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Short communication

Detection of gastric carcinoma-associated antigen MG7-Ag in human sera using surface plasmon resonance sensor

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ABSTRACT

Background: MG7-Ag is a kind of gastric cancer-specific tumor-associated antigen and has been investigated to serve as a marker of gastric cancer for early diagnosis. *Methods*: Surface plasmon resonance (SPR) sensor was used for the detection of MG7-Ag in the sera of gastric cancer patients to develop an innovative, simple and rapid assay method for early diagnosis. The specific monoclonal MG7 antibodies were used as capture and detection receptors which were immobilized on the surface of SPR sensor chips for MG7-Ag identification in the human sera. The measurements include 9 cases of gastric cancer patients and 2 cases of healthy blood donors and a MKN45 cancer cell lysate solution sample for positive control. *Results*: The binding of MG7-Ag onto the sensor surface was observed from SPR spectra. The sera of most gastric cancer patients revealed much higher expression level of MG7-Ag than healthy human sera did in SPR measurement. *Conclusion*: The initial results demonstrate that the SPR biosensor has the potential for its application in the early diagnosis of gastric cancer. However, more tests need to be done to confirm the detection limitation and the criterion for cancer risk evaluation in early diagnosis.

1. Introduction

Gastric carcinoma is one of the most prevalent malignant tumors in China and the second most common cause of cancer-related death in the world [1–3]. It has a high mortality rate because of the lack of effective methods for early diagnosis. The gastric cancer patients in early stage generally have no clinical symptoms. Once diagnosed, most of the patients are at advanced stages. In order to improve the survival rate of gastric cancer patients, many efforts have been made to explore simple and practical methods for early detection of gastric cancer [4,5].

Several monoclonal antibodies had been produced by immunizing the BALB/C mice directly with poor-differentiated gastric cancer cell line MKN-46-9 [6], among which MG7-Ab is of the highest sensitivity and specificity for detecting gastric cancer. The correspondent antigen MG7-Ag in the human serum is a kind of gastric cancer-specific tumor-associated antigen. It was found expressed in most gastric carcinoma tissues and in the sera of 82.8% patients with gastric cancer [8]. Thus, it might serve as a marker of gastric cancer for early diagnosis. At present, several methods have been investigated for immunoassay of MG7-Ag using antibody MG7-Ab, such as immunopolymerase chain reaction (Immuno-PCR) [7], immunohistochemistry stain (ABC) [8], and enzyme-linked immunosorbent assays (ELISA) [9]. However, these methods require labeling, and are time-consuming. Compared with the above methods, SPR-based assays need no labels and have no requirement of laborious sample preparation and are relatively less costly and less time-consuming.

Surface plasmon resonance (SPR) is an optical phenomenon occurred in total internal reflection of light at a metal film–liquid interface [10]. When the incident light is totally reflected, a component of the incident light momentum, the so-called evanescent wave, penetrates into the liquid medium near the metal (generally Au) surface. The evanescent wave interacts with surface plasmon (longitudinally oscillating free electrons) in the thin metal film surface. When SPR occurs, energy from the incident light is absorbed to the metal film, resulting in a decrease in light intensity. When the angle of incidence light is fixed, the resonance phenomenon occurs only at a precisely defined wavelength which is dependent on the refractive index of the medium adjacent to the metal surface. The refractive index changes in direct proportion to the mass and the dielectric permittivity of the medium present. If antibodies are immobilized on the metal surface, the corresponded antigens would specifically bonded on the surface when the surface is torched with liquid samples. The binding process can be observed by monitoring the SPR wavelength which depends on the amount of antibody–antigen binding [11]. The SPR biosensor is sensitive to the changes in the thickness or refractive index of

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biomaterials at the interface between a thin Au film and an ambient medium. Thus, using antibodies specific to pathogens of interest, it is able to characterize biomolecular interactions on the surface in real time without labeling and to measure the amount of pathogenic bacteria present in a sample by measuring the change in refractive index [12]. SPR methods have also been employed to investigate the thermodynamics of sugar ligand–lectin (R) interactions and the screening of lectin sources [13,14]. In our work, a SPR device is established for innovative, simple and rapid detection of MG7-Ag in human sera. To our knowledge, this is the first attempt to apply the SPR biosensor to the detection of MG7-Ag in the human sera for immunoassay.

2. Materials and methods

2.1. Chemicals and reagents

The mouse monoclonal antibody MG7-Ab was produced and purified in State Key Laboratory of Cancer Biology, Institute of Digestive Diseases, Xijing Hospital, Fourth Military Medical University, PRC. 3-Mercaptopropionic acid (MPA, 99%) and *N*-hydroxysuccinimide (NHS) were purchased from Alfa Aesar, USA. *N*-ethyl-*N*-(dimethylaminopropyl)-carbodiimide (EDC·HCl, 99%) was purchased from Shanghai Medpep Co., Ltd. Bovine serum albumin (BSA) and other chemicals were purchased from standard commercial sources and were of analytical grade.

2.2. Methods

2.2.1. Establishment of surface plasmon resonance sensor

The spectral SPR sensor was established based on the principle of Kretschmann–Raether attenuated total reflection (ATR) configuration [15]. A schematic diagram of the SPR sensors is shown in Fig. 1A.

A quartz tungsten halogen lamp was used as a white light source. A collimator was positioned in front of the prism to form a parallel light beam. A polarizer was positioned at the input light path to obtain transverse magnetic polarized light. A SF10 prism combined with sensor chip by index matching fluid was mounted on a goniometer. The reflected light from the sensor chip was collected into the optical fiber of which the diameter was 200 μm, and then was analyzed by a fiber spectrometer (Avantes, the Netherlands, AvaSpec-2048TEC). The resolution of AvaSpec-2048TEC spectrometer was 0.4 nm in the range of 400–740 nm. The incidence angle was adjusted to a proper angular to obtain the resonance wavelength in 600–740 nm range. The SPR measurement was performed in liquid ambient.

2.2.2. Sensor chip preparation

Glass slides were sonicated in soapy water, deionized water, acetone, ethanol, respectively, for 10 min in an ultrasonic bath. Then, slides were sufficiently rinsed with deionized water and dried with a stream of nitrogen. Au films (50 nm thickness) were deposited on the slides using a DC sputter. Au deposited fresh chips were incubated in a solution of MPA (5%, v/v) in ethanol for 12 h at room temperature to form a MPA self-assembled monolayer on Au films with its carboxyl group outward. After the surface self-assembly process, the sensor chips were washed in methanol and deionized water respectively. Then the carboxyl groups on the surface were activated by incubating the surface in a solution of 50 mM *N*-hydroxysuccinimide and 200 mM *N*-ethyl-*N*-(ethylaminopropyl)-carbodiimide hydrochloride in deionized water for 30 min. Washed with deionized water, the activated surface was incubated with antibody MG7 solution (0.5 mg/ml MG7 in 10 mM sterile phosphate buffer, pH 7.4) at 35 °C for an hour, and followed by washing with PBS and deionized water in sequence. The residual carboxyl groups of MPA on the sensor surface were blocked by incubation with 1 M ethanolamine (pH 8.6) for 30 min to prevent non-specific absorption. After the incubation period, the sensor chips were rinsed with PBS (pH 7.4) solution and then stored in 4 °C for later SPR measurements.

3. Results and discussion

3.1. Detection of MG7-Ag in the human sera using SPR sensor

MG7-Ag in the serum samples were analyzed by the established SPR system using the above prepared sensor chips. Human sera specimens were obtained from the blood samples collected from gastric cancer patients and healthy blood donors in Xijing Hospital. In SPR measurements, all human serum albumin specimens were diluted to 1:300 (v/v) in sterile phosphate buffer solution (pH 7.4).

The shift of the resonance wavelength was monitored by the spectral SPR sensors during successive incubation of MPA, MG7 and MG7-Ag subsequently. As shown in Fig. 1B, SPR resonance wavelength, corresponding to the resonance dip of the SPR spectra curve, increased in response to the successive incubations.

It is well understood that the increase of resonance wavelength is caused by the change of refractive index in response to the MPA self-assembly, the immobilization of MG7, and the binding of MG7-Ag, respectively. It demonstrated that the antibody MG7 had been well immobilized on the gold surface of sensor chip and the binding process of MG7-Ag could be well monitored using the SPR sensor.

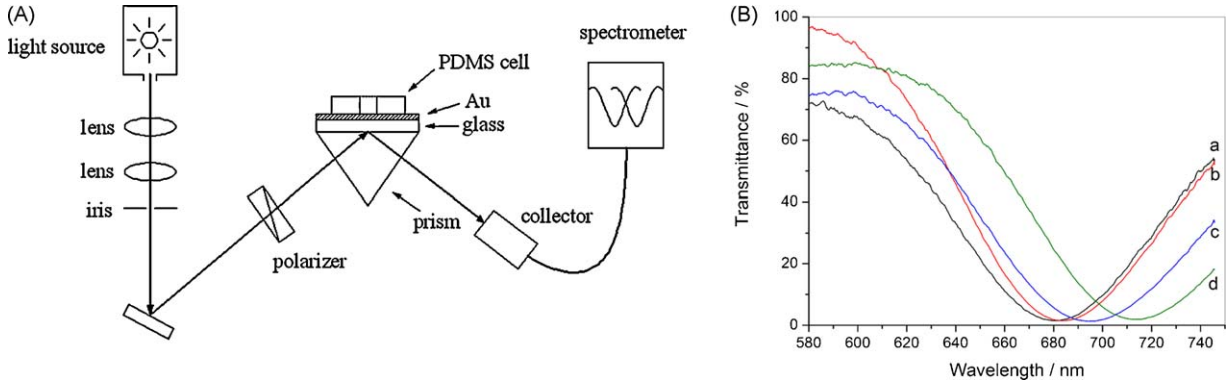

Fig. 1. (A) Schematic diagram of the established spectral SPR sensor system. The collimated white light beam passes through a polarizer and enters a SF10 prism at a proper incidence angle. The prism is contacted with a sensor chip by index matching fluid. The reflected light is collected into an optical fiber and analyzed by a spectrometer. (B) SPR spectra curves in every step of successive incubation with MPA, MG7 and MG7-Ag on the surface of sensor chip. (a) Fresh Au surface in deionized water; (b) modified by MPA and washed with deionized water; (c) MG7 in PBS and washed with deionized water; (d) serum specimen of a gastric cancer patient and washed with deionized water.

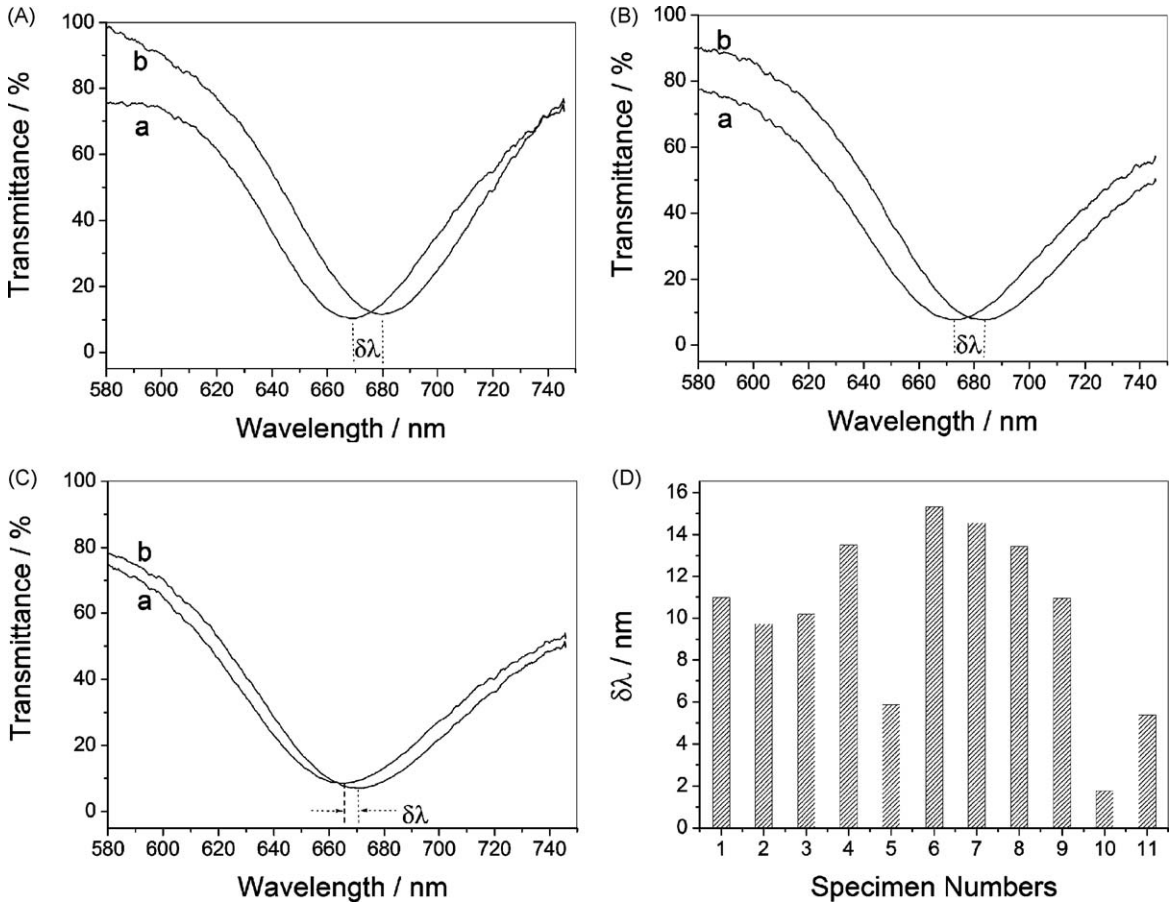

Fig. 2. (A) SPR spectra curves for sequent injections of PBS (curve a) and MKN45 gastric cancer cell lysate solution followed by a washing step with PBS solution on sensor surface (curve b). The net shift of resonance wavelength $\delta\lambda$ = 11.9 nm; (B) SPR spectra curves of PBS (curve a) and serum specimen of a gastric cancer patient followed by a washing step with PBS solution (curve b). The net shift of resonance wavelength $\delta\lambda$ = 10.95 nm; (C) SPR spectra curves of PBS (curve a) and serum specimen from a healthy blood donor followed by a washing step with PBS solution (curve b). The net shift of resonance wavelength $\delta\lambda$ = 5.4 nm; (D) net shifts of resonance wavelengths ($\delta\lambda$) of 11 specimens. Specimen 1 is MKN45 gastric cancer cell lysate solution. Specimens 2–9 are the sera of gastric cancer patients. Specimens 10 and 11 are the sera of healthy blood donors.

Fig. 2A shows the SPR spectrum curves for sequent injections of PBS (curve A) and MKN45 gastric cancer cell lysate solution followed by a washing step with PBS solution (curve B) on sensor surface. The final washing step with PBS removed most of the unbound MG7-Ag. The binding of MG7-Ag to immobilized MG7 antibody led to an increase in the SPR wavelength. Net shifts of resonance wavelengths $\delta\lambda$ were obtained by subtracting the resonance wavelength of PBS from that of MKN45 cell lysate solution. For MKN45 cell lysate solution, the average wavelength shift $\delta\lambda$ is 11.9 nm.

Fig. 2B shows the SPR spectra curves of PBS (curve a) and the serum specimen of a gastric cancer patient followed by a washing step with PBS solution (curve b). The $\delta\lambda$ of the serum of a gastric cancer patient is 10.9 nm. Fig. 2C shows the SPR spectra curves of the serum specimen from a healthy blood donor (curve a) and PBS solution (curve b) for reference. The wavelength shift $\delta\lambda$ is 5.4 nm for the healthy serum. Comparing the above three $\delta\lambda$, the $\delta\lambda$ of MKN45 cell lysate solution and that of the sera of gastric cancer patient were much larger than the $\delta\lambda$ of the sera of healthy blood donor, indicating that the shift of resonance wavelengths $\delta\lambda$ can be used to characterize the MG7-Ab concentration in human sera.

The histogram Fig. 2D shows the net shift of resonance wavelengths ($\delta\lambda$) of 11 specimens. Specimen 1 is MKN45 cell lysate solution. Specimens 2–9 are sera of gastric cancer patients. Specimens 10 and 11 are sera of healthy blood donors. Three measurements were taken for every specimen using three sensor chips and average shift of resonance wavelength was calculated for every specimen. It can be seen that the $\delta\lambda$ of the sera of most patients are much larger than those of healthy blood donors, except specimen 5. Those results indicate that SPR is capable of characterizing MG7-Ab expression in human sera. It demonstrates that SPR technique might be a potential method for early detection of gastric cancer. Further works are being carried out for such practical application. More sera specimens are being collected from adult health donors and gastric cancer patients. The SPR measurements are being carried out to statistically determine a critical value of the resonance wavelengths shift ($\delta\lambda$) which is expected to be used for early gastric malignance risk assessment.

In order to make this method applicable to rural clinics for widely diagnostic testing, we are developing a dip type of SPR sensor which will be convenient and cheap than the present system. The sensor details and the characterization results will be reported in later publication.

The specific interaction process between antibody MG7 and MG7-Ag had been monitored by recording the resonance wavelengths against reaction time. The kinetic process is shown in Fig. 3.

It consisted of a 100 μl injection of 1:300 diluted serum specimen of a gastric cancer patient and that of a healthy blood donor, respectively. Following up the injection, incubating for a time, we injected the PBS solution to wash the non-specific binding on the surface. The experiment was performed at 22 °C. Dynamic

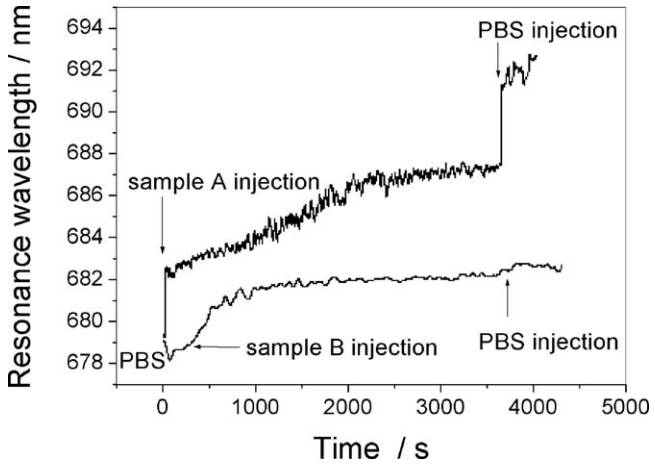

Fig. 3. Resonance wavelengths versus interaction time after 100 μl injections of 1:300 diluted sera specimens of patient (sample A) and healthy blood donor (sample B).

binding interaction was observed for the patient's sera (sample A), indicating intense existence of MG7-Ag in the sera. It can be seen from the interaction curve of sample A that 2400 s of incubation is sufficient after injection of diluted sera specimen because the wavelength shift reached an almost stable value, implying the completion of most specific binding of MG7-Ag on the sensor surface in 40 min. It can also be seen from Fig. 3 that a weak binding on sensor surface is also observed for the healthy sera. It implies that there might be still a little bit expression of MG7-Ag in healthy sera, even though much less than that of gastric cancer patient. Perhaps, there might be a little bit non-specific absorption on the sensor chip.

It will save cost and time if detection can be performed on the same chip with regeneration. For this purpose, a 0.2 M glycine/HCl buffer (pH2.5) solution was used to dissociate MG7-Ag antigens from the sensor surface for next assay. The PBS buffer solution was injected over the sensor surface after the regeneration. The result is shown in Fig. 4.

It can be seen that the regeneration process lasted 9 h and could only partially make the dissociation of the bound MG7-Ag from the surface. The resonance wavelength could not return to the original wavelength after the regeneration. It means that the employed generation process is not able to make the surface thoroughly reset. More effort should be made to look for a suitable regeneration process. In another hand, it implies that the affinity of MG7-Ab and MG7-Ag complex is very high.

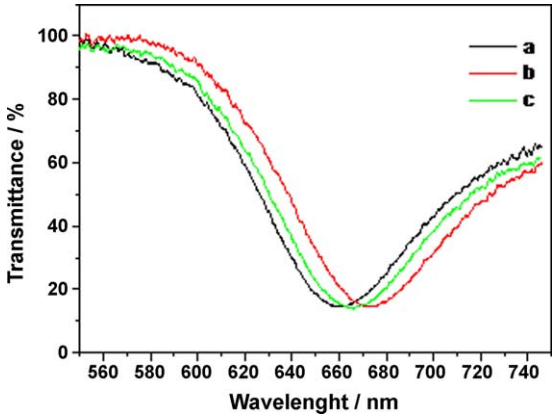

Fig. 4. SPR spectra curves of a regeneration cycle. (a) MG7-Ab immobilized surface in PBS. (b) Injecting serum specimen of a gastric cancer patient followed by a washing step with PBS solution. (c) Regenerated in 0.2 M glycine/HCl buffer (pH 2.5) for 9 h and followed by a washing step with PBS solution.

4. Conclusions

The spectral SPR biosensor is used for rapid detection of antigen MG7-Ag in the human sera. Monoclonal antibodies MG7 were immobilized on the sensor surface as the specific bio-probes. Three different types of specimens: MKN45 cancer cell lysate, the sera of gastric cancer patients and that of the healthy donors were tested to determine the difference of MG7-Ag expression intensity. It was found that most of the patient's sera have intense expression of MG7-Ag. At the same time, it was also observed that there is still a weak expression of MG7-Ag in the healthy sera. No laborious sample preparation is required in this method. The sample volume requires only 100 μl less diluted solutions. The above results demonstrate that the surface plasmon resonance biosensor has potential use in rapid, real-time detection and identification of MG7-Ag in the human sera. With further researches, the SPR biosensor would be useful as an evaluation method for early gastric cancer diagnosis.

Conflict of interest

The authors claim no financial or intellectual conflicts of interest in the preparation and submission of this manuscript.

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