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Hemagglutination detection for blood typing based on waveguide-mode sensors

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ABSTRACT

ABO and Rh(D) blood typing is one of the most important tests performed prior to blood transfusion. Although on-site blood testing is desirable for expedient blood transfusion procedure, most conventional methods and instruments lack the required usability or portability. Here, we describe a novel method, based on the detection of hemagglutination using an optical waveguide-mode sensor, for on-site use. The reflectance spectrum of blood alone and that of blood mixed with antibody reagents was measured using the waveguide-mode sensor. Differences in reflectance by agglutinated and non-agglutinated blood samples were observed at the bottom of the spectral dips; due to differences in the manner in which red blood cells interacted with the surface of the sensor chip. Following the addition of the antibody, blood types A, B, O, and AB were clearly distinguishable and Rh(D) typing was also possible using the waveguide-mode sensor. Furthermore, the waveguide-mode-based measurement exhibited the potential to detect weak agglutination, which is difficult for human eyes to distinguish. Thus, this method holds great promise for application in novel on-site test instruments.
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1. Introduction

Blood type, represented by the ABO and Rh(D) systems, is an immunoreactive feature of red blood cells (RBCs) that is determined by the antigenic moieties of sugar chains on the RBC surface. Determination of the blood type is especially important before blood transfusion, because transfusion of mismatched blood types can lead to hemolysis within patient's blood vessels, which mediated by antibodies to the RBCs in blood plasma. Blood typing tests have traditionally been conducted manually, using techniques such as slide and tube agglutination tests. In the last 20–30 years, fully automated blood testing instruments have been developed and these are operational at blood centers and major hospitals. These instruments have advantages such as high throughput and high sensitivity. However, these advantages are somewhat offset by the large size and high cost of the instruments. These are major drawbacks, especially because on-site blood testing is an important requirement during an emergency or natural calamity. Development of a portable, low-cost, and sufficiently sensitive instrument for blood typing is therefore required to make on-site blood testing feasible.

Agglutination of RBCs (hemagglutination) is caused by an immune reaction between the RBCs and antibodies against the corresponding blood type. In conventional blood typing methods, hemagglutination caused by antibodies is detected by human eyes or by imaging techniques. Alternate methods of blood typing using optical techniques have also been reported. Quinn et al. first reported the use of a surface plasmon resonance (SPR) sensor for blood typing [1]. The SPR sensor is a sensitive biosensing instrument based on electrical field enhancement by SPR excitation [2–4]. SPR-based blood typing has been previously performed using the Biacore system [1,5,6] or an SPR imaging technique [7]. Narayanan et al. have reported a technique for absorbance measurement-based blood typing [8]. This group reported the detection of a weak agglutination reaction of A_2 subtype and weak-D. Robb et al. demonstrated fluorescence-based blood typing on a planar microarray

Abbreviations: RBCs, red blood cells; SPR, surface plasmon resonance; PBS, phosphate-buffered saline; LED, light-emitting device.

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platform [6]. In another approaches, blood typing have been performed using a microchannel [9–11] or paper [12,13].

In this study, we propose a blood typing method using an optical waveguide-mode sensor that detects changes in absorbance properties of the blood sample. The waveguide-mode sensor utilizes electric field enhancement in the sensor chip, similar to the SPR sensor, and is, therefore, more sensitive than a reflectance absorption spectrometer. The waveguide-mode sensor-based method provides a simple and sensitive blood typing technique, and utilizes a portable, small-sized instrument. Hemagglutination detection using the waveguide-mode sensor in this study was examined both theoretically and experimentally, using human blood and blood typing antibody reagents. ABO and Rh(D) blood typing was conducted using the waveguide-mode sensor-based hemagglutination detection method.

2. Materials and methods

2.1. Materials

Reagents were used as received with no further purification. Human whole blood containing the anticoagulant ethylenediaminetetraacetic acid dipotassium salt (EDTA 2K) was purchased from Tennessee Blood Service. Tubes containing fresh blood samples were shipped by air and used within 2 weeks. Before using the sample, we checked for hemolysis and used only non-hemolyzed samples. Blood types were confirmed by the supplier. Monoclonal anti-A and anti-B reagents (Neo Kokusai) were purchased from Sysmex Corporation. Monoclonal anti-D reagent (Monoclonal Anti-D Wako), Rh control reagent, and phosphate-buffered saline (PBS) was purchased from Wako Pure Chemical Industries, Ltd. Refractive index matching liquid for fused silica (Fused Silica Matching Liquids 50350) was purchased from Cargille Laboratories. Waveguide-mode sensor chips were supplied from Shin-Etsu Chemical Co., Ltd.

2.2. Experimental details

Hemagglutination was detected using a spectral readout-type waveguide-mode sensor [14]. Fig. 1(a) shows a schematic drawing of the experimental setup. The waveguide-mode sensor is based on the Kretschmann configuration containing a white light-emitting device (LED), collimator lens, polarizer, trapezoidal prism, sensor chip, and spectrometer. All the components of the waveguide-mode sensor are enclosed within a $30 \times 20 \times 15$ cm^3 box, making the device portable. The sensor chip consists of a surface SiO_2 glass waveguide layer and an embedded silicon reflectance layer on silica glass [15]. The chips are placed on the prism at base angle 38°, which corresponds to an incident angle of 70.6° and the index matching liquid is introduced in between. S-polarized incident light from the LED is irradiated onto the chip from the bottom, and the spectrum of the reflected light is measured by the spectrometer. Dips in reflectance were observed because of excitation of waveguide-mode propagation. By monitoring alteration in the dips, changes in the complex refractive index in the vicinity of the sensor chip surface can be detected. The wavelength of the spectral dip is controlled by adjusting the thickness of the SiO_2 waveguide layer and the silicon reflectance layer. In this study, the thickness of the waveguide layer and reflectance layer of the chips were approximately 360 and 45 nm, respectively. Those chips exhibited spectral dip around 540 nm during blood measurements, which were calibrated as per the peak wavelength for the RBC absorbance spectrum. For the hemagglutination detection measurements, a drop of the blood sample was directly placed onto the calibrated chip without surface modifications.

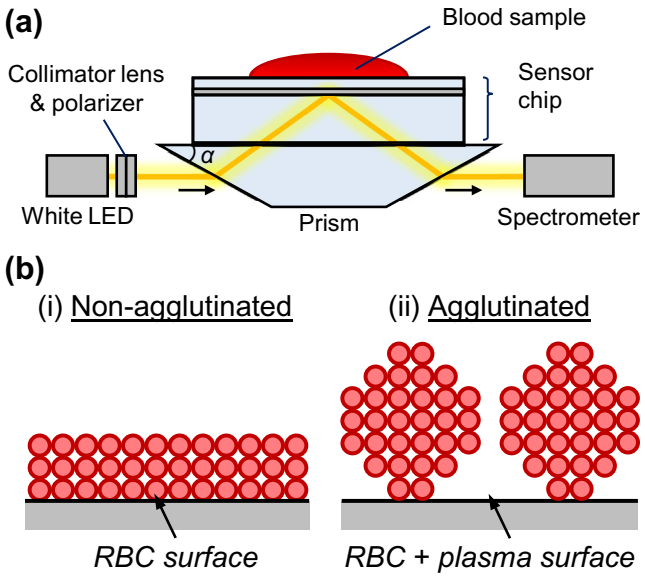

Fig. 1. (a) A schematic diagram of the experimental setup to detect hemagglutination. α represents base angle of the trapezoidal prism. (b) Schematic diagrams of the sensor chip surface with blood samples containing (i) non-agglutinated and (ii) agglutinated RBCs. Red circles represent individual RBCs.

Fig. 1(b) shows a schematic diagram of the sensor chip surface during analysis of blood samples. In case of non-agglutinated blood samples, RBCs will sediment uniformly and form a dense layer on the chip surface. By contrast, agglutinated RBCs (e.g., blood A mixed with anti-A) will sediment discretely onto the chip surface. Consequently, according to the ratio of contact area of RBCs to the surface, the complex refractive indices of the chip surface differ between the agglutinated and non-agglutinated blood samples. Since RBCs exhibit absorption around 540 nm while plasma does not, we focused on the change in extinction coefficient due to hemagglutination. The spectral response of the waveguide-mode sensor to hemagglutination was calculated using the transfer matrix method. To represent hemagglutination in the calculation, we assumed uniformly mixed layers that contained RBCs and plasma in various ratios. The extinction coefficient k of both RBCs and plasma, was calculated from the absorbance A as follows:

$$k = \ln 10 \frac{A\lambda}{4\pi d} \quad (1)$$

where λ is the wavelength and d is the optical path length. The absorbance was measured using NanoDrop 2000c (Thermo Fisher Scientific Inc.). The values of k of RBCs and plasma derived using the measured absorbance and Eq. (1) are shown in Supplementary Fig. S1. The k value of the mixed layer was derived from the weighted average k of RBCs and plasma according to the composition ratio. For the refractive index, n, a previously reported value for RBCs, $n = 1.40$, was used [16].

For detection of hemagglutination using the waveguide-mode sensors, samples of human whole blood were diluted with PBS to 5% of the RBC volume. First, the spectral responses for agglutinated and non-agglutinated blood samples were examined using the diluted blood and blood-antibody mixture in a 1:1 ratio by volume. Second, ABO forward blood typing was conducted using blood types A, B, O, and AB. Diluted blood was placed onto the sensor chip surface, antibody reagents were added and mixed gently by pipetting. The spectra of the blood and blood-antibody mixture were measured and compared to identify any antibody-dependent change. Rh(D) blood typing was also performed. All reactions and measurements were conducted at room temperature.

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