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ORIGINAL ARTICLE

Development of a Strategy Based on the Surface Plasmon Resonance Technology for Platelet Compatibility Testing

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SUMMARY

Background: **This study was aimed to establish a novel strategy based on the surface plasmon resonance (SPR) technology for platelet compatibility testing.**
Methods: **A novel surface matrix was prepared based on poly (OEGMA-*co*-HEMA) via surface-initiated polymerization as a biosensor surface platform. Type O universal platelets and donor platelets were immobilized on these novel matrices via amine-coupling reaction and worked as a capturing ligand for binding the platelet antibody. Antibodies binding to platelets were monitored in real time by injecting the samples into a microfluidic channel. Clinical serum samples (n = 186) with multiple platelet transfusions were assayed for platelet antibodies using the SPR technology and monoclonal antibody-immobilized platelet antigen (MAIPA) assay.**
Results: **The novel biosensor surface achieved nonfouling background and high immobilization capacity and showed good repeatability and stability after regeneration. The limit of detection of the SPR biosensor for platelet antibody was estimated to be 50 ng/mL. The sensitivity and specificity were 92% and 98.7%. It could detect the platelet antibody directly in serum samples, and the results were similar to MAIPA assay.**
Conclusions: **A novel strategy to facilitate the sensitive and reliable detection of platelet compatibility for developing an SPR-based biosensor was established in this study. The SPR-based biosensor combined with novel surface chemistry is a promising method for platelet compatibility testing.**
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KEY WORDS

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INTRODUCTION

Transfusion of platelets is an effective way to prevent and treat bleeding resulting from immune thrombocytopenic purpura (ITP), neonatal alloimmune thrombocytopenia, or platelet function defects. Platelets are the cellular components of the blood coagulation system. Some important proteins [e.g., human platelet antigen (HPA)-1a/b] are found on the surface of the platelet plasma membrane. Multiple platelet transfusions can easily result in immunized antibodies, giving rise to platelet transfusion refractory (PTR) [1]. Therefore, the platelet antibody screening and cross-matching play an

important role before platelet transfusion. The conventional methods used for detecting platelet antibody are monoclonal antibody-based antigen-capture assays, such as solid-phase agglutination (SPA) and monoclonal antibody-immobilized platelet antigen (MAIPA) assays [2]. In addition, flow cytometry, mixed passive hemagglutination assay, and modified antigen-capture enzyme-linked immunosorbent assay (ELISA) are also used for detecting platelet antibodies. At present, the MAIPA assay represents the standard technique for the serologic diagnosis of platelet antibodies. However, it is time consuming and limited by the availability of monoclonal antibodies (MoAbs). The SPA assay has been modified to improve its sensitivity and specificity, but several inherent problems still exist, such as the short lifetime of indicating erythrocytes, complex process (several washing steps), and huge time consumption [3,4].

The surface plasmon resonance (SPR) technology has gained widespread recognition in the study of molecular interactions between different biomolecules in real time, without any labeling or washing procedure [5]. It provides information on kinetic processes, binding affinity, analyte concentration, and real-time detection [6]. Socher et al. demonstrated that the SPR method could facilitate the diagnosis of clinically relevant low-avidity HPA-1a antibodies [4]. Although the label-free SPR method for detecting biomolecular interactions was attractive, it had limited sensitivity and ability to prevent protein nonspecific adsorption in complex media, such as serum or cell lysis [6]. These limitations of the label-free SPR method for platelet antibody screening led to the development of a three-dimensional (3D) surface matrix consisting of poly (ethylene glycol) (PEG) through surface-initiated polymerization as the basis for an ultralow fouling surface chemistry platform enabling sensitive and specific detection of platelet antibodies in complex media. Besides its excellent nonfouling properties, this 3D matrix has abundant functional groups for ligand immobilization [7]. The ligands to detect platelet antibodies, including antigens, and peptide aptamers, contain just one activation site per molecule [8]. Platelets are an interesting ligand to detect the amount of platelet antibodies in the sample due to the number of antigens present on their membrane. However, so far, they have rarely been used for diagnostic purposes. Type O universal platelets and donor platelets were immobilized on the 3D matrix via amine-coupling reaction in this study. Platelet antibodies were detected in serum in real time using the SPR technology. The analysis was performed on a significantly larger number of samples, and the results obtained by the MAIPA assay were compared to confirm that the new method accurately detected platelet antibodies.

MATERIALS AND METHODS

Patients' serum and platelet antigens

Serum samples were collected from 186 patients (86 males and 100 females; including patients with ITP and neonatal alloimmune thrombocytopenia, with more than 2 platelet transfusions) at the Second People's Hospital of Shenzhen (Guangdong Shenzhen, China). The average age of patients was 32.3 years (ranging from 5 months to 65 years). Universal type O platelet mixtures [for platelet antibody screening, including most platelet surface antigens, HPA, and human leukocyte antigen (HLA)] were purchased from Changchun Bode Company (Changchun, China). Donor's platelets (for cross-matching tests) were gifted by the municipal blood center (Shenzhen, Guangdong, China). AB sera were taken from healthy nontransfused and nonpregnant donors (negative control). HLA antibodies containing sera were used as positive samples as the platelets expressed the HLAs.

Preparation of the 3D matrix

The 3D matrix was prepared as described in a previous study [7]. Briefly, the mixed self-assembled monolayers (SAM) of initiators and 11-(mercaptoundecyl) triethylene glycol were prepared by immersing the SPR chips into a 1 mM mixed solution (total concentration) of two thiols for 15 hours at room temperature. Surface-initiated polymerization was started by transferring the mixture (including OEGMA, HEMA, and bipyridine) (OEGMA: poly (2-oligo (ethylene glycol) monomethyl-ether methacrylate), HEMA: poly (2-hydroxyethyl methacrylate)) to an SPR chip presenting an atom transfer radical polymerization (ATRP) initiator in an inert gas glove box. The terminal hydroxyl groups of side chains in the matrices were converted into terminal carboxyl groups by immersing the chips into a dimethylformamide (DMF) solution. The chips were rinsed with DMF and methanol several times to remove salt particles and dried with nitrogen.

Nonfouling property of the 3D matrix

In biosensor applications, it is essential for the biosensor matrix to reduce or prevent nonspecific protein adsorption from complex samples. For example, the comprehensive proteins of human blood consist of thousands of proteins. Protein nonspecific adsorption not only causes significant background noise but also blocks the analyte binding sites. SPR was used in this study to investigate the nonspecific adsorption from serum proteins and other biomolecules onto the chip coated with poly (OEGMA-*co*-HEMA) polymer, and the results were compared with those of a chip modified with PEG SAM.

Regeneration of the SPR chips

Regeneration not only helps to reduce the difference between the chips but also extends the lifetime of these chips. This procedure requires removing the binding an-

alyte without affecting the activity of the ligand. It involves five injections of the positive standards and then regeneration each time by injecting glycine (10 mM, pH 2.0) to study the repeatability and stability of SPR chips.

Specificity and detection limit of the analyte
The specificity of the analyte was confirmed using the SPR technology. The platelet antigen (including HPA-1a) and nonstructural protein 1 (NS1, used as reference) antibody were printed on the same chip face. After the chip was treated with 1 mL of ethanolamine (1 M, pH 8.0) to deactivate the matrix, a serum sample containing the platelet antibodies anti-HPA-1a and NS1 was injected to display the specific interaction and nonspecific adsorption. Different concentrations of positive immunoglobulin G (IgG) samples [5, 10, 20, 30, 40, and 60 μL of positive standards were diluted with phosphate-buffered saline (PBS) to prepare a 500 μL solution, corresponding to the concentrations of 50, 100, 200, 300, 400, and 600 ng/mL] were injected over the chip at a flow rate of 2 μL/s to investigate the detection limit of the platelet antibodies. The limit of detection (LOD) of the SPR biosensor for platelet antibody was estimated.

Platelet antibody screening by the SPR assay
The 3D matrix was activated with an aqueous mixture of N-hydroxysuccinimide (NHS) (0.4 M) and 1- [3- (dimethylamino) propyl] -3-ethylcarbodiimide (EDC) (0.1 M) for 30 minutes. Universal type O platelets were printed on the SPR chip using the automated Genetix Q Array mini printer. The platelets were incubated for 1 hour. The amino groups on the platelets interacted with the carboxyl groups on the modified chip by carboxyl group coupling reaction, and the platelets were coupled to the SPR chip (Figure 1 and 2). Then, the SPR chip was loaded on the SPR instrument following the manual. PBS (10 mM) was introduced at a rate of 2 μL/s as a running buffer until a stable baseline was achieved. After deactivation with an aqueous solution of 1 mL of 1 M ethanolamine (GWC Technologies company, Madison, WI, USA), aliquots of 20 μL of standards in PBS (dilution 1:50) or 100 μL serum samples in PBS (dilution 1:10) were serially injected at a rate of 2 μL/s. The sample was replaced with PBS after the association phase of 400 seconds. The dissociation phase was then recorded for 400 seconds. In all experiments, the SPR chip was regenerated with 2 mL of 10 mM glycine (pH 2.0) after each run.

Platelet antibody screening by MAIPA
The MAIPA assay was performed as described in the kit manual. Briefly, 50 μL of positive and negative control sera or sample sera were added to the microtiter plate wells (including platelet antigens). Antigen and antibody reacted at 37°C for 45 minutes. After five washes with an isotonic solution, 50 μL of indicating erythrocytes (red blood cells carrying anti-human IgG antibody) were added to each well. The microplates were centrifuged at 1500 rpm for 1 minutes 30 seconds before reading.

Clinical cross-matching by the SPR assay
The direct technique for platelet antibody detection was employed according to the aforementioned description for the SPR assay: donor platelets were printed on the activated SPR chip surface with an automated Genetix Q Array mini printer and incubated for 1 hour at room temperature. One spot was printed with PBS buffer without platelets as a negative control. The SPR technology was used to perform matched platelet infusion for 34 patients with platelet antibody positivity, and the clinical effectiveness of platelet transfusions was evaluated using the 24-hour corrected count increment (CCI) value.

Statistical analysis
Statistical analysis of the data was performed using SPSS version 16.0 (SPSS, IL, USA). The counts of positive and negative sera were compared using the chi-square test. A p-value less than 0.05 was considered to be significant. The sensitivity, specificity, positive and negative predictive values (PPV and NPV), and accuracy of detection were determined using the following formulas: sensitivity = TP/(TP + FN); specificity = TN/(TN + FP); PPV = TP/(TP + FP); NPV = TN/(TN + FN); and accuracy = (TP + TN)/n.

RESULTS

Properties of the novel 3D matrix
Figure 3 shows that the PEG SAM formed on the thermo-evaporated gold (Au) film was prone to nonspecific protein adsorption. The SPR resonance value due to the nonspecific adsorption was about 100 RU. In contrast, the nonspecific adsorption was not found on introducing the serum on the poly (OEGMA-*co*-HEMA) matrix-modified Au film; the curves reverted to the baseline. The experimental results demonstrated that the 3D matrix could reduce the nonspecific protein adsorption to the level below the detection limit of SPR. It was suitable for biochip application in a complex environment.

Repeatability and stability of SPR chips
After regenerating the SPR chip using glycine five times, the signal nearly reverted to its value before the injection, indicating that the antibodies were completely removed from the surface without a loss of platelet antigens. Figure 4 presents the typical binding curves reconstructed from typical SPR sensorgrams. The curves were nearly overlapping, indicating that the regeneration of the chip was reproducible and good for reuse.

Specificity and detection limit of the analyte
The specificity of the analyte was confirmed using the SPR technology. Figure 5A shows a typical SPR sensorgram of an injection of anti-HPA-1a antibody, where

Table 1. Platelet antibody detection results of 186 samples using the SPR and MAIPA methods.

SPR method	MAIPA method		Total
	Positive	Negative	
Positive	34	2	36
Negative	3	147	150
Total	37	149	186

χ^2 = 0.200, p = 0.655, (MAIPA - monoclonal antibody-immobilized platelet antigen, SPR - surface plasmon resonance).

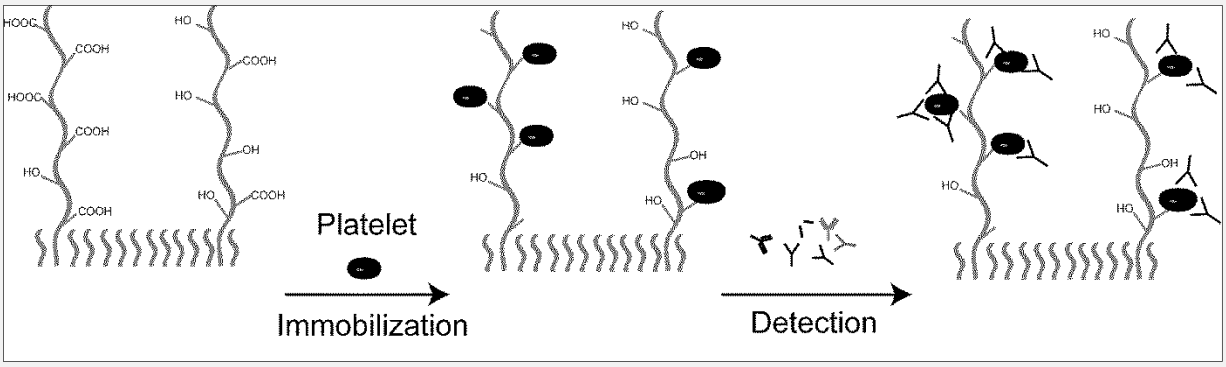

Figure 1. Schematic illustration for detecting platelet antibody using the SPR method.

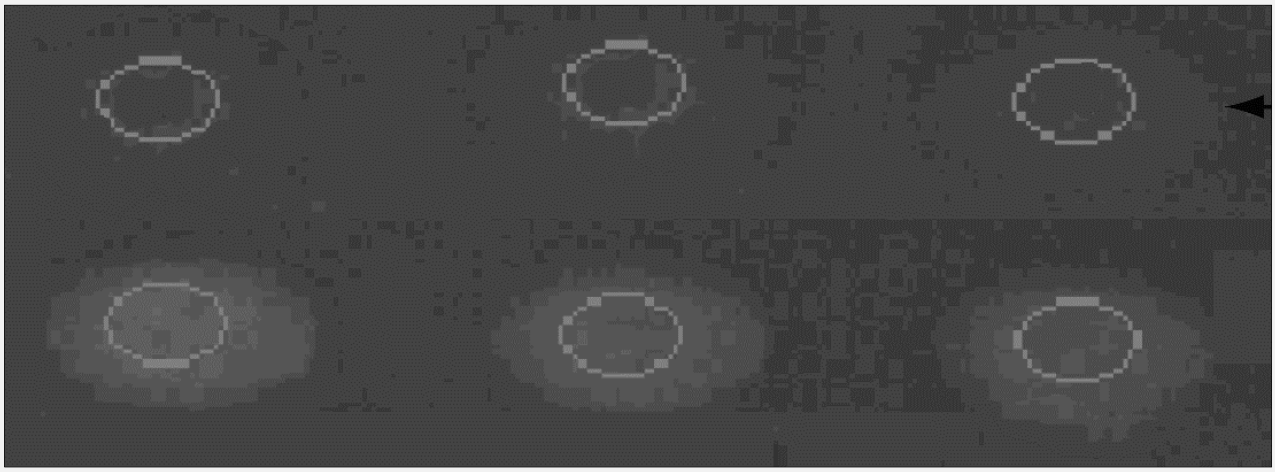

Figure 2. Results of coupling the PLT antigens on the SPR chip (PBS on the first row, platelet antigens on the next row).

Universal type O platelets were printed on the SPR chip. After incubating the platelets for 1 hour, the amino groups on the platelets interacted with the carboxyl groups on the modified chip by carboxyl group coupling reaction, and the platelets were coupled to the SPR chip.

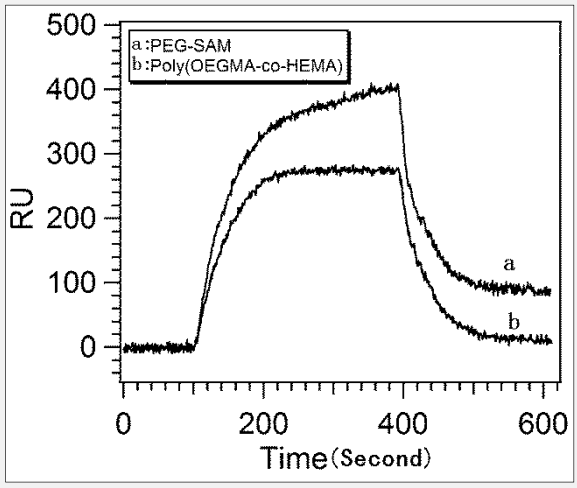

Figure 3. Typical SPR sensorgrams showing nonspecific adsorption from 10% serum to poly (OEGMA-*co*-HEMA) matrix; the curves reverted to the baseline.

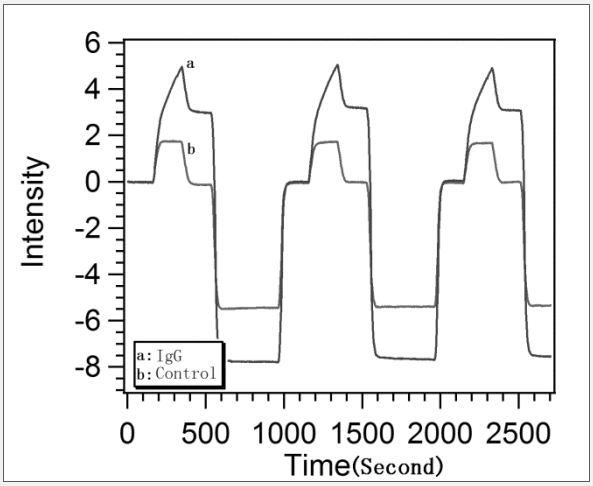

Figure 4. Repeatability test results of platelet antibody-positive samples (n = 3); 20 µL of positive samples in PBS (dilution 1:50) were injected at a rate of 2 µL/s.

The sample was replaced with PBS after the association phase of 400 seconds. The dissociation phase was then recorded for 400 seconds. This procedure was repeated three times, which showed the same SPR value. In all experiments, the SPR chip was regenerated with 2 mL of 10 mM glycine (pH 2.0) after each run.

serum samples containing anti-HPA-1a antibody interacted specifically with platelet antigen HPA-1a (shown as curve c), but nonspecifically with NS1 antibody ligand (shown as curve d). Figure 5B shows an SPR sensorgram for a similar experiment, where NS1 was used as the analyte. It was a clear, specific response of the

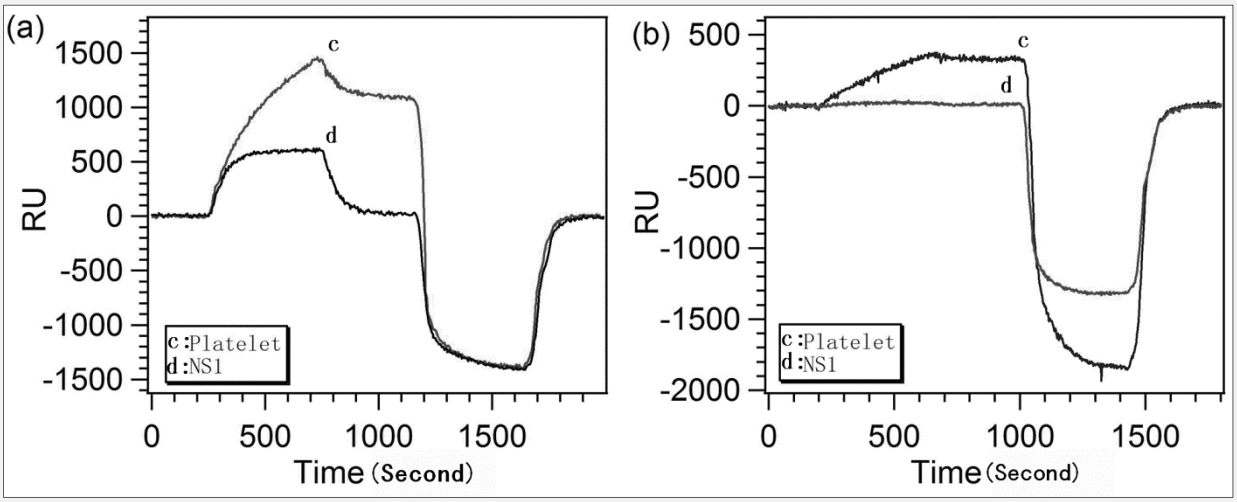

Figure 5. Specific binding was evaluated by injecting specific antibody into the SPR sensor chip. (a) Serum sample containing anti-HPA-1a; (b) serum sample containing NS1 antibody.

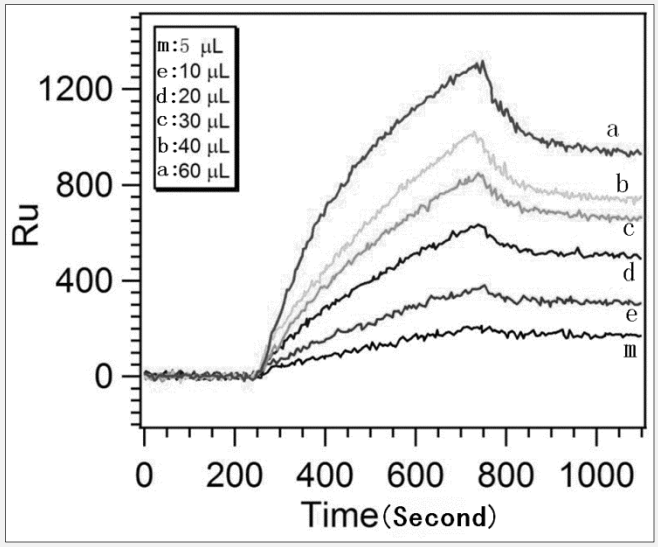

Figure 6. SPR response to a series of concentrations of positive sample.

The curves showed that the SPR response values decreased with the decrease in concentration.

two analytes in the respective ligand spots. Figure 6 shows the change in SPR shift rates at various concentrations. The curves show that the SPR response values decreased with the decrease in concentration. The LOD of the SPR biosensor for platelet antibody was estimated to be 50 ng/mL.

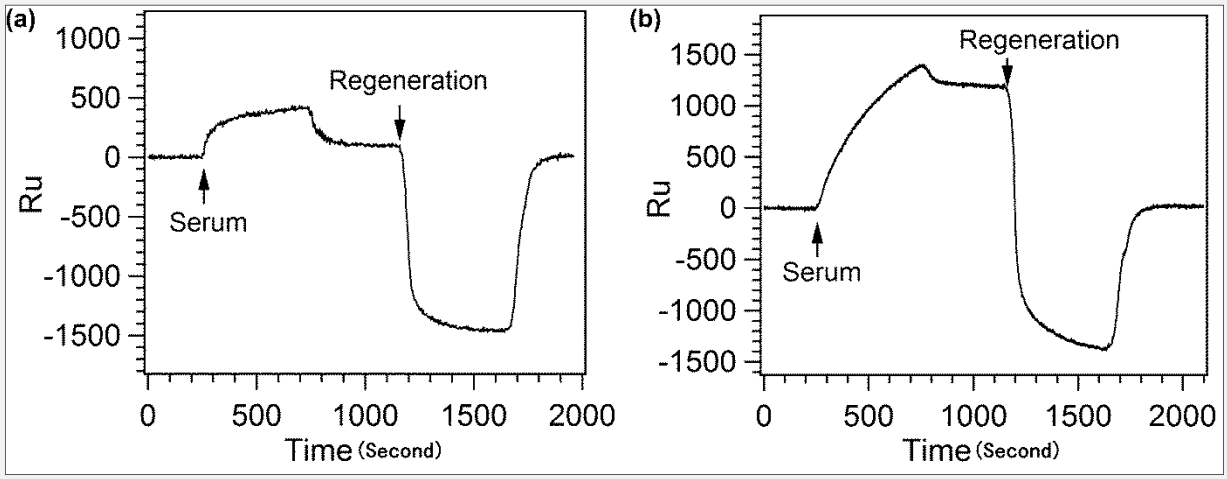

Figure 7. (a) Detection results of negative serum samples; 100 µL of serum samples in PBS (dilution 1:10) were serially injected at a rate of 2 µL/s. The sample was replaced with PBS after the association phase of 400 seconds. The dissociation phase was then recorded for 400 seconds; the curves reverted to the baseline. In all experiments, the SPR chip was regenerated with 2 mL of 10 mM glycine (pH 2.0) after each run. (b) Detection results of positive serum samples; 100 µL of serum samples in PBS (dilution 1:10) were serially injected at a rate of 2 µL/s. The sample was replaced with PBS after the association phase of 400 seconds. The dissociation phase was then recorded for 400 seconds. The curves did not revert to the baseline, showing the same SPR resonance value. In all experiments, the SPR chip was regenerated with 2 mL of 10 mM glycine (pH 2.0) after each run.

Comparison of platelet antibody screening using SPR and MAIPA assays

The aim of this study was to evaluate the sensitivity and specificity of the SPR technology and compare the results with those of the MAIPA assay. A total of 186 sera collected from hospital patients were tested using the SPR technology. The results were further investigated using the MAIPA assay performed according to the manufacturer's instructions. The results are shown in Table 1. Of the 186 sera tested, 36 were found to be positive and 150 were found to be negative for platelet antibody using SPR. However, 37 sera samples were found to be positive and 149 were found to be negative for platelet antibody using MAIPA. The overall concordance between SPR and MAIPA for detecting platelet antibody was 97.3% with 34 concordant positives and 147 concordant negatives. The sensitivity and specificity of the SPR assay were 92% and 98.7%, respectively. These results indicated that the sensitivity and specificity of SPR and MAIPA were similar (chi-square test, $\chi^2 = 0.200$, $p = 0.655$). These data further supported the sensitivity and specificity of the SPR technology, which was capable of detecting the platelet antibody in serum directly.

Effect of cross-matching test

The direct technology for platelet compatibility testing was employed for clinical samples. Figure 7 shows the typical SPR sensorgrams for negative and positive sera, respectively. A cross-matching test using the SPR technology was performed for 36 patients with platelet antibody positivity and matched platelet transfusion was conducted from donor platelets. After a 24-hour transfusion, 28 cases showed CCI > 4.5 according to the clinical follow-up analysis, indicating effective infusion, and the patients were in good condition; however, the other eight cases showed CCI < 4.5, implying other common diseases resulting in clinical PTR.

DISCUSSION

It is estimated that the refractory state of approximately 25% - 30% of patients undergoing multiple platelet transfusions is caused by platelet-specific antibodies [9]. The specific and sensitive detection of platelet-specific antibodies is essential for adequate treatment of patients suffering from multiple platelet transfusions. One strategy to solve the issue of autoimmune factors for PTR may be to choose the fully matched platelet infusion of human platelet-specific antigen (HPA) and HLA for donors and recipients [10,11]. An alternative is to directly supply compatibility platelets for recipients by a cross-matching test. The former needs to establish a known HLA and HPA gene library for donors, but the procedure is large, expensive, and time consuming. The

second method lacks these limitations, but it needs a simple and applicable method to reduce alloimmunization and refractory rates [12,13]. A number of methods have been developed for detecting platelet-specific antibodies, such as monoclonal antibody-immobilized PLT antigen and ELISA. The low cost of the MAIPA method allows many laboratories to use platelet antibody screening and cross-matching. However, all the methods used at present for PLT antibodies include washing steps, leading to the dissociation of low-avidity antibodies. Furthermore, the lifespan of indicating erythrocytes is short. These tests are insensitive due to high background values and involve a complex operational process.

A novel SPR method allowing direct analysis of platelet antibodies without any washing steps, use of capture MoAbs, or a labeling step was devised in this study to avoid these problems. This study aimed at developing a simple and rapid system for monitoring platelet antibodies and selecting effective platelets for patients refractory to random donors as a consequence of platelet alloimmunization [14]. An SPR-based method combined with surface chemistry was developed to overcome the aforementioned limitations and accomplish the goal. A detailed description of preparing a 3D poly (OEGMA-*co*-HEMA) matrix as a platform for SPR biosensor was provided in this study. This matrix had the advantages of both nonfouling property and high immobilization capacity. The results indicated that the 3D matrix was suitable for detecting platelet antibodies in a serum sample. The results were in accordance with those derived from MAIPA. The overall concordance between SPR and MAIPA for detecting platelet antibodies was 97.3%. The results demonstrated that the SPR method could be a valuable tool for detecting platelet antibodies and cross-matching for platelet transfusion. This work was significant owing to the following aspects. First, this study afforded a novel chemical strategy for designing an ideal matrix for biosensor applications in complex media, such as serum. Second, it described a novel assay based on SPR monitoring of platelet antibodies in serum samples and cross-matching; IgG was not purified from sera. Third, the procedure was not time consuming and could be completed in 3 hours.

Despite the advantages of the SPR method over other assays, it had a few drawbacks. First, the purity and stability of platelet antigens are of prime importance for platelet antibody detection [4]. Second, the 3D matrix should be prepared to prevent nonspecific protein adsorption, which is suitable for biochip application in a complex serum environment. Moreover, due to the defect in the type O platelet antigen spectrum, leakage of some rare platelet antibodies may occur during detection. Finally, SPR chip regeneration is risky. These limitations need to be resolved in the future.

CONCLUSION

This study showed that the SPR method could facilitate platelet compatibility testing. The ability of the developed SPR biosensor to directly detect platelet antibodies in serum samples without the use of any label illustrates the potential of this technology for medical diagnostics [15,16]. Further studies are required to establish the impact of the new method in determining the binding characteristics of platelet antibodies as predictive parameters for the clinical severity of PTR.

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Declaration of Interest:
All authors declared that they have no conflict of interest.

References:

1. Hatakeyama N, Hori T, Yamamoto M, et al. Platelet transfusion refractoriness attributable to HLA antibodies produced by donor-derived cells after allogeneic bone marrow transplantation from one HLA-antigen-mismatched mother. Pediatr Transplant 2011; 15:E177-82 (PMID: 20880381).

2. Lin JS, Lyou JY, Chen YJ, et al. Screening for platelet antibodies in adult idiopathic thrombocytopenic purpura: a comparative study using solid phase red cell adherence assay and flow cytometry. J Chin Med Assoc 2006;69:569-74 (PMID: 17182350).

3. Thibaut J, Merieux Y, Rigal D, Gillet G. A novel assay for the detection of anti-human platelet antigen antibodies (HPA-1a) based on peptide aptamer technology. Haematologica 2012;97: 696-704 (PMID: 22133781).

4. Socher I, Andrei-Selmer C, Bein G, Kroll H, Santoso S. Low-avidity HPA-1a alloantibodies in severe neonatal alloimmune thrombocytopenia are detectable with surface plasmon resonance technology. Transfusion 2009;49:943-52 (PMID: 19175553).

5. McDonnell JM. Surface plasmon resonance: towards an understanding of the mechanisms of biological molecular recognition. Curr Opin Chem Biol 2001;5:572-7 (PMID: 115789320).

6. Olmsted IR, Kussrow A, Bornhop DJ. Comparison of free-solution and surface-immobilized molecular interactions using a single platform. Anal Chem 2012;84:10817-22 (PMID: 23173653).

7. Ma H, He J, Liu X, Gan J, Jin G, Zhou J. Surface initiated polymerization from substrates of low initiator density and its applications in biosensors. ACS Appl Mater Interfaces 2010;2:3223-30 (PMID: 20942448).

8. Bakchoul T, Meyer O, Agaylan A, et al. Rapid detection of HPA-1 alloantibodies by platelet antigens immobilized onto microbeads. Transfusion 2007;47:1363-8 (PMID: 17655579).

9. Slichter SJ, Davis K, Enright H, et al. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. Blood 2005; 105:4106-14 (PMID: 15692069).

10. Xia WJ, Ye X, Deng J, et al. [Study of the platelet GP specific antibodies and HLA antibodies expression in platelet transfusion refractoriness patients]. Zhonghua Xue Ye Xue Za Zhi 2010;31: 594-8 (PMID: 21122318).

11. Heikal NM, Smock KJ. Laboratory testing for platelet antibodies. Am J Hematol 2013;88:818-21 (PMID: 23757218).

12. Bonstein L, Stemer G, Dann EJ, Zuckerman T, Fineman R, Haddad N. Alloimmune platelet transfusion refractoriness circumvented by allogeneic stem cell transplantation. Transfusion 2013; 53:1019-23 (PMID: 22897696).

13. Wiita AP, Nambiar A. Longitudinal management with cross-match-compatible platelets for refractory patients: alloimmunization, response to transfusion, and clinical outcomes (CME). Transfusion 2012;52:2146-54 (PMID: 23113654).

14. Moncharmont P, Rigal D. [Prevalence of platelet-specific antibodies in the recipients of platelet units with transfusion adverse event]. Transfus Clin Biol 2012;19:333-7 (PMID: 23103423).

15. Fontao-Wendel R, Silva LC, Saviolo CB, Primavera B, Wendel S. Incidence of transfusion-induced platelet-reactive antibodies evaluated by specific assays for the detection of human leucocyte antigen and human platelet antigen antibodies. Vox Sang 2007; 93:241-9 (PMID: 17845262).

16. Peterson JA, Kanack A, Nayak D, et al. Prevalence and clinical significance of low-avidity HPA-1a antibodies in women exposed to HPA-1a during pregnancy. Transfusion 2013;53:1309-18 (PMID: 23003125).