAL STRUCTURES OF MICROCHIP

To improve blood typing technology, different microchip functional structures have been developed. The most common structures are microfluidic systems, such as conventional microfluidic devices, microfluidic paper-based analytical devices (μPADs), and thread-based microfluidic systems.

The second most common category of structures includes monolayers of cells with antigens or antibodies which form a solid phase to improve test sensitivity. Examples such as microplate monolayer technique, microplate agglutination method and solid-phase adherence method are given below to demonstrate the variety and utility of solid phase technology in blood typing.

What´s more, by using monolayers, there are advantages in fabricating arrays to facilitate the automation processes. These may take the form of microtiter trays, microplates, or active spots on the surface. Since this useful technology has been developed for a long time and is rather mature, products with the same principle can be found easily on the market for routine test and diagnostics.

### Conventional microfluidic device

For straightforward, accurate, and precise analysis, the utilization of microfluidic systems in modern procedures has become one of the most promising approaches among emerging technologies[10]. In the early stage, conventional microfluidic systems are made of common materials such as glass or plastic materials for ease of fabrication.

With an autonomous microfluidic device, the fast analysis of the ABO blood group system was demonstrated through the characterization of blood flow[11]. In this model experiment, the accumulation of red blood cells immobilized on the capillary wall led to increased lateral movement of the flowing cells, resulting in the overall selective deceleration of the red blood cell flow column compared to the plasma fraction. By monitoring the flow rate characteristics in capillaries coated with blood type reagents, it is possible to identify red blood cell types. With the support of the analysis of hydrodynamic effects governing blood flow by Finite Element Method based modeling, the construction of a simple quantitative device for blood group determination can be fulfilled. This proof-of-concept chip is illustrated in ***Fig. 1***.

Based on the advantages of microfluidic devices, there is another methodology with more integration elements[12]. Here, after separating blood cells from

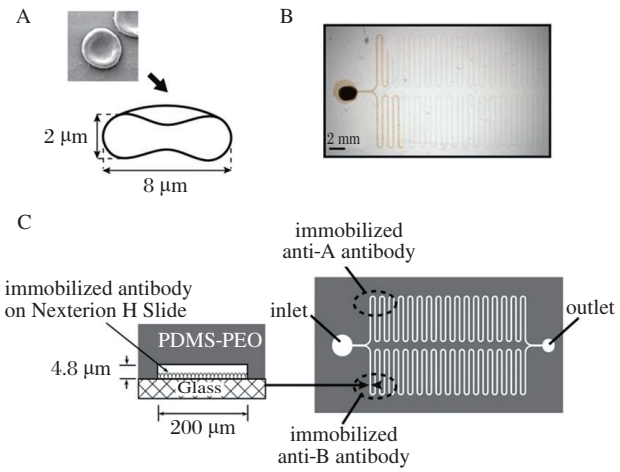


***Fig. 1* RBC dimensions and design of microfluidic chamber.** A: A scanning electron microscopic picture and a cross-sectional view of a red blood cell that indicates its shape and average dimensions. B: The photo shows the capillary system filled by blood. C: The microfluidic device was formed by aligned attachment of PDMS-PEO based flow cell containing microfluidic channels onto slides functionalized by anti-A and anti-B reagents in the indicated regions. The left panel presents the cross-sectional view of the winding channel. (Images courtesy of K. Papp, *et al.*[11])

plasma by applying hydrodynamic forces, the plasma and RBCs are collected respectively and tested. This portable device which includes a separator and a detector is capable of detecting agglutination in both antigens of RBCs (forward) and antibodies of plasma (reverse). The typing results are distinguishable by the naked eye and microscope images. Besides, in high-throughput microfluidic devices, it is also possible to sort healthy and pathological human RBCs with different biomechanical characteristics.

A plug-based microfluidic device was designed to perform analyses on multiple agglutination assays in parallel without cross-contamination, using only microliter volumes of blood[13]. Proof-of-concept ABO and D (Rh) blood typing and group A subtyping were successfully performed by screening against multiple antigens. On-chip subtyping distinguished common A and A (2) RBCs by using a lectin-based dilution assay.

By using a microfluidic thermoplastic chip, a portable and cost-effective colorimetric diagnostic device was fabricated for rapid ABO and Rh blood typing[14]. Unambiguous blood typing tests can be distinguished by the naked eye in 1 minute with only 1 microliter of blood. In blood typing, for hematocrit determination and coagulation analysis, the travel distance of the color can be measured after a certain time period instead of a change of color. The results are shown below in ***Fig. 2***.

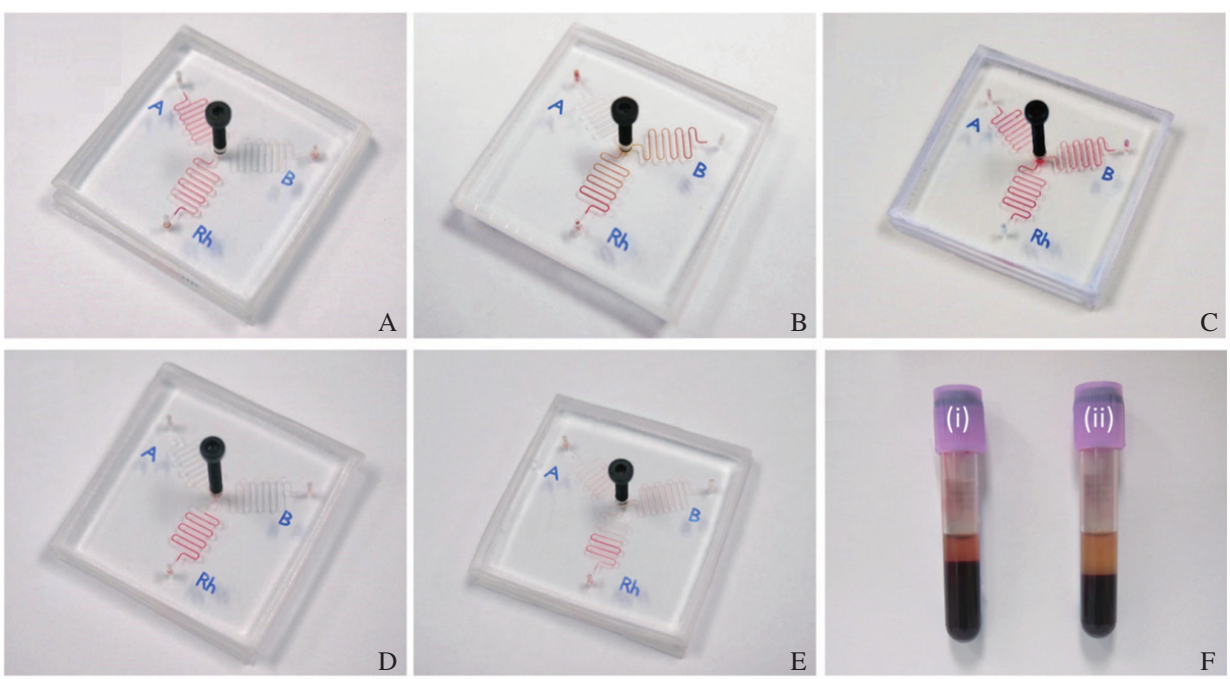


***Fig. 2* Representative chip test results.** A: A Rh+. B: B Rh+. C: AB Rh+. D & E: O Rh+. These visualized results are clearly indicated by the red lines and the corresponding A, B, and Rh symbols on the top of the chip which label the antibody locations. D: healthy blood sample. E: Thalassemia samples with smaller RBCs and lower hematocrit were also successfully verified, displayed as a clear but shorter agglutinated RBC line compared to healthy blood sample in image d. 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°C refrigerator for 1 hour. (Images courtesy of S. Chen, *et al.*[14])

These days, blood typing devices are expected to be portable and energy saving. With a finger-actuated smart microfluidic device, the typing result can be displayed by means of channels bearing the letter and the symbol of the corresponding blood type[15]. By seven button-pushes using less than 10 μL of blood within 30 s, blood typing can be successfully performed. Another example features a finger-powered agglutination lab chip with CMOS image sensing[16]. In this case, the blood antibodies are first preloaded into the antibody reaction chamber with the fine control of driving sample volume via finger push. This initiates a hemagglutination reaction. Finally, the blood type is identified using a CMOS image sensing system.

Microfluidic devices can be shaped into different forms of channels and even disks. These localized areas are suitable for further surface modification to improve their hydrophilicity and blood compatibility. With a kind of antifouling material, polyethylene glycol methacrylate (PEGMA), microfluidic disks were developed for blood-typing and irregular antibody screening[17]. This showed an extremely high consistency with the traditional tube method in the identification of ABO and RhD blood types.

It is also important to control the liquid flow in microfluidic devices. To hold the injected microfluid in the valve position, a chamfer-type capillary stop valve was designed. As a flow regulator, this helps to better discriminate the agglutination results from non-agglutination cases[18]. In another work, a portable micro-mixing device was designed to avoid the common backflow[19]. Through a bypass connected between two inlet ports, the initial pressure difference can be balanced. Subsequent typing tests of ABO/Rh blood groups demonstrated consistent results and demonstrated its great potential for future use in agglutination-based assays or lab-on-a-chip testing applications.

Microfluidic devices also have the potential to perform tests simultaneously with some integrated automatic manipulation apparatus, such as a lab-on-disc blood-typing system with red blood cell (RBC) agglutination in the microchannels[20]. According to the experiment, a total of eight assays including forward-typing, reverse-typing, and irregular-antibody tests were conducted successfully with high accuracy up to 97.5%, again demonstrating the capability of automation and high throughput of microfluidic devices.

## Microfluidic paper-based analytical device (μPAD)

The microfluidic devices presented above are made of polymer materials such as polydimethyl siloxane (PDMS), polyethylene oxide (PEO), polymethyl methacrylate (PMMA) and polyethylene glycol methacrylate (PEGMA). Paper as another category of material is also explored for its convenience and low cost.

Since the first report on the fabrication of a microfluidic paper-based analytical device (μPAD) by using the photolithographic technique to create a hydrophobic and hydrophilic barrier[21], lots of relevant applications in biochemistry and environmental sciences have been introduced. These paper-based microfluidic devices are useful in the detection of glucose and cholesterol, measurement of enzymatic markers, and separation of blood[22], etc. This method is promising for a class of low-cost, portable, and technically simple platforms for running multiplexed bioassays with microliter volumes of a single biological sample.

As a powerful diagnostic platform, the commercialization of paper-based microfluidics for medical diagnostics is discussed in detail[23]. In fact, paper-based POC devices are widely used as a powerful platform for cell analysis[24] and point-of-care blood-based analysis and diagnostics[25]. With hydrophilic letters A and B representing the pre-immobilized anti-A and anti-B respectively, a simple and low-cost portable paper-based ABO blood typing device was constructed for point-of-care testing[26]. Well-controlled procedures such as adsorption and washing steps help to achieve 100% accuracy.

In addition, the behavior of RBCs on porous paper substrates was also studied to understand the underlying mechanism and for engineering blood analysis devices[27]. The ring-shaped RBC stain formed on paper is found to be mainly from the combined effects of capillary wicking, filtration, and evaporation. However, the fiber structure, RBC incubation time, relative humidity, and paper additives were also found to change the results. Based on these discoveries a paper-based microfluidic device was fabricated to detect hematocrit level and demonstrated the controllable spatial distribution of RBCs on porous materials.

Since agglutinated RBCs cannot travel through the porous structure of the paper, it indicates a positive antibody-antigen interaction. For red blood cell antigens in blood group systems such as Rh, Kell, Duffy, and Kidd, typing was also explored on paper material. By testing reaction time and reagent concentration, most blood groups with antibodies available such as IgM were identified[28]. Some interesting aspects, such as reduced sample volume, test time, detection limit, quantification method, and the communication of information for these paper-based systems are still

undergoing research and development.

Clinically, except for ABO and Rh blood group, other less common blood groups can also cause fatal blood transfusion accidents and should be identified. To fulfill this purpose, a paper-based device also exists for the rapid typing of other human blood groups[29]. In a similar way, the visual observation of agglutination patterns on paper also proved to be effective for given examples such as E, e, S, s, K, k, etc.

Though paper-based diagnostics are reliable and cost-effective, the shelf-life of such products is of concern to many users. A study was done to examine the effects of aging on antibody activity on paper and in solutions under different aging and drying conditions such as air-dried, lyophilized, and kept as a liquid[30]. It showed that paper kept wet with undiluted antibodies has the longest shelf-life and the clearest reading.

In a word, microfluidic methods have gained popularity and have various applications in medical and diagnostic devices. From the cases given above, it can be seen that microfluidic methods are very useful and promising in blood grouping.

## Thread-based microfluidic system

Unlike the devices based on surfaces, a easily stainable thread can transport liquid via capillary wicking without the need for a barrier. These advantages are suitable for fabricating low-cost and low-volume microfluidic devices. An inexpensive thread-based system for simple and rapid blood grouping was developed[31]. Using this thread-based device, ABO and Rh groups can be successfully determined from only two microliters of whole blood from a pricked fingertip within one minute and without blood sample pre-treatment. It does this by utilizing flow resistance sensitivity to large particles in narrow capillary channels to separate agglutinated red blood cells (RBCs) from plasma. The results are shown in ***Fig. 3***.

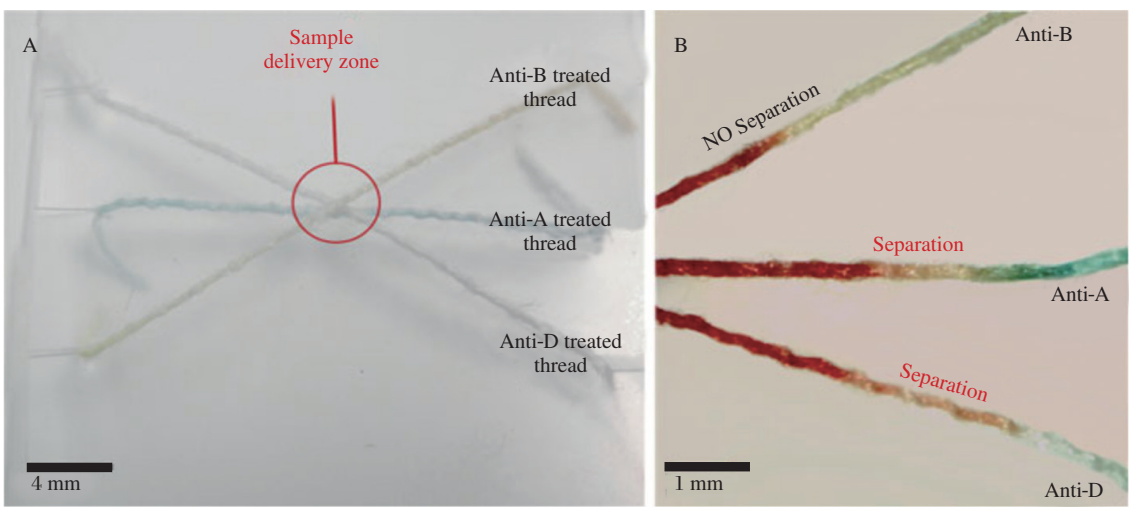


***Fig. 3*** **A single-step blood grouping test prototype sharing an intersecting sample delivery zone.** A: The unused device with sample delivery zone indicated within a red circle. B: An enlargement of the right-hand side of the device after whole blood has been introduced into the delivery zone in the center via needle eye. The separation occurring on threads anti-A treated and anti-D treated indicates that the blood is of type $A^+$. (Images courtesy of W. Shen, *et al.*[31])

## Microplate monolayer technique (MMT)

By immobilizing red blood cells in wells of microplates using a cell fixation buffer, a monolayer was formed and employed for detecting and identifying RBC antibodies[32]. This method has greater sensitivity than hemagglutination antiglobulin tests, without loss of specificity, therefore decreasing the quantity of erythrocytes and relevant reagents required. This was subsequently applied to a microplate coagglutination method (MCM)[33]. When compared with an automated hemagglutination system in 34 519 samples, it has been shown to be more sensitive, free of false-positive results, and cost-effective.

## Microplate agglutination method (MAM)

Based on the formation of an RBC monolayer on a microplate, a novel agglutination method for blood grouping and reverse typing was introduced[34]. Without the need for centrifugation and washing, this microplate agglutination method (MAM) has the possibility of being integrated into a fully automated system.

## Solid-phase adherence method (SPAM)

Due to the above agglutination methods lacking an objective endpoint easily distinguished by automated instruments, as an alternative, the solid-phase adherence method (SPAM) for ABO grouping and

Rh typing of RBCs was developed[35]. A or B RBCs were immobilized on wells and formed monolayers as the solid phase. The adherence reactions were read by spectrophotometer and interpreted by a computer. The efficiency is the same as conventional microplate agglutination methods but the accuracy is reported to be higher.

### Microtiter tray method

V-bottom microtiter tray method, a red blood cell typing technique which has proven to be many times more sensitive than tube or slide methods, can be used for direct agglutination, antiglobulin tests and mixed field agglutination[36].

This very sensitive solid-phase antiglobulin test, widely used in blood group serology, has an inherent disadvantage: the sedimentation variability of indicator cells during centrifugation. However, with the "length measurement of the absorption curve" technique, the solid-phase microtiter plate assay has since proved to be more discriminating and safe[37]. By combining microtiter plates and solid-phase red cell adherence (SPRCA) assays for antibody detection, a fully automated blood typing system was fabricated[38]. This system automates routine blood bank tests with an accuracy comparable to that of hospital transfusion services´ standard manual methods.

### Microarray technology

Microarray technology utilizes an array of active spots on a solid surface. This technology is widely used in the fields of genomics and proteomics. Solid-phase antibody microarrays were fabricated for ABO and RhD blood typing[39]. Using image recognition, this array chip demonstrated good accuracy and precision for rapid blood grouping. Undoubtedly, it is quite useful in large blood donation centers for its high throughput.

Microarrays were developed to collect a huge amount of information for screening and diagnostics. The main benefit of this technology lies in its capabilvity for the simultaneous analysis of thousands of analytes. The application of microarray technology may revolutionize efficiency in blood testing. It provides an integrated platform for comprehensive donor and donation testing and may replace multiple individual assays. It is also possible to integrate it with microfluidic structures to offer more complicated applications.

## PATTERNING METHODS FOR MICROCHIP

Patterning methods and microfabrication approaches are the basis for the construction of variable microchips. The most frequently used microfabrication approaches are photolithography, wet etching, and soft lithography. Photolithography and wet etching are popular in microelectronics and are practical for fabricating structures on glass or silicon oxide. Soft lithography or replica molding is also well known and widely used. Here we emphasize some less practiced but nonetheless promising patterning methods like wax printing and molecular imprinting.

### Wax printing

Wax is used to form hydrophobic parts on raw materials and is especially suitable for paper-based devices. To adapt to the surface properties, wax printing has been adapted for paper patterning[40] and forms a simple and inexpensive method for fabricating microfluidic devices using a commercially available printer and hot plate[41].

For example, by printing six parallel channels with wax onto filter paper, a new platform for a paper-based analytical device was fabricated for simultaneous forward and reverse ABO blood group typing[42]. The results were then identified by hemagglutination of the corresponding antigen and antibody. The forward and reverse patterns of blood groups A, B, AB, and O were barcode-like and since can be visually analyzed by machine.

A paper-based analytical device (PAD), fabricated by using a combination of wax printing and wax dipping methods, allows for the simultaneous determination of ABO and Rh blood groups on the same chip[43]. This PAD has excellent reproducibility and higher accuracy in comparison with the conventional slide test method.

Wax printing as a straightforward, timesaving, and inexpensive method is undoubtedly promising for use in developing countries and presents itself to be an ideal method for large-scale production of μPADs.

### Molecular imprinting technology

Molecular imprinting technology (MIT) is a technique for the creation of molecularly imprinted polymers (MIPs) with tailor-made binding sites complementary to the template molecules in shape, size, and functional groups[44]. Different microchips for sensing and analysis can be made based on their unique features of structure predictability, recognition specificity, and application universality. The advantages of using MIPs are their high binding selectivity, straightforward synthesis in a short time, and high thermal/chemical stability. Molecularly imprinted polymers have good physical and chemical robustness, high stability, low-cost, and reusability features. MIPs based sensors have lots of applications owing to good sensitivity to minute structural changes on the scale of the molecule[45].

By introducing molecularly imprinted polymeric nanoparticles, it is possible to conduct antibody-free blood typing[46]. This novel approach presents an alternative to widely used conventional agglutination methods and its results can be acquired using a standard microtiter plate reader.

## CHARACTERIZATION TECHNIQUES IN MICROCHIP DEVICES

Since microchips are quite small, the signals from minimal samples and reagents are normally weak, so special techniques and characterization methods are required. Several examples are given in the following paragraph such as video recording, dielectrophoretic response, quartz crystal microbalance (QCM) technology, surface-enhanced Raman scattering (SERS), and surface plasmon resonance (SPR).

### Video recording and image acquisition

After drying anti-A or anti-B typing reagents inside the microchannel of a passive microfluidic chip, real–time time observation of RBCs agglutination was realized[47]. This agglutination process was monitored by video recording which allowed for the research on the kinetics of agglutination. Within less than two minutes, 100% correct discrimination between positive and negative agglutinations was performed. An injection-molded microfluidic chip was then designed for point-of-care blood typing[48]. With anti-A or anti-B dried reagents contained inside its microchannel, enhanced capillary flow facilitated blood handling and process automation.

For point-of-care applications, it is also interesting to observe the process and record images with a smartphone[49]. To this aim, a built-in light source was firstly converted into uniform backlight illumination in an imaging box. Then, a multi-piece orthoscopic lens was embedded for magnification. Finally, a microfluidic chip was fabricated for ABO blood typing. With this system, clear images can be observed in a 3 μL sample without additional light sources. This example presents great potential for portable smartphone-based test devices.

### Dielectrophoretic response

Dielectrophoretic manipulation of erythrocytes/red blood cells was investigated as an effective tool to identify blood type for medical diagnostic applications[50]. With a platinum electrode microdevice and video microscopy, positive blood types of the ABO typing system ($A^+$, $B^+$, $AB^+$, and $O^+$) were tested and cell responses quantified. Cells of $O^+$ type showed a relatively attenuated response to the dielectrophoretic field and were distinguished with greater than 95% confidence from all other three blood types. $AB^+$ cell responses differed from $A^+$ and $B^+$ blood types because $AB^+$ erythrocytes express both A and B glycoforms on their membrane. This research suggests that dielectrophoresis of untreated erythrocytes beyond simple dilution depends on blood type, so it could be used in portable blood typing devices.

### Quartz crystal microbalance (QCM) technology

Notable features of quartz crystal microbalance (QCM) devices include low-cost fabrication, room temperature operation and ability to monitor extremely low mass shifts. The combination of MIPs with QCM has turned out to be a prominent sensing system for the label-free recognition of diverse bioanalytes. Label-free bioanalyte detection from nanometer to micrometer dimensions was demonstrated[51]. The development of an efficient MIP-QCM platform for offering rapid analysis is expected sometime in the future, providing suitable accuracy at a reduced cost.

As revealed by sensor responses using quartz crystal microbalance, erythrocyte-specific interactions are achieved with recognition sites on surface-imprinted polyurethane[52]. This technique is applicable for the blood group typing of main ABO antigens. This selective recognition is likely to be driven by hydrogen bonds to sugar residues which define the major blood group type on the cell membrane. In the future, a highly integrated microchip with arrays of recognition sites will be routinely made using surface imprinting. An example of this kind of sensing has already been achieved. By combining a two-dimensional molecularly imprinted polymer film with copper oxide nanoparticles, a trypsin imprinted polymer-based electrochemical sensor on 96-well microplates was constructed and a good dynamic response was observed[53]. Detections of trypsin in fetal bovine serum were demonstrated using the imprinted polymer thin films.

### Surface-enhanced Raman scattering (SERS)

To study the molecular bases of ABO blood types, the membrane electrophoresis-based surface-enhanced Raman scattering (SERS) method was employed. It provided a rapid, convenient and, accurate way to identify ABO blood types[54].

### Surface plasmon resonance (SPR)

Surface plasmon resonance (SPR) is the resonant oscillation of conduction electrons at the interface between adsorption materials and a thin planar metal (typically gold or silver) layer stimulated by incident

light. This forms the basis for many applications such as biosensors, lab-on-a-chip sensors, and photosynthesis. Surface plasmon resonance imaging (SPRi) technology measures modifications of the mass variation correlated refractive index at the surface, which enables users to visualize the working area and monitor and analyze biomolecular interactions in real-time. For the detection of biomolecular interactions, SPR and SPRi immunosensors have several advantages: they can operate in real-time, and are label-free, highly sensitive, easy for automation, quantitative, etc. Here are several examples.

The quantitative detection of erythrocytes through surface plasmon resonance (SPR) was studied by covalently immobilizing blood group-specific antibodies and monitoring the cell-binding response[55]. Firstly, anti-human IgG antibodies were immobilized onto the sensor surface and then incubated with RBCs. Via SPR detection, this platform for quantitative blood group typing proved to be effective and fully regenerable. For the RhD blood group, a clear distinction between positive and negative D antigen RBCs was observed. Later, a sensor chip was prepared by immobilizing a monoclonal antibody (anti-A or B) on a gold thin layer[56]. Through the measurement of SPR signal, the A-type sample responded to the sensor-chip immobilizing anti-A monoclonal antibody. For the B-type, AB-type, and O-type samples, the chip responded correspondingly. Thus, it proved to be a rapid, sensitive, and simple method for inspecting the ABO blood type for saliva and blood samples.

In another study, SPRi based sensor was developed for ABO blood typing[57]. Red blood cell samples flowed into the multichannel flow cell, which was orthogonal to the detection line arrays immobilized by blood group antibodies. Analyzing the interaction between RBCs, antibody A, and antibody B, blood typing of four RBC samples was shown to be rapid, effective, and sensitive with the lowest detection limit of $2\times10^6$ cells/mL for A and B RBCs.

A biochip prototype including a fluidic system and an optical absorbance detection module was demonstrated in an automated ABO compatibility test[58]. At critical red blood cell concentrations of $1\times10^8$ cells/mL, this optical detection proved to be efficient.

In the case of low antigenic expression of the ABO subgroup system on red blood cells, SPRi has advantages. An antibody array was designed and immobilized onto a sensor surface to specifically and quantitatively detect the ABH antigen on RBCs[59]. This technique was able to indicate different ABH antigen densities on the RBCs and ABH substances in the saliva of strong and weak subgroups.

In addition, a SPR biosensor can be combined with an ultraviolet-visible (UV-vis) spectrometer to construct a simple, rapid, and reliable assay for ABO blood group typing and erythrocyte counting[60]. Gold nanoprisms (GNPs) were deposited on glass substrates and then antibodies were immobilized on the GNPs surface. Coupled with an UV-vis spectrometer, the sensors were further integrated into a microfluidic chip containing two independent sample cells with anti-isoagglutinins A and anti-B antibodies. They were applied for the identification of blood type A, B, AB, and O, respectively. Compared with results from conventional gel column assay on seven different blood samples, the accuracy of blood typing was 100%.

## MOLECULAR TYPING AND FUTURE TRENDS

Molecular blood group typing is indispensable when there is a lack of erythrocytes or antisera, or in certain other cases, where serological testing is not conclusive. With the development of molecular technology, the majority of blood group genes are identified and sequenced. Single nucleotide polymorphisms (SNPs) are assigned to blood group antigen specificities. Nucleic acid amplification techniques (NAT), usually based on the polymerase chain reaction (PCR), have been successfully applied to blood grouping to support routine serological testing. Many methodologies are also available to genotype blood groups from laboratory-developed assays to commercially available platforms[61]. For example, direct PCR from plasma or serum with melting point analysis has been developed for faster red cell genotyping to identify blood group antigens within 40 min[62]. A real-time based blood-group genotyping approach was also introduced with automation potential and rapid cycling time[63].

In combination with microarrays for automation, molecular typing methods will make large-scale screening a cost-effective reality. The evolution of molecular methods will certainly increase the use of red cell genotyping. In the era of personalized medicine, it is possible that all patients requiring transfusion could be genotyped for blood group antigens. In this way donor units could be chosen based on genotype matching rather than a serologic crossmatch[64]. It is also possible to conduct on-chip PCR with microfluidic devices. This procedure was proved by the amplification of genomic and viral templates in unprocessed whole blood for clinical testing, without the need for DNA extraction[65].

Sequencing technology has evolved rapidly. With the development of next-generation sequencing (NGS) technology, blood group genotyping is becoming

easily accessible and more popular[66]. Sequencing technology has the advantage that it can sequence complete genes with high efficiency. For instance, by using overlapping long-range polymerase chain reaction (LR-PCR) amplicons, the complete RHD gene in the Rh blood group system was sequenced[67]. Through the analysis of the correlation between intronic SNPs and their specific Rh haplotype, a novel diagnostic approach can be established to investigate variants of the RHD and RHCE genes.

Accordingly, microchip technology and even nanotechnology are utilized more frequently in sequencing. Oxford Nanopore Technologies invented the nanopore sequencing method to determine the nucleotide sequence of single-stranded DNA molecules through measuring the electrical current over the pore. Several studies of RNA sequence and structure using nanopores were reported[68]. It is possible to sequence the almost complete long read genes in the ABO system and RHD and RHCE genes without the prior step of PCR in the future.

## CONCLUDING REMARKS

Blood grouping and typing are indispensable in clinical trials and tests. The demand for accurate and fast typing with a large number but a small quantity of samples is growing rapidly. Microchip technology is a possible solution to meet these requirements, especially at the POC.

Microchip technologies are providing: (1) superior sensitivity; (2) high throughput; (3) automated analytical systems. These characteristics are essential for monitoring and quantifying reactions between antigen and antibody. Therefore, commonly used classical types of hemagglutination and solid phase assays are in the process of being updated with new microchip technologies. Different methods of microchip applications reviewed here show the effectiveness of this technology. Further innovations of microchip designs will certainly arise for blood typing in the near future, particularly in higher sensitivity and efficiency, lower energy consumption and cost for specific usages in laboratories and clinics.

Serological assays, though still widely used, may be inaccurate in certain cases of multiply transfused patients. Molecular typing using microchip-based techniques is emerging as a supplement to the standard serology. Microchip technology will absolutely play a key role in supporting blood group analysis at the molecular scale by using microliters of blood samples for extremely sensitive, quantitative, and high throughput analyses. An increasing number of professionals have noticed this trend and are bringing more resources to this field, in order to accelerate its applications. We expect with certainty that many more related research results will be delivered in the near future.

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