Blood-typing and irregular antibody screening through multi-channel microfluidic discs with surface antifouling modification

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ABSTRACT

A novel surface modification technology for microfluidic disks was developed for multichannel blood-typing detection and irregular antibody screening. The antifouling material, poly(ethylene glycol) methacrylate (PEGMA), was used to modify the surface of the microfluidic disk for improving its hydrophilicity and blood compatibility. With the modification of PEGMA, the hydrophilicity was sufficiently improved with a 44.5% reduction of water contact angle. The modified microfluidic disk also showed good biocompatibility with a reduction of hemolytic index (from 3.4% to 1.2%) and platelet adhesion (from $4.6 \times 10^4/\text{cm}^2$ to $1.9 \times 10^4/\text{cm}^2$). Furthermore, the PEGMA modification technique conducted on the microfluidic disk achieved successful adjustment of burst frequency for each chamber in the microchannel, allowing a sequential addition of reagents in the test protocol of manual polybrene (MP) blood typing. Clinical studies showed that the proposed MP microfluidic disk method not only performed at extremely high consistency with the traditional tube method in the identification of ABO/RhD blood types, but also accomplished an effective screening method for detecting irregular antibodies. In conclusion, this study demonstrated that the easily mass-produced MP microfluidic disk exhibited good blood-typing sensitivity and was suitable for clinical applications.

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I. INTRODUCTION

Human blood is a complex media which can be categorized into 35 blood subgroups based on the existence of more than 300 antigens on the number of red blood cells (RBCs).¹ Blood mismatching occurs when the antigens on the surface of the donor's RBCs react with the corresponding antibodies in the recipient's serum, thereby causing severe RBC agglutination, intravascular hemolysis, renal failure, and shock.¹ Typing and cross-matching of blood groups are, therefore, critical processes that need to be carried out before the clinical blood transfusion process, which certify the blood compatibility between the donor and the intended recipient.^{2–4} In substantial routine blood tests of blood banks, an automatic, simple, and accurate blood-typing system is essential to avoid laborious operations.^{5,6}

The traditional microplate method for blood-typing assay and irregular antibody screening requires a visual determination of RBC agglutination levels by highly trained personnel. In addition, its procedure is tedious and time-consuming. Several commercial devices coupled with automated detection systems are available currently for blood typing and for screening irregular antibodies based on the indirect antiglobulin test (IAT) method. However, no commercial devices have yet to be developed for the manual polybrene (MP) method. Microfluidic systems have been reported as a useful and facile platform for clinical diagnosis.^{7–16} Through introducing microfluidic chips, a parallel biomedical analysis on a single chip was achieved with less consumption of reagents and shorter reaction time.⁷ A variety of microfluidic blood-typing methods have been previously reported, including disposable biochip,⁷ droplet technique,⁸

speed-measured microchannel,⁹ RBC-trapped biochip, and paper-based microfluidic bioships.^{10–16} A novel time-saving lab-on-a-disk blood-typing system has been developed in our previous works, which was a centrifugal microfluidic platform composed of a multi-channel microfluidic disk and a mechanical apparatus for operating forward typing (red cell typing), reverse typing (serum typing), and MP irregular antibody screening simultaneously.¹⁷ However, the multichannel microfluidic disk system still had room for improvement, as program errors occurred during centrifugation and there was poor accuracy while screening for irregular antibodies.¹⁸

Unexpected fouling of biomaterials may lower the sensitivity and accuracy of a biosensor during biochemical analysis. Therefore, antifouling technology plays an important role for nonspecific biomaterials of biosensors or bio-analysis techniques. Several hydrophilic materials such as hydroxyethyl methacrylate (HEMA), poly(ethylene glycol) (PEG), and zwitterionic chemicals exhibit an excellent antifouling property on nonspecific microorganisms.^{19–21} Among them, PEG is a relatively low-cost and widely used antiprotein-fouling material with great resistance to biomaterials. Chang *et al.* showed that a well-grafted PEGylated surface could sufficiently resist nonspecific materials in the human blood, including proteins, platelets, RBCs, and white blood cells.^{22,23} Cong *et al.* demonstrated the addition of PEG to a microfluidic chip through sandwich photolithography.²⁴ Moreover, PEG has served as an additive agent for improving the sensitivity of blood typing in the manual test tube method.^{25,26} By means of improving the detection level for irregular antibodies as well as reducing the errors emanating from nonspecific bindings, PEG exhibited a higher sensitivity for blood typing.²⁷

In our previous works, a PEG structure was modified on the polystyrene (PS) multiwell plate to inhibit the nonspecific agglutination of RBCs during the blood-typing process, which reduced the possibility of typing error and increased the accuracy rate.²⁷ In this study, a poly(ethylene glycol) methacrylate (PEGMA) hyperbrush-grafted PS substrate was introduced into the fully automated lab-on-a-disk microfluidic system for sufficiently resisting the adhesion of blood cells and thereby reducing the amount of blood required from donors. Moreover, the hydrophilic interface produced by PEG modification could also enhance the fluid behavior in the microchannel, improving the accuracy of blood typing. In addition to exploring the surface properties, biocompatibility, and centrifugal burst frequencies of the microfluidic disk, the disk was also applied clinically to standard pretransfusion blood tests for forward typing and reverse typing of ABO and D grouping as well as MP irregular antibody screening and was compared with those obtained by standard manual test tube methods.

II. MATERIALS AND METHODS

A. Materials

The PS plastic pellets used for injection molding were purchased from Formosa Plastics Co. Polyethylene terephthalate (PET) film was sold by 3M Company. Chemicals such as polybrene, glutaraldehyde, and PEGMA (average molecular weight of 500 Da) were purchased from Sigma-Aldrich Co. Ltd. Phosphate-buffered saline (PBS) and ethanol were purchased from Thermo Fisher scientific Inc. The purple-headed blood collection tube containing

K2-EDTA was sold by Becton, Dickinson, and Company. Oxygen gas for an ozone generator was purchased from Sinda Gases Co. Ltd. Anti-A antibody, Anti-B antibody, Anti-D antibody, A cell, B cell, and DiaPanel cell were purchased from DiaMed AG. Screen cells (SC-I, SC-II, and SC-III RBCs) and antihuman globulin (AHG) reagents used for the indirect anti-globulin test (IAT) as well as low ionic medium (LIM), polybrene, and resuspending solution used for the MP method were purchased from Formosa Biomedical Inc. Deionized water was produced using a Millipore water purification system with a minimum resistivity of 18.0 MΩ cm. Human blood samples were from Taiwan Blood Service Foundation and processed following the protocol approved by the Institutional Review Board (IRB; approval number: MMH-TP 99050).

B. Thermally induced surface PEG brush graft polymerization

Our previous works concluded that 30 wt. % of PEGMA was the optimized condition for grafting PEGylated hyperbranch brushes on a PS microplate interface for blood typing, and the surface PEGylation process was followed.²⁷ The circular PS plate, the reaction chamber of the PS microfluidic disk (referred to as the PS microfluidic disk), and the upper PET film for disk packaging (referred to as the PET film) were treated with a O₃/O₂ mixture with an O₃ concentration of approximately 46 g/l at a flow rate of 6 l/min for 30 min at 25 °C via an ozone generator (model OG-10PWA, Ray-E Creative Co., Ltd., Taiwan). The PS plate, the target chamber of PS microfluidic disk, and the PET film were loaded with PEGMA macromonomer solution (30 wt. % in deionized water) for uniform coverage of all surfaces and were incubated at 80 °C for 24 h. The surface grafting density of the PEGylated hyperbrush was determined by weight gain after grafting.

For further biocompatibility studies mentioned below, the PEGylated and the non-PEGylated PS plates, the PS microfluidic disk, and the PET film were cut into 1 cm².

C. Surface hydrophilicity measurement

The hydrophilicity of surfaces before and after PEGylated hyperbrush grafting was determined by measuring water contact angles. A droplet of deionized water was dropped onto the surface of the materials, and the water contact angle over 10 s at 25 °C was detected via an automatic contact angle meter (model FTA1000, First Ten Ångströms Co, Ltd., USA).

D. Hemolysis analysis

Human whole blood samples were centrifuged for isolation of RBCs, which were then washed three times with 0.15M normal saline solution, counted, and transferred into a tube to yield a homogeneous solution containing 1×10^9 RBCs.

Prior to the study, the surface of the materials was equilibrated by 150 μl of PBS at 37 °C for 24 h. The equilibrated materials were then immersed into a 100 μl RBC solution for 1 h at 37 °C, followed by the collection of RBC solution. The viable RBCs were removed by centrifugation at 2000 rpm for 5 min, and the resulting supernatant containing released hemoglobin (Hb) from the dead RBCs was

quantified by measuring the absorbance at 540 nm via a microplate spectrophotometer (model PowerWave XS, BioTek Instruments Inc., USA). A calibration curve ranged from 50 to 800 $\mu\text{g}/\text{ml}$ was constructed for calculating the concentration of Hb ($R^2 = 0.9923$). Incubation of RBC samples with deionized water and PBS was served as positive control (i.e., 100% hemolysis) and blank control, respectively. Each data point of hemolysis ratio reported was the numerical average of six independent measurements ($n = 6$).

E. Platelet adhesion test

To observe the activated platelet adhesion on the PEGylated surfaces, platelet-rich plasma (PRP) containing 1×10^5 platelet/ml was prepared by centrifuging the human whole blood samples at 120 rpm for 10 min. PRP (150 μl) was then allocated into the PEGylated and non-PEGylated PS plates, the PS microfluidic disk, and the PET film for a 120 min incubation at 37 °C. The surfaces were then washed with 150 μl of PBS two times and fixed in 2.5% glutaraldehyde at 4 °C for 48 h. The fixed platelet on the surfaces was washed again with 1 ml of PBS. After sequential dehydration treatment [0%, 10%, 25%, 50%, 90%, and 100% (v/v) ethanol in PBS] with 20 min incubation at each step, the platelet attached samples were air dried, cut, sputter coated with gold, and quantified

using a scanning electron microscope (SEM; model JSM-5410, Jeol Ltd., Japan) operating at 7 keV.

F. Automatic blood-typing lab-on-a-disk system

A multichannel optical disk detection system composed of a robotic arm, an automatic dispensing system, a centrifugal equipment with a speed control platform, a charge-coupled device (CCD) coupled with a camera, and an image analysis software (made by the Department of Mechanical Engineering, Chung Yuan Christian University) was used in this study.¹⁹ The design of the microfluidic disk is illustrated in Fig. 1 (see also the [supplementary material](#) for its photo image); each disk contained eight microfluidic units (channels A–H) each with five reagent chambers (chamber 1–5). Channels A–B, channels C–D, channel E, and channels F–H were designated for AB forward typing, AB reverse typing, Rh D phenotype identification, and irregular antibody screening by the MP method, respectively. Chamber 1 acted as the reagent chamber, the reaction chamber, and the detection chamber.

G. Measurement of burst frequency of microfluidic disk

In order to achieve the automation of the integrated system and to ensure the fluency of the reagent addition, the burst frequency

FIG. 1. Schematic illustration of the developed microfluidic disk design. (a) Disk surface pattern design; (b) capillary valve design; and (c) microchannel design. Note: See the [supplementary material](#) for the photo image of the multichannel microfluidic disks.

(i.e., an instantaneous frequency driven by centrifugal force to trigger the fluid flow out from the reagent chamber to the reaction chamber) of each chamber on the microfluidic disk was examined and optimized.¹⁵ It is noteworthy that the reagents should be introduced to the reaction chambers following a particular order for the blood-typing procedure, and, therefore, a unique and dispersive burst frequency should be given to each chamber. To facilitate automatic CCD shooting, the reagent such as LIM, 0.05% polybrene, and resuspending solution was mixed with 10 μ l of watercolor paint. By gradually increasing the rotation speed of the motor, the flow-out of reagents was observed, and the burst frequency of each channel was recorded. The results were confirmed by repeating the measurement 30 times.

H. Microfluidic disks method for ABO blood typing and irregular antibody screening

The blood-typing tests and irregular antibody screening using PS microfluidic disks followed the protocol as described by Chang *et al.*¹⁹ A 3 μ l of 3% RBC solution was dispensed into chambers A, B, and E, and 3 μ l of serum samples were dispensed into chambers C, D, E, F, G, and H, separately. The microfluidic disk was then loaded onto the automatic blood-typing lab-on-a-disk system.

For forward ABO blood typing, Rh D phenotype identification, and reverse ABO blood typing, a 5 μ l of anti-A and anti-B antibody, anti-D antibody, and A cell and B cell was introduced to chamber 2 of channels A and B, channel E, and channels C and D by the dispensing module, individually. The microfluidic disk was centrifuged at 400 rpm for 5 s under room temperature to drive the antibody or cell flow to the reaction chamber and was rotated within 40° back and forth three times by an electrical motor. The RBC aggregation was observed at 30 s after mixing.

For irregular antibody screening, a 5 μ l of screen cells (SC-I, SC-II, and SC-III for channels F, E, and H, separately), 6 μ l of LIM, 2 μ l of 0.05% polybrene, and 2 μ l resuspending solution were introduced to chambers 2, 3, 4, and 5 by dispensing module, respectively. The microfluidic disk underwent a series of mixing and incubation processes as follows: centrifuge at 700 rpm for 5 s to drive screen cell antibodies and LIM flow to the reaction chamber, rotate within 40° back and forth three times for mixing, rest for 30 s, centrifuge at 1100 rpm for 3 s to drive 0.05% polybrene flow to the reaction chamber, rotate within 40° back and forth three times for mixing, centrifuge at 1400 rpm for 5 s to drive the resuspending solution flow to the reaction chamber, and rotate within 640° back and forth ten times for dispersing the aggregated clots. The results were automatically captured by the CCD camera mounted atop the whole system from the detection chamber.

I. Manual test tube methods for ABO/D blood typing and irregular antibody screening

The manual test tube method is the standard method for blood tests in the blood banks according to the AABB guidelines. Briefly, a 100 μ l of anti-A, anti-B, or anti-D antibody was mixed with 50 μ l of 3% RBC for forward typing of ABO/D groups. For reverse typing, a 100 μ l of A cell, B cell, or D cell was mixed with 50 μ l of serum samples. The mixture was then centrifuged at 3400 rpm for 15 s at room temperature. The blood type was then determined by observing the level of RBC agglutination.

The IAT method for irregular antibody screening was performed through incubating standard saline IAT at 37 °C for 30 min, followed by washing and the addition of AHG.²⁸ The presence of irregular antibodies was confirmed by observing the agglutination of RBCs.

J. Statistical analysis

Data were expressed as the arithmetic mean $\pm$ 1 SD from at least five repetitions. One-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range tests for correcting multiple comparisons. A *P*-value less than 0.05 was considered to be significant.

III. RESULTS AND DISCUSSION

The recent biomedical development of lab-on-a-disk platforms focused on liquid mixing, valving, flow switching, metering, and sequential loading.²⁹ Through the design of the driven centrifugal force and the valves, the sequential loading of samples and reagents on the centrifugal microfluidic and lab-on-a-disk platforms can be manipulated, achieving microdetection and high-throughput detection.³⁰ The advantages of the technique include efficient removal of bubbles and residual volumes in microfluidics that could interfere in the result interpretation, as well as controllable transportation and separation of samples between valves, and therefore, the technique is considered important in clinical IVD (*in vitro* diagnostic) detection.²⁹

However, critical factors for the commercial application of lab-on-a-disk platforms included improving accuracy (i.e., achieving sequential loading of reagents), lowering manufacturing cost, and prolonging long-term stability during storage (for prefilled microfluidics). This study aimed to introduce the thermally induced surface PEG brush graft polymerization technique on easily mass-produced PS microfluidic disks for (1) developing an automatic blood-typing platform, (2) modifying sequential loading (i.e., improving detection accuracy) through the optimization of specific control valves, and (3) improving sensitivity for clinical blood typing.

A. Surface treatment of PEG brush

To improve the hydrophilicity and anti-biofouling properties of the PS microchannel, the PEGMA polymer was grafted onto the surface of PS microfluidic disks (Fig. 2). The thermally induced polymerization with ozone treatment was divided into two steps. Firstly, the peroxidation reactive sites on the PS surface were activated by ozone treatment. The overall peroxidation sites were controlled by tuning the O₃/O₂ ratio. Here, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to determine the density of surface peroxide and optimize the concentration of peroxide on the treated surfaces to around 2.85 nmol/cm².^{22,31} In general, prolonging ozone treatment time could increase the concentration of accumulated peroxide; however, long-term ozone treatment may cause adverse reactions such as etching or degradation on the surface of the PS microplate. Therefore, controlling the ozone treatment period is a crucial step that affects the roughness and grafting efficiency of the subsequent

FIG. 2. Schematic process of PEGMA grafted onto PS substrates via the ozone treatment and thermal polymerization.

thermal polymerization on the surface, and a 30-min ozone treatment was selected as the optimized condition in this study. Secondly, the PEGMA macromolecules were grafted onto the material via thermally induced radical graft polymerization with a heat treatment on the surface. During the thermally induced radical polymerization, the reaction temperature and time were crucial to affect the decomposition of peroxide and the polymerization of PEGMA macromonomers on the ozone-treated PS surfaces. In this study, the optimized polymerization condition for PS substrates was set at 80 °C for 24 h.

The surface hydrophilicity of the PS microfluidic disk after PEGylation was analyzed based on the static water droplet contact angle measurement. Both the PET film and the PS microfluidic disk contacted directly with blood samples during the blood-typing processes and were, therefore, analyzed prior to and posterior to PEGylated modification. The virgin (unmodified) PET film, the optical PS disk (a bulk PS plate), and the PS microfluidic disk had a contact angle of $108^\circ \pm 4.6^\circ$, $93.9^\circ \pm 3.3^\circ$, and $88.1^\circ \pm 1.4^\circ$, respectively. After the surface PEGylation process, the contact angle measured on the surface of the modified PET film, PS disk, and PS microfluidic disk was reduced to $79.2^\circ \pm 0.4^\circ$, $76.7^\circ \pm 0.2^\circ$, and $77.4^\circ \pm 1.0^\circ$, respectively (Fig. 3 and Table I).

B. Blood compatibility of microfluidic disks

The hemolysis properties and human platelet adhesion assay were performed to assess the hemocompatibility of the prepared biomaterials.^{32,33} The former indicates the RBCs' rupture through the presence of hemoglobin, while the latter relates considerably to the further adsorption of reagents to the prepared materials during blood typing and irregular antibody testing.³⁵

In this study, the hemolysis rate of virgin PET film, PS disk, and PS microfluidic disk was $5.49 \pm 0.08\%$, $3.47 \pm 0.12\%$, and $1.83 \pm 0.01\%$, respectively. After surface PEGylation, the hemolysis rate

in the modified PET film, PS disk, and PS microfluidic disk was decreased to $4.62 \pm 0.05\%$, $1.82 \pm 0.02\%$, and $1.32 \pm 0.03\%$, respectively (Fig. 4 and Table I).

The attachment of platelets on the surface of materials was also observed by SEM in this study (Fig. 5). The adhesion of the platelet was 9.7×10^4 cell/cm², 5.6×10^4 cell/cm², and 2.8×10^4 cell/cm² on the virgin PET film, PS disk, and PS microfluidic disk, respectively. A large amount of platelet adhesion observed on the PET film

FIG. 3. Hydrophilic characterization of the PET film and PS substrates with and without PEGMA modification by the static water droplet contact angle test. ^{a-c}Different letters indicate significant difference ($P < 0.05$) compared with different groups; i.e., symbol "a" marked above PET film means that there is significant difference between the PET film and all the other groups.

TABLE I. Hydrophilicity and hemocompatibility of the PET film and PS substrates with and without PEGMA modification.

Sample name	Water contact angle (deg)		Hemolysis ratio of RBC solution (%)		Numbers of platelet adhesion ($10^4/\text{cm}^2$)	
	Virgin	Surface PEGylation	Virgin	Surface PEGylation	Virgin	Surface PEGylation
PET film	108.2 ± 4.6^a	53.2 ± 2.4^c	6.4 ± 0.8^a	1.4 ± 0.2^c	9.4 ± 1.2^a	3.4 ± 0.5^c
PS disk	96.6 ± 5.2^b	49.7 ± 5.0^c	3.7 ± 0.4^b	1.3 ± 0.3^c	5.8 ± 0.8^b	2.3 ± 0.7^d
PS microfluidic disk	94.3 ± 3.3^b	52.3 ± 3.7^c	3.4 ± 0.5^b	1.2 ± 0.2^c	$4.6 \pm 0.9^{b,c}$	1.9 ± 0.4^d

^{a-d}Different letters in the same column indicate significant difference ($P < 0.05$) compared with different groups; i.e., symbol a mark on PET film of the water contact angle test mean that have significant difference between the PET film and all the other groups.

was mainly due to its hydrophobic surface. After PEG brush grafting, the adhesion of platelets reduced, since the formation of the hydrophilic interface, in the modified PET film, PS disk, and PS microfluidic disk to 8.3×10^4 cell/ cm^2 , 2.8×10^4 cell/ cm^2 , and 1.38×10^4 cell/ cm^2 , respectively (Fig. 6). Ishihara *et al.* have indicated that the shape change of platelets represented the interaction between materials and the blood as well, which induced the release of cytokines that further affected the blood analysis and led to an indirect effect on the blood-typing test.³⁴ In Fig. 5, no pseudopodia or morphological changes of platelets were found after their contact with the modified materials.

Together with the results of contact angle measurement, the hydrophilicity of the PET film, PS disk, and PS microfluidic disk was improved through PEG brush grafting, while the hemolysis ratio was decreased, which was consistent with the previous literature.³⁵ Besides, the improved surface hydrophilicity reduced the platelet adhesion on the materials, which might avoid the thrombosis in the microchannels.³³

FIG. 4. Hemolysis ratio of RBC solution in the presence of the PET film and PS substrates with and without PEGMA modification. ^{a-d}Different letters indicate significant difference ($P < 0.05$) compared with different groups; i.e., symbol “a” marked above DI water means that there is significant difference between DI water and all the other groups.

C. Optimization of centrifugal burst frequency with surface treatment of microchannels

In this PS microfluidic disk system, according to the blood-typing protocol, the addition of different reagents sequentially from the reagent chambers to the reaction chamber was driven by centrifugal force. In order to introduce the reagents in the correct order, different depths and patterns of the microfluidic disk which affect the degree of the capillary phenomenon have been studied,³⁶ and a unique and dispersive burst frequency for each chamber should be designed. That is, the reagents could be added in a specific sequence according to the blood-typing procedure used in this study.

Table II(a) shows the burst frequency of virgin chambers 2–4 (without PEG brush grafting) in the PS microfluidic disk (with PEGylation only in the reaction chamber) measured from 30-time replicates. By gradually increasing the centrifugal speed (at intervals of 100 rpm) from 300 rpm, the successful flow-out of reagents from chambers 2 and 3 into a reaction chamber under 600 rpm was recorded as 24 and 13 times, respectively, with the remaining 6 and 17 samples flowed out under 700 rpm, and thus the burst frequency of both chambers was considered as 700 rpm. Similarly, in virgin chambers 4 and 5, the burst frequency under 30-time replicates was measured as 1300 and 1400 rpm, individually.

To avoid the overlap of burst frequency between chambers 2 and 4, which hindered the clinical MP blood-typing procedures, the surface treatment of PEGMA was performed. The PEGylation

FIG. 5. SEM images of platelets adhered onto the surfaces of non-PEGylated (a) PET film, (b) PS disk, (c) PS microfluidic disk, as well as PEGylated (d) PET film, (e) PS disk, and (f) PS microfluidic disk.

FIG. 6. Quantitative results of platelet adhesion on the surface of materials. ^{a-d}Different letters indicate significant difference ($P < 0.05$) compared with different groups; i.e., symbol “a” marked above PET film means that there is significant difference between the PET film and all the other groups.

process was evaluated to increase the hydrophilicity of the PS microfluidic disk, reducing the resistance between its surface and the specimen/reagents. Table II(b) demonstrates the adjustment of burst frequency after PEG grafting on the surface of chambers 2 and 4. As expected, the burst frequency of PEG-modified chambers dropped significantly, and the final burst frequency of chambers 2–4 was recorded as 400, 700, 1100, and 1400 rpm, individually.

The timing and sequence of the reagents addition and mixing time impact the detection accuracy considerably, and the

control of sequential loading is a critical element for microfluidic systems. Besides, the dimensional tolerance of the mold for producing microfluidic disks and that of the designed patterns of injection molded microfluidic disks might result in the failure of sequential loading and are almost unavoidable. In recent years, the design of advancing valves for regulating sequential loading of microfluidics has become a research trend.²⁹ The valving platform development includes basic capillary valves,^{37–40} siphon valves, novel membrane deformation-based valves, and dissolvable film-based valves.^{41,42} However, these novel valving techniques have many disadvantages in commercial applications, for instance, the feasibility, cost, and quality control of mass production.

In this study, the siphon valves were utilized for designing blood-typing microfluidic disks. Although the hydrophilic siphon valves are generally produced by surface etching through O₂ plasma treatment, the O₂ plasma-etched surface aged easily in an ambient environment.⁴³ Katerina *et al.* examined the aging phenomenon of poly(methyl methacrylate) (PMMA)- and poly(ether ether ketone) (PEEK)-based microfluidics after O₂ plasma-etching, and the microfluidics exhibited a hydrophilic property in the atmosphere for 30 and 17 days, respectively. The thermal polymerization with ozone treatment was utilized for grafting PEGylated hyper-branch brushes on a PS microplate interface in this study. Due to the ionic bonding between the PEG brush and the ozone, the hydrophilic property could be maintained for several years in the atmosphere. Through the hydrophilic modification of PEGMA, the burst frequency of the specific chamber was adjusted under control, improving the detection accuracy of the lab-on-a-disk platform. The PEGMA grafting also enhanced the detection sensitivity of blood typing under a minimal amount of samples.²⁷ It is noteworthy that this technique proposed for modifying sequential loading of the microfluidic system in this study realized mass production and lowered production costs easily.

TABLE II. Summary of burst frequency of microchannels in the microfluidic disk with and without PEGMA modification.

(a) Virgin PS microfluidic disk					(b) 30 wt. % PEGylated microfluidic disk				
Rotating speed (rpm)	Microreagent chamber #				Rotating speed (rpm)	Microreagent chamber #			
	2	3	4	5		2	3 (PEGylated)	4	5 (PEGylated)
300	0	0	0	0	300	28	0	0	0
400	0	0	0	0	400	2	0	0	0
500	0	0	0	0	500	0	0	0	0
600	24	13	0	0	600	0	11	0	0
700	6	17	0	0	700	0	19	0	0
800	0	0	0	0	800	0	0	0	0
900	0	0	0	0	900	0	0	21	0
1000	0	0	0	0	1000	0	0	8	0
1100	0	0	0	0	1100	0	0	1	0
1200	0	0	14	0	1200	0	0	0	0
1300	0	0	9	21	1300	0	0	0	19
1400	0	0	0	9	1400	0	0	0	11
1500	0	0	0	0	1500	0	0	0	0
1600	0	0	0	0	1600	0	0	0	0

FIG. 7. Schematic illustration of MP method using a PEGMA-modified PS microfluidic disk platform. Step 1, Mixture of antibodies, erythrocytes, and screening cells; step 2, decreasing the zeta potential of RBCs; step 3, nonspecific condensation of RBCs; step 4, neutralization of polybrene with the PEGMA-modified surfaces.

D. Blood testing of an automatic blood-typing lab-on-a-disk system

The automatic lab-on-a-disk system provided a simple technique for blood typing and was optimized and applied in a clinical setting as shown in Fig. 7. The flow-out of SC-I, II, and III antibodies from chamber 2 to chamber 1 was driven under 400 rpm of rotation speed; then, the LIM reagent in chamber 3 was introduced under 700 rpm. After homogeneous mixing, 0.05% polybrene in chamber 4 was flown into chamber 1 under 1000 rpm. Finally, the resuspending solution in chamber 5 was added into chamber 1 with 1400 rpm of centrifugation. The whole detection procedure took approximately 5 min.

Blood samples ($n=101$) were analyzed and cross-assayed using the standard manual test tube method (Table III), of which 23.77% (23 samples) were type A, 35.64% (36 samples) were type B, 9.47% (9 samples) were type AB, and 32.67% (33 samples) were type O. Moreover, within the 101 samples, 97.03% (98 samples) were Rh D-positive, 1.98% (2 samples) were Rh D-negative, and

0.99% (1 samples) were weak D-type. The designed PS microfluidic disk showed extremely high accuracy and consistency with the traditional manual test tube method.

For further irregular antibody recognition by the IAT method, 2/101 serum samples (1.98%) had Mi^a antibody, 1/101 (0.99%) had Di^a antibody, 2/101 (1.98%) had Le^b antibody, 1/101 (0.99%) had C + e antibody, and 1/101 (0.99%) had D + Fy^a + s antibody. However, by using the PS microfluidic disk method, only 2/101 serum samples were recognized containing Mi^a antibody, 1/101 sample containing C + e antibody, and 1/101 sample containing D + Fy^a + s antibody.

The identification differences resulted from the absence of a temperature-controlling device in the proposed automatic lab-on-a-disk system, leading to an insignificant response of temperature responsive Le^b antibody during the screening process.²⁸ Besides, the lesser demand for blood specimens and the shorter agglutination time for detection may also affect the screening outcomes. More tests such as cell-antibody contact time were suggested for modifying the testing conditions of the system.

TABLE III. Comparisons of clinical results (N = 101) of blood typing and irregular antibody screening between the multichannel microfluidic disk method and the standard IAT tube method.

	Standard IAT [N (%)]	MP virgin microfluidic disk [N (%)]	MP PEGylated microfluidic disk [N (%)]
ABO typing			
A	23 (23.10)	22 (21.78)	23 (23.10)
B	36 (35.64)	35 (34.65)	36 (35.64)
AB	9 (9.01)	9 (9.01)	9 (9.47)
O	33 (32.67)	35 (34.65)	33 (32.67)
Rh D typing			
Rh D (+)	98 (97.03)	98 (97.03)	98 (97.03)
Rh D (−)	2 (1.98)	2 (1.98)	2 (1.98)
Rh D _w	1 (0.99)	1 (0.99)	1 (0.99)
Irregular antibody			
Mi-a ^a	2 (1.98)	1 (0.99)	2 (1.98)
Di-a	1 (0.99)	0	0
Le-a ^a	2 (1.98)	0	0
C + e	1 (0.99)	0	1 (0.99)
D + Fy ^a + s	1 (0.99)	0	1 (0.99)

^aCold antibodies.

IV. CONCLUSION

The critical factors for the commercial application of lab-on-a-disk platforms include improving accuracy, lowering manufacturing cost, and prolonging long-term stability during storage (for prefilled microfluidics). This study aimed to introduce the thermally induced surface PEG brush graft polymerization technique on easily mass-produced multichannel injection PS microfluidic disks for (1) developing an automatic blood-typing platform, (2) modifying the sequential loading due to dimensional tolerance of the mold for producing microfluidic disks and that of the designed patterns of injection molded microfluidic disks, and (3) improving the sensitivity for clinical blood typing. The surface PEGylation process not only improved the hydrophilicity of the PS microfluidic disk, but also elevated blood compatibility. The adhesion of platelets on the surface of PS microfluidic disk was reduced, preventing the occurrence of thrombosis. In addition, the hydrophilic properties of PEGMA-modified material helped control the centrifugal burst frequency of the reagent reservoir, so that the centrifugal device could smoothly control the sequence of the liquid flow out of the reagent chamber. Thus, sequential loading of reagents was important to ensure that the blood-typing method on the microchannel optical disc was successfully carried out. The results of the microfluidic disk method and the standard manual test tube method were compared, and both methods were highly consistent in ABO typing and Rh D type identification. However, in the screening of irregular antibodies, two blood samples could not be identified by the microfluidic disk method due to the lesser demand for blood specimens and the lack of a temperature-controlling module. It is suggested that the microfluidic disk method for irregular antibody screening should aim at the optimization of reagent concentration, reaction time, and the development of a temperature-controlling system in future studies.

In conclusion, the proposed surface treatment method with easy operation procedure and good biocompatibility opens a new

chapter for mass production of IVD microfluidic detection systems. It could be applied not only to the MP blood-typing system for commercial purpose, but also to the injection molded microfluidic disks for nucleic acid and immune detections.

SUPPLEMENTARY MATERIAL

The photo image of the multichannel microfluidic disk could be found in Fig. S1 of the [supplementary material](#) and was published with this paper on the internet.

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