Sensors & Actuators: B. Chemical 273 (2018) 703–709

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Polymethyl methacrylate (PMMA) point of care for ABO-Rh(D) blood typing

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ARTICLE INFO

Keywords:
Blood grouping
POC
PMMA
Strip test
Surface modification

ABSTRACT

Polymethyl methacrylate (PMMA) based point of care (POC) for ABO-Rh(D) blood grouping by the naked eye was investigated. PMMA substrate was covalently linked with carboxyl methyl dextran (CMD) and coupled to the blood group specific antibodies anti A, anti B, and anti D, which were patterned into "A", "B", and "+". The red read out color originated from the color of hemoglobin in the RBCs. The major human blood group, ABO-Rh (D), was successfully sorted using these patterns. PMMA strip-based assays for blood grouping by the naked eye resulted in correct blood group identification of all 74 samples in comparison to slide agglutination test. The determination of captured RBC Rh(D) required a longer reaction time with immobilized anti D antibody on the PMMA substrate than other blood groups to produce sufficient color for unambiguous visual identification. The proper RBC concentration was found to be higher than 1% v/v for naked eye detection. The dilution of blood sample is playing a crucial role in reducing interference from blood proteins during detection. A used of undiluted blood sample for the testing should be avoided. The proper blood dilution was found to be 10% or 1:10 dilution. The proposed technique presents a quick, cheap, simple, and almost instantaneous assay for blood grouping.

1. Introduction

Hemagglutination is an essential technique used for blood typing where the agglutination reaction between specific antibodies against the red blood cell antigen (RBC) is induced in the liquid phase [1]. This assay has long been used for blood typing via the serological method. Blood groups have been divided into thirty five systems by the International Society of Blood Transfusion (ISBT) [2]. ABO and RhD are two of the most clinically important major blood types in the field of transfusion medicine because they can lead to fatal hemolytic transfusion reactions (HTRs) or hemolytic disease in fetuses and newborns (HDFN) [3]. In the clinical laboratory, tube or micro-column and microplate agglutination are two of the most frequently used assay for blood typing [4]. Recently, many articles on blood typing techniques have been published, such as flow cytometry-based assays [5], genes sequencing of DNA [6,7], microarray genotying [6], etc. These technologies provide highly specific, sensitive, and reliable blood phenotyping. However, they suffer from the use of special laboratory equipment, experienced laboratory personnel, and high costs for initial setup [1,4].

Point-of-care (POC) testing is often required for blood transfusion, where blood type testing can be done at the bed side or at the point of care. The slide agglutination assay, which involves mixing of a RBC specific antibody with RBC on the slide and observing the agglutination reaction, is an inexpensive and rapid blood typing technique which is considered as an early POC testing option in the field of transfusion medicine. The technique requires a skilled technician to interpret the results [8]. Recently, POC devices for blood typing have been reported. This technique is developed based on the principles of simple, rapid, easy to handle, and reliable detection for blood grouping. Bedside compatibility testing is needed during blood typing in fieldwork, emergency cases, and in locations that laboratory facilities are not available, such as rural areas, military facilities, and in local areas that lack a laboratory [9]. A microfluidic, paper-based analytical device (μPAD) was first published by Whiteside et al., which used a photolithographic technique to fabricate hydrophobic and hydrophilic areas for the reaction [10]. Paper-based devices for blood typing were

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https://doi.org/10.1016/j.snb.2018.06.074
Received 3 February 2018; Received in revised form 4 June 2018; Accepted 15 June 2018
Available online 18 June 2018
0925-4005/

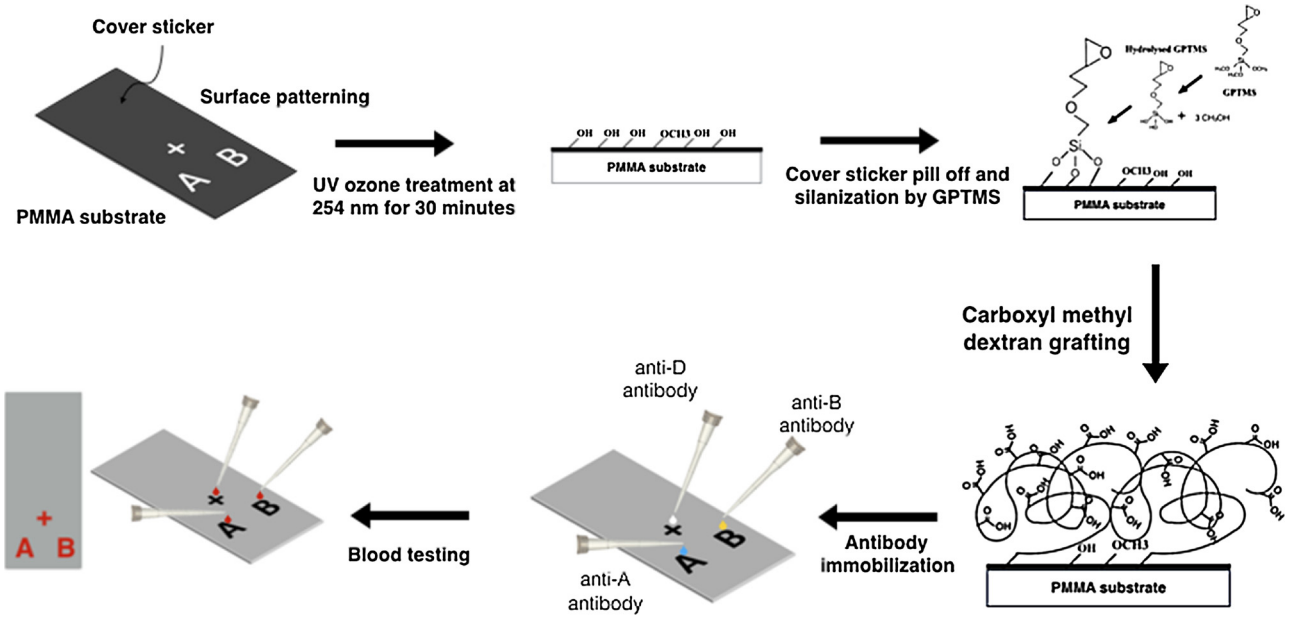

Fig. 1. The process of surface modification and patterning by UV-ozone treatment, silanization, carboxyl methyl dextran (CM-dextran) grafting on PMMA surface, antibody immobilization, and blood test, respectively.

fabricated by soaking filter paper with RBC specific antibodies that can induce the formation of a plasma separation band on paper by the agglutinated of blood [11]. Furthermore, they reported that RBCs fixed on paper embedded with antibodies recognized with them and thereby validated their proposed assay [12]. Although these assays are reliable and useful for blood grouping, they do not show explicit results [13,14]. The pale red color that is often observed originates from several layers of paper fiber stacked on top of each other. Moreover, these devices are difficult to handle and are prone to environmental damage, such as from humidity. An alternative to paper-based blood typing are more robust polymeric based devices [15,16] which are inexpensive, easily processed, and can be scaled up for mass production.

In this study, we develop a novel POC for ABO and Rh (D) blood typing fabricated by UV-ozone induced hydroxylation of PMMA into a pattern of "A", "B", and "+". Epoxy-silane was reacted onto hydroxylated PMMA yielding the epoxy terminated, which was reacted with dextran, and subsequently converted into carboxy methyl dextran (CMD), Fig. 1. The patterned CMD was reacted with anti A, anti B, and anti D generating the pattern of immobilized antibodies. Blood grouping can be recognized by the naked eye, as RBCs adhered on immobilized antibodies against RBC antigens form patterns "A" (immobilized anti-A), "B" (immobilized anti-B) for the ABO blood group, and "+" (immobilized anti-D) for the blood group RhD. A combination of the read out pattern represents the ABO-Rh(D) blood group. The following parameters were investigated: (1) optimization of antibody dilution to find a suitable dilution for immobilization, (2) the effect of RBCs dilution to find a suitable RBC concentration for detection, (3) the time-dependence of the antibody-antigen reaction of RBC capture to determine a suitable reaction time, and (4) accuracy of the test under optimized conditions compared to slide agglutination.

2. Materials and method

2.1. Materials

Commercial PMMA (Towapex, 1 mm thick and 100 cm × 100 cm, Bangkok Tokyo Polymer, Thailand), 3-glycidoxypropyltrimethoxysilane (GPTMS) (Z-6040, Dow Corning, Thailand), and deionized (DI) water (Millipore, USA) were used. In addition, 1- ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N- hydroxy-succinimide (NHS), glycine, casein, sodium chloride (NaCl), sodium phosphate dibasic (Na_2HPO_4), potassium chloride (KCl), potassium hydroxide (KOH), potassium phosphate monobasic (KH_2PO_4), 2-Aminoethanol (ethanolamine), and StabilCoat®, an immunoassay stabilizer, were purchased from Sigma-Aldrich (Singapore). Dextran T500 with a molecular weight of 500 KDa was purchased from Pharmacosmos (Denmark). Antibodies were purchased from the National Blood Centre, Thai Red Cross society (Thailand), including anti A monoclonal IgM antibody (Lot: 57,014), anti B monoclonal IgM antibody (Lot: 57,033), and anti D (Rh) monoclonal IgG/IgM mixed antibodies (Lot: 57,011). A total of 74 RBC samples in ethylenediamine-tetraacetic acid (EDTA) blood collecting tubes were provided by Blood Bank Laboratory, Department of Pathology, Faculty of Medicine-Ramathibodi Hospital. Blood grouping was identified by the Blood Bank laboratory using micro-column (gel) and the slide agglutination assay.

2.2. Surface modification and patterning

PMMA sheet with a surface protected paper layer was laser cut into 2.5 cm x 7.5 cm strips by LASER cutting machine with 50 W of LASER power and 0.5 mm/s of speed. The paper layer that covers on the surface of PMMA sheet was cut by LASER cutting machine with 20 W of LASER power and 10 mm/s of speed as "A", "B", and "+". After that, the patterns "A", "B", and "+" were made by peel off the protecting layer as shown in Fig. 1. The substrate was washed with water until free of debris. The slide was sonicated in 10% (v/v) KOH (Ultrasonic cleaner 575TH, Crest ultrasonics Crop.) for 15 min at room temperature, rinsed thoroughly with DI water, and dried with a stream of nitrogen gas. UV-ozone (model 42, UVO-CLEANER®, Jelight) treatment was carried out by irradiation of the PMMA for 30 min to generate residual hydroxyl groups on the surface. The substrate was then immersed in a solution of 10% (v/v) GPTMS in 95% ethanol in water for 6 h at room temperature and incubated overnight in an oven at 70 °C [17]. GPTMS was grafted by the condensation reaction between the surface OH groups and the silanol groups of hydrolyzed GPTMS to create epoxy-modified PMMA. Dextran was grafted by immersing the substrate in dextran solution (0.1% of 100 mM NaOH) for 18 h at room temperature, washing with DI water, and drying under a nitrogen stream. The substrate was immersed in a 100 mM solution of bromoacetic acid ($BrCH_2COOH$) to generate the carboxydextran [18]. Finally, the carboxyl methyl dextran (CM-