



Reuse of sensor chip using UV/ozone method for surface plasmon resonance biosensor

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Abstract

This work presents a novel method to reuse the gold (Au) sensor chip for surface plasmon resonance (SPR) spectroscopy. The SPR chip, used in ABO blood group typing, was regenerated with a functional layer and biomolecule layer on the SPR surface by UV/ozone method. The layer of used SPR consisted of a functional layer of carboxyl methylate dextran (CMD) and a layer of biomolecules (antibody and red blood cell (RBC)). After regenerating, the reused SPR chip was monitored by the SPR imager, including SPR image and SPR curve. The ability of the reused SPR chip was again tested in ABO blood typing and compared to that of the new SPR chip. For the construction of the blood group typing biosensor, the antibodies—anti-A and anti-B—were covalently immobilized on the CMD surface of the reused or new SPR chip. The RBCs—RBC A, RBC B, RBC AB, and RBC O—were passed over the immobilized antibodies' surface for analyzing blood types. The result of the SPR curve shifted left and equaled to that of the new SPR chip, and the obtained SPR images from the used/new SPR chip were similar. The SPR signal obtained from the reused SPR chip for blood typing was correctly grouped, same with the new SPR chip. Thus, this method is feasible to regenerate the surface of the SPR chip for its prolonged reuse.

Keywords: Reused SPR chip, Surface plasmon resonance (SPR), Blood group typing, Carboxyl methylate dextran (CMD)

1. Introduction

Surface plasmon resonance (SPR) spectroscopy is a powerful technique for label-free bioanalysis with real-time monitoring. SPR has been widely used in analytical biomolecule interactions such as antigen-antibody binding, DNA hybridization, whole cell detection, and so on [1-4]. In the SPR technique, the sensor chip (SPR chip) plays an important role on costly consumables for the construction of the SPR sensor. Generally, the SPR chip (a 50-nm gold (Au) coated on BK7) must be modified by a functional layer on the surface for biomolecular probe immobilization, such as self-assembled monolayer (SAM) or carboxyl methylate dextran (CMD), mostly [5-6]. However, the SPR sensor chip, after bioanalytical detection, can be reused by regenerating the analytes on their immobilized biomolecular probe surface. This capture layer, the immobilized biomolecular probe surface, is still retained. For example, in an immunosensor, the regenerating method uses an acid or alkaline solution for antigen-antibody interaction; this interaction would be broken. However, the reuse of the SPR sensor chip by regeneration relies on disturbing the bioactivity. This result will lead to the loss of bioactivity of the immobilized antibody in each number of regeneration time. Therefore, the developed methods will be important to regenerate the biomolecule layer and functional layer

because they will enable the prolonged reuse of the SPR chip. This will increase the cost-effectiveness of the SPR biosensor. Moreover, it will also allow the same SPR chip to be used for other bioanalytical applications instead of restricting its use to only a specific bioassay.

Several methods have been studied for the regeneration of the gold-coated substrate. As examples, Ref [7] can successfully use a piranha solution (concentrated H₂SO₄/30% H₂O₂ =3:1 v/v) to regenerate the 3-aminopropyltriethoxysilane (APTES) on the SPR sensor chip for use in the immunosensor of human fetuin A. Ref [8] regenerated the SAM of 6-mercapto-1-hexanol and adsorbed biomolecules on the gold surface using UV-photooxidation technique. Moreover, Ref [9] also used HCl solution and oxygen plasma to clean the APTES on the gold surface for detection of human fetuin A. It can be seen that these methods have developed to regenerate SAM or APTES for the prolonged reuse of the SPR chip. However, the SPR surface has not been reported for regeneration of CMD on the surface.

Thus, this work was to regenerate the CMD surface of the SPR chip used in ABO blood group typing (denoted as the used SPR chip). The used SPR chip was exposed to UV/ozone light for regeneration of CMD on the SPR surface. The reused SPR chip was characterized by SPR technique (SPR image/curve) for confirmation of the cleanliness of the

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SPR surface. The Kolmogorov Smirnov test was used as a tool for testing the consistence of the SPR curves obtained from used- and reused SPR chips. Moreover, the ability of the reused SPR chip for construction of SPR-biosensor ABO blood group typing was explored and compared to that of a new SPR chip.

2. Materials and methods

2.1. Materials and reagents

The SPR sensor chip (one inch in diameter) coated on BK7 with an adhesive layer of titanium and a 50-nm-thick layer of gold was purchased from Ssens (The Netherlands). 11-mercapto-1-undecanol (11-MUD), N-ethyl-N-(3-diethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) was purchased from Sigma. Dextran 500 kDa MW was purchased from Pharmacosmos A/S, Denmark, and Sodium Dodecyl Sulfate (SDS) was purchased from Thermo-Fisher scientific, USA. Anti-A (284 mg/dL) and Anti-B (382 mg/dL) were purchased from National Blood Centre, Thai Red Cross Society. The RBC sample was prepared as following report [10]. Other reagents were used without purification.

2.2 Biosensor construction for blood group typing

The SPR imager with multichannel flow cell, constructed by the National Electronics and Computer Technology Center (NECTEC), was used in this work. Details of SPR imager was similar to that reported previously [10]. The new or reused SPR chip were used as a surface to construct the biosensor for blood group typing. This biosensor was prepared according to the previous report [10] with some modifications. The experiment was carried out at room temperature. Phosphate buffer saline (137 mM NaCl, 2.7 mM KCl, 1.4 mM KH_2PO_4 , 8 mM Na_2HPO_4 , PBS pH 7.4) was used as a running buffer. The antibodies, anti-A and anti-B, were covalently immobilized on the CMD surface using a freshly mix of EDC/NHS solution. The carboxyl group on the CMD surface was firstly activated with 0.4/0.1 M of EDC/NHS solution for changing the carboxyl group to N-ester molecule. The antibodies (anti-A and anti-B) were diluted in sodium acetate buffer (10 mM CH_3COONa , pH 5.3) to 5% of concentration. The 100 μL of antibodies solution were passed through the activate surface with 10 $\mu\text{L}/\text{min}$. The remaining available active groups were blocked by 1.0 M of ethanolamine pH 8.5. The unbound or loosely bound antibodies on the surface were flushed out by 5 mM of NaOH followed by PBS running buffer. The experiments of blood group typing were performed by injecting 100 μL of a 5% RBC samples at a flow rate 10 $\mu\text{L}/\text{min}$. After sample injection for 12 min, during which the SPR signal reached a plateau, running buffer was passed over the surface to remove the unbound RBCs from the surface. The blood type was determined from the change of the SPR signal in the region of interest (ROI). Regeneration of the surfaces was performed using 10 mM of NaOH with 100 $\mu\text{L}/\text{min}$ of flow rate, followed by running buffer.

2.3. UV/ozone cleaning and surface characterization

The used SPR chip from blood group typing was carefully removed from the SPR instrument and rinsed with ethanol and deionized water (DI water). It was then immersed in 10% SDS solution for 24 hours (approximately) and rinsed with DI water and dried with nitrogen gas. It was

then exposed to UV/ozone in five-minute intervals for 30 minutes. After exposure in each interval, the SPR chip was rinsed with ethanol and DI water for several times and dried with nitrogen gas. The surface of the reused SPR chip was characterized by SPR technique and was compared to that of the new SPR chip. The characterization was performed by dropping 30 μL of DI water on the SPR surface. The region of interest (ROI) was selected about six to eight regions over the SPR surface. The SPR image was analyzed in every 10 nm of wavelength and chosen at a high-contrast image. The SPR curve was scanned at a 700–1,000 nm range with 5 nm of resolution. The reused surface was again used in the blood group typing. The biosensor for blood group typing was constructed as described above.

3. Results and discussion

The sensor surface was analyzed by both SPR curve and SPR imaging. Figure 1A shows an example of SPR curves for reusing the SPR sensor chip using the UV/ozone method. The SPR images at different states were shown in Figure 1A (top). The DI water was dropped on the SPR surface, and the SPR surface was then determined the SPR curves and SPR images. The ROIs (red rectangular shape in Figure 1A) were selected on the surface for measuring signal intensity as a function of wavelength from 700 nm to 1,000 nm at the fixed incident angle. The signal intensity was then plotted as an SPR curve. The SPR curve of the used SPR chip shifted to the right because of the adsorbed CMD and biomolecule layer on the surface (see Figure 1A, 1B). The SPR wavelength of the used SPR chip was approximately 810 nm. This was consistent to the SPR image of the used SPR chip that showed the line of adsorbed biomolecule on the surface. The used SPR chip was then cleaned by UV/ozone technique for 30 minutes. The SPR curve shifted left (see Figure 1A, reused SPR chip) and the line of adsorbed biomolecule obtained from the SPR imager disappeared as shown at the middle of the SPR image in Figure 1A. The SPR wavelength decreased from 810 nm to 760 nm because biomolecules and the CMD layer were eliminated by photodegradation of hydrocarbon using UV/ozone. The SPR curve of the reused SPR chip was compared to that of the new SPR chip. These SPR curves do not seem significantly different. These SPR curves between new SPR chip and reused chip were analyzed by Kolmogorov Smirnov test. The results found that the D value had 0.030, corresponding to significant level of $<0.001\delta$. This suggests that these curve is similar or is not different significantly. This suggests that this curve is similar or is not different significantly. This implies that the biomolecule layer and CMD layer were removed from the surface. Moreover, the optimization of exposure time for UV/ozone treatment was 30 minutes (data not shown).

Figure 2 shows the SPR signal of immobilized antibodies on the new and reused SPR surface. The 5% of anti-A and anti-B were covalently immobilized on the sensor surface. The SPR signal of the new sensor chip was 1,720 and 1,250 μRIU for immobilized anti-A and anti-B, respectively. In the case of the reused SPR chip, the SPR signal was 2,050 and 1,270 μRIU for immobilized anti-A and anti-B, respectively. It can be seen that the SPR signals obtained from the new and reused SPR chip were similar or were not significantly different. Moreover, the control was the surface without an immobilized antibody for testing the nonspecific binding signal. The immobilized antibody surfaces were further used to analyze the blood typing.

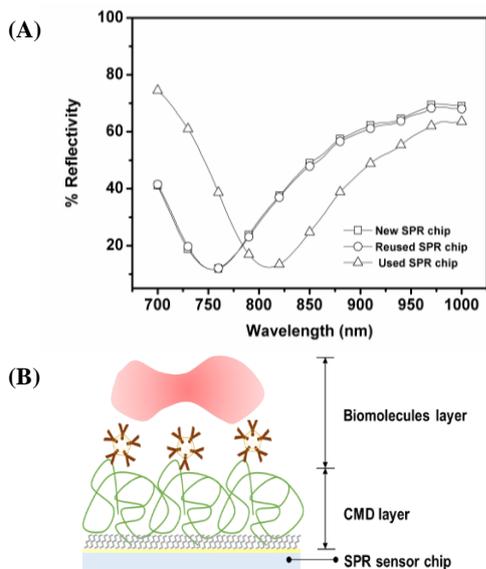


Figure 1 (A) SPR curves of the new and reused SPR chip. (B) Schematic of used SPR chip.

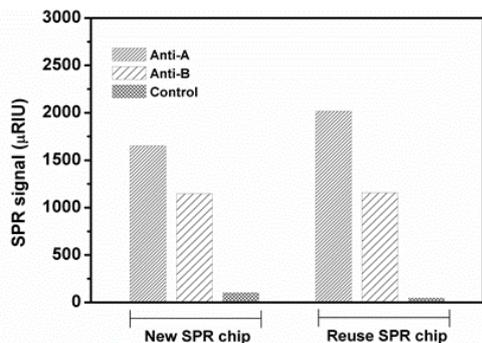


Figure 2 SPR signals of immobilized antibodies on the surface.

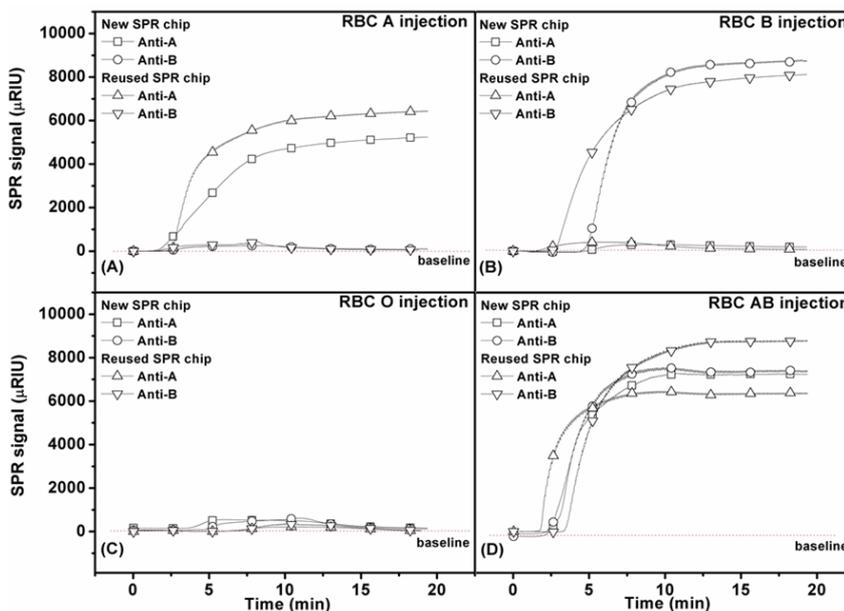


Figure 3 SPR sensorgram for analyzing blood typing. (A) RBC A injection. (B) RBC B injection. (C) RBC O injection. (D) RBC AB injection.

Figure 3 shows the example of the SPR sensorgram for detection of RBCs. The RBCs were passed over the immobilized antibodies' surface. The specific binding would occur when the anti-A or anti-B can capture antigen A or B on their membranes leading to an increase of SPR signal. Figure 3A shows the injection of RBC A over the immobilized antibody surfaces. The SPR signal dramatically increased and reached a steady state because of capturing RBC A by the immobilized anti-A surface on both the new and reused surfaces (only the immobilized anti-A surface, Figure 3A). However, the signal obtained from the new sensor chip was higher than that of the reused sensor chip. This is because of the different amount of immobilized antibody on the surface. The nonspecific binding was also observed on the immobilized anti-B surface. The signal of nonspecific binding was negligible. It can be seen that the reused SPR chip can be used for detection of RBC A as well as the new SPR chip. In the case of RBC B, the SPR result was similar to the detection of RBC A. The SPR sensorgram presented that the signal of RBC B injection was increased only on the immobilized anti-B surfaces (for both the new and reused surfaces, see Figure 3B). For the RBC O injection in Figure 3C, the SPR signal was equal to the baseline because of the absence of antigen A or B on the RBC membrane. The SPR signal obtained from the injection of RBC AB was shown in Figure 3D. The SPR signals shifted increases for both immobilized anti-A and anti-B surfaces because the RBC AB had both antigens A and B on the RBC membrane. Blood typing can correctly group in each blood type with the reused SPR chip equipped with the SPR biosensor. Furthermore, the signals from the new SPR chip were similar to those of the reused SPR chip. These results suggest that the ability of the reused SPR chip is not significantly different compared to that of the new SPR chip.

4. Conclusions

The used SPR chip was successfully cleaned by using the UV/ozone method for reusing the SPR chip. The SPR chip was measured by SPR image/curve to confirm the

elimination of the biomolecule layer and CMD layer on the used SPR surface. The optimization of exposure time was 30 minutes for removing those layers out of the SPR surface by UV/ozone. The reused SPR chip was also used in the application of blood group typing. The results found that the reused SPR chip can be used to analyze blood groups as well as the new SPR chip. The limitation of this work was the loss of Au layer on the glass substrate after a few times of recycles. Moreover, the other techniques for surface characterization should be used to investigate the cleanliness of the reused surface.

5. Acknowledgements

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6. References

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