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RESEARCH ARTICLE

High-throughput analysis of mumps virus and the virus-specific monoclonal antibody on the arrays of a cationic polyelectrolyte with a spectral SPR biosensor

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We investigated the potential use of a spectral surface plasmon resonance (SPR) biosensor in a high-throughput analysis of mumps virus and a mumps virus-specific mAb on the arrays of a cationic polyelectrolyte, poly(diallyldimethylammonium chloride) (PDDA). The PDDA surface was constructed by electrostatic adsorption of the polyelectrolyte onto a monolayer of 11-mercaptoundecanoic acid (MUA). Poly-L-lysine was also adsorbed onto the MUA monolayer and compared with the PDDA surface in the capacity of mumps virus immobilization. The PDDA surface showed a higher adsorption of mumps virus than the poly-L-lysine surface. The SPR signal caused by the virus binding onto the PDDA surface was proportional to the concentration of mumps virus from 0.5×10^5 to 14×10^5 pfu/mL. The surface structure of the virus arrays was visualized by atomic force microscopy. Then, a dose-dependent increase in the SPR signal was observed when various concentrations of the antimumps virus antibody in buffer or human serum were applied to the virus arrays, and their interaction was specific. Thus, it is likely that the spectral SPR biosensor based on the cationic polyelectrolyte surface may provide an efficient system for a high-throughput analysis of intact virus and serodiagnosis of infectious diseases.

Keywords:
Mumps virus / PDDA / Protein arrays / Serodiagnosis / SPR biosensor

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Abbreviations: AFM, atomic force microscopy; **dH_2O,** deionized water; **MUA,** 11-mercaptoundecanoic acid; **PDDA,** poly(diallyldimethylammonium chloride); **SPR,** surface plasmon resonance

1 Introduction

There has been a great demand for protein arrays, since the technologies allow a large-scale and high-throughput analysis of biomolecular interactions in cells, tissues, and organisms [1]. A number of techniques have been reported for the

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analysis of protein interactions on arrays with various methods such as fluorescence labeling, MS, atomic force microscopy (AFM), and surface plasmon resonance (SPR) [2]. The fluorescence labeling method has been frequently used because of its high sensitivity and compatibility with the standard DNA microarray scanners [2, 3]. However, the fluorescence labeling method has disadvantages including the heterogeneity of fluorescence labeling of proteins [4, 5]. Surface-enhanced laser desorption and ionization MS was used to identify proteins captured on the metal surface of arrays [6]. SPR method is advantageous to the analysis of protein interactions as compared with other methods, since it does not require labeling of proteins and allows real-time monitoring of biomolecular interactions [7, 8]. The SPR method has been predominantly used to analyze various biomolecular interactions including protein–protein interactions [9, 10], protein–DNA interactions [11], and protein–carbohydrate interactions [12].

Recently, there have been an accumulating number of reports to demonstrate the value of protein arrays in the serodiagnosis of infectious diseases [13–15]. There are reports on the parallel detection of infections caused by various viral pathogens including rubella virus, herpes simplex virus, and hepatitis B virus on peptide or protein arrays [13, 14]. Protein arrays have been used to screen specific antigens for the diagnosis of severe acute respiratory syndrome [15, 16]. In these reports, the protein arrays were prepared with various GST-fusion proteins from severe, acute respiratory syndrome-associated coronavirus, and the arrays were applied to the diagnosis of the syndrome by analyzing seral antibodies directed against the coronavirus based on the fluorescence detection method. *In situ* SPR method has been also used to detect antibodies against virus in human serum [17–19]. It was reported that antiadenoviral antibodies were detected and isotyped by the SPR method among the patients dosed with an adenoviral-based gene therapy vector [17]. A possible diagnosis of human hepatitis B virus infection by SPR measurement was demonstrated with the Spreeta™ chip [19]. However, there is no report on the analysis of antibodies in human serum for diagnosis of viral infections with an array-based SPR biosensor.

Several methods have been reported to detect viral pathogens such as AFM [20, 21], quartz crystal microbalance [22], MS [23], and SPR [24]. AFM is a direct and label-free imaging method, and this method was used to analyze various viruses including bacteriophage, canine parvovirus, and coxsackievirus captured on their antibodies in a nanoarray format [21]. In addition, AFM has been used to directly visualize fully hydrated and intact intracellular mature vaccinia virus and intraviral structure [20]. Detection of pathogens by MS, termed TIGER (triangulation identification for the genetic evaluation of risks) based on polymerization chain reaction of broadly conserved regions of pathogen genomes, was used to identify DNA and RNA viruses [23]. There have been a couple of reports on the detection of viruses by *in situ* SPR biosensors [24, 25]. However, there is no report on the analysis of viruses in an array format with SPR biosensors.

In this manuscript, we presented a potential use of a cationic polyelectrolyte, poly(diallyldimethylammonium chloride) (PDDA), in the high-throughput analysis of mumps virus and its mAb by using a spectral SPR biosensor. Mumps virus is a single-stranded negative sense RNA virus and an etiologic agent of mumps, an illness characterized by fever and parotitis [26]. Gold arrays were modified with the polyelectrolyte and used to immobilize intact mumps virus. The spectral SPR biosensor provided a quantitative detection tool for the mumps virus on the polyelectrolyte surface. The virus arrays prepared on the surface were successfully applied to the analysis of the virus-specific mAb in PBS and human serum. Thus, the cationic polyelectrolyte is a useful surface for a high-throughput analysis of mumps virus and antimumps virus antibody on arrays with the spectral SPR biosensor.

2 Materials and methods

2.1 Chemicals and reagents

Octadecyltrichlorosilane, carbone tetrachloride, cyclohexane, BSA, 11-mercaptoundecanoic acid (MUA), and PDDA were obtained from Sigma (St. Louis, MO). Monoclonal anti-GST was provided by Boditech (Chuncheon, Korea).

2.2 Preparation of mumps virus and monoclonal antimumps virus antibody

Mumps virus (strain 98–40), obtained from the School of Medicine, Korea University (Seoul, Korea), was prepared in VeroE6 cells (American Type Culture Collection, CRL 1585). The cells were cultured with Dulbecco's modified Eagles medium supplemented with 5% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 9 mM $NaHCO_3$ in culture flasks at 37°C under humidified 5% CO_2. The viral titer was determined by a plaque assay.

The hybridoma cell line producing a mAb against mumps virus was established by a fusion of Sp2/0-Ag14 mouse myeloma cells (ATCC, CRL-1581) with spleen cells of Balb/c mice immunized with mumps virus strain 98–40 [27]. The specificity of the mAb was established by immunofluorescence staining and immunoblotting.

2.3 Surface modification of gold arrays

Fabrication of gold arrays and hydrophobic modification of glass surface were performed according to the previous report [28]. Briefly, gold arrays with 50 spots (a diameter of 2 mm each spot with 1 mm distance between spots) were fabricated by depositing Ti/Au (50/450 Å) films on the pyrex glasses by an RF-magnetron sputtering apparatus at a vacuum of 3×10^{-6} Torr. After washing them with a cleaning solution of $H_2O_2/NH_4OH/dH_2O$ (deionized water) (1:1:5, v/v/v) at 70°C for 10 min, the arrays were incubated with a mixture

of cyclohexane/carbon tetrachloride/octadecyltrichlorosilane (40:10:0.08 v/v/v) to generate hydrophobic glass surface at 45°C for 30 min. And the arrays were washed with a mixture of cyclohexane and carbon tetrachloride (4:1 v/v), carbon tetrachloride, and ethanol in order. Then, the array surface was cleaned with the cleaning solution at 70°C for 10 min.

The surface of gold arrays was modified with PDDA as described in Fig. 1. Gold arrays were incubated with 1 mM MUA in ethanol for 16 h and washed with ethanol to remove the excess of MUA. After incubating with 50 mM NaOH in dH_2O for 10 min to make the MUA surface negative, the modified arrays were incubated with 1 mg/mL PDDA in 0.5 M NaCl or 1 mg/mL poly-L-lysine in dH_2O for 30 min, and the arrays were washed with dH_2O.

2.4 Analysis of surface topology by AFM

The surface of gold-coated silicon wafer (thickness of gold layer ≈50 nm) was modified with the PDDA layer, and mumps virus was immobilized onto the surface. AFM imaging was performed under air using a Nanoscope IIIa with a J-type scanner (125 × 125 μm) (Digital Instrument, USA) in the contact mode. Cantilevers with Si_3N_4 oxide-sharpened tips (spring constant of 0.12 N/m) were used for the imaging. Applied force was varied from several to tens of nN. A section analysis was performed to measure the size of virus particles.

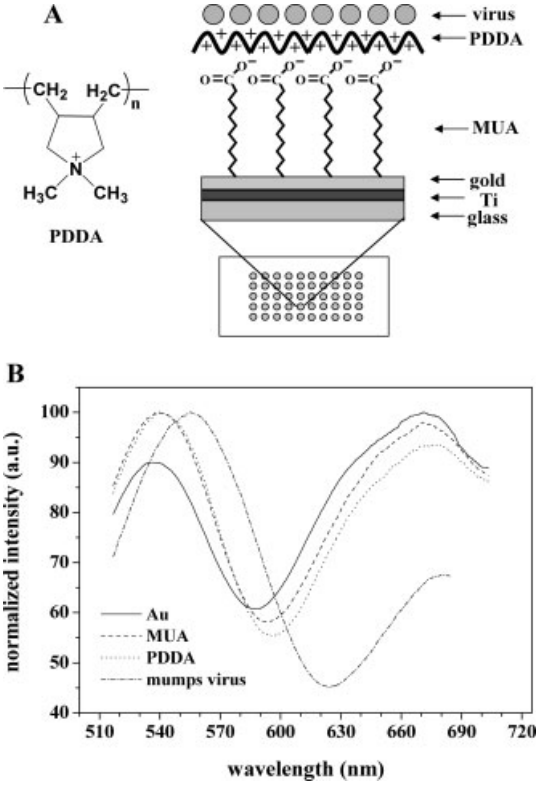

Figure 1. Schematic diagram of surface modification of gold arrays (A), and changes in SPR spectra caused by a successive modification with MUA, PDDA, and mumps virus (B).

2.5 Analysis of protein arrays by the line-scanning mode of a spectral SPR biosensor

Immobilization of mumps virus and its mAb onto the polyelectrolyte surface of the arrays was analyzed by the line-scanning mode of a self-developed spectral SPR biosensor [28], configured by using Kretschmann geometry of the attenuated total reflection method [29]. A 20 W quartz tungsten halogen lamp was used as the light source and a polarizer was positioned at the input light path to obtain transverse magnetic polarized light. Protein arrays were coupled with a fused-silica prism *via* an index matching fluid and mounted on an *x-y* linear stage. The arrays were automatically scanned every 100 μm by the line-scanning mode of the spectral SPR biosensor. The reflected light from the arrays was collected into an optical fiber, and analyzed by using an AVS-S2000 spectrometer, which provides a spectral resolution of 0.4 nm in the range of 500–700 nm (Avantes, Netherlands). The net shift of SPR wavelengths obtained from the line-scanning analysis and displayed by color spectra or 3-D images represents the amount of mumps virus and its mAb bound to the surface of arrays.

2.6 Analysis of interaction of mumps virus with antimumps virus antibody on the PDDA surface

Interaction of mumps virus with its mAb was analyzed with the spectral SPR biosensor. The mumps virus was immobilized onto the PDDA surface at 37°C for 2 h. The mumps virus arrays were incubated with 3.7% formaldehyde in PBS (8.1 mM Na_2HPO_4, 1.2 mM KH_2PO_4, 2.7 mM KCl, 138 mM NaCl, pH 7.4) for 30 min, and then permeabilized with 0.2% Triton X-100 in PBS for 30 min. Following washing with PBS, the arrays were incubated with 10 mg/mL of BSA in PBS for 30 min to reduce nonspecific interactions. After washing with 0.1% Tween 20 in PBS, the virus arrays were incubated with 2 μL of 100 μg/mL antimump virus or anti-GST antibody in PBS and human serum for 2 h at 37°C. The arrays were then washed with 0.1% Tween 20 in PBS (pH 7.4) and dH_2O, dried under N_2 gas, and analyzed by the line-scanning mode of the spectral SPR biosensor.

3 Results

3.1 Immobilization of mumps virus on the PDDA surface

For a high-throughput analysis of antimumps virus antibody on protein arrays with the spectral SPR biosensor, first, we designed a new surface using a cationic polyelectrolyte, PDDA, for immobilization of mumps virus. Viruses have been immobilized onto the cationic surface such as poly-L-lysine [30–32] or diethylaminoethyl [24]. However, protein interactions on these cationic surfaces can be affected by pH environment [1]. Thus, we modified the surface of gold arrays

with the PDDA monolayer to provide a quaternary ammonium surface, which is not affected by buffer environment, and used the polyelectrolyte layer to immobilize mumps virus. As shown in Fig. 1A, the gold arrays were successively modified with MUA and PDDA by the self-assembled monolayer method, and intact mumps virus was immobilized on the PDDA surface. The layer formation of MUA, PDDA, and mumps virus was demonstrated by the shift of SPR wavelengths which was analyzed under air with the spectral SPR biosensor (Fig. 1B). In addition, the immobilization of mumps virus onto the polyelectrolyte surface was shown by AFM imaging. Mumps virus is a single-stranded negative sense RNA virus in the Paramyxovirus family, and its size is known to be approximately 100–600 nm. As shown in Fig. 2, the shape of mumps virus was pleomorphic and roughly spherical, and the size determined by the section analysis of AFM was approximately 107–134 nm. These results depicted that the cationic PDDA layer can be used as a useful surface for immobilizing mumps virus.

3.2 High-throughput analysis of mumps virus on the PDDA surface with the spectral SPR biosensor

As the previous results showed that the PDDA surface was appropriate for immobilizing the mumps virus, we tested whether mumps virus could be analyzed on the PDDA surface in a high-throughput manner by the spectral SPR biosensor. Various concentrations of mumps virus from 0.5×10^5 to 14×10^5 pfu/mL in PBS were, initially, applied to the surface of PDDA arrays, and the arrays were analyzed by the line-scanning mode of the spectral SPR biosensor. Figure 3A shows the relative number of mumps virus

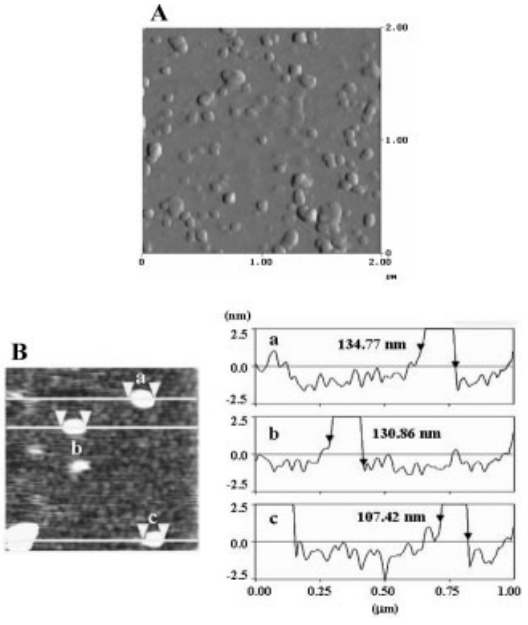

Figure 2. AFM imaging of mumps virus. (A) Imaging of mumps virus by the contact mode. (B) Section analysis of mumps virus.

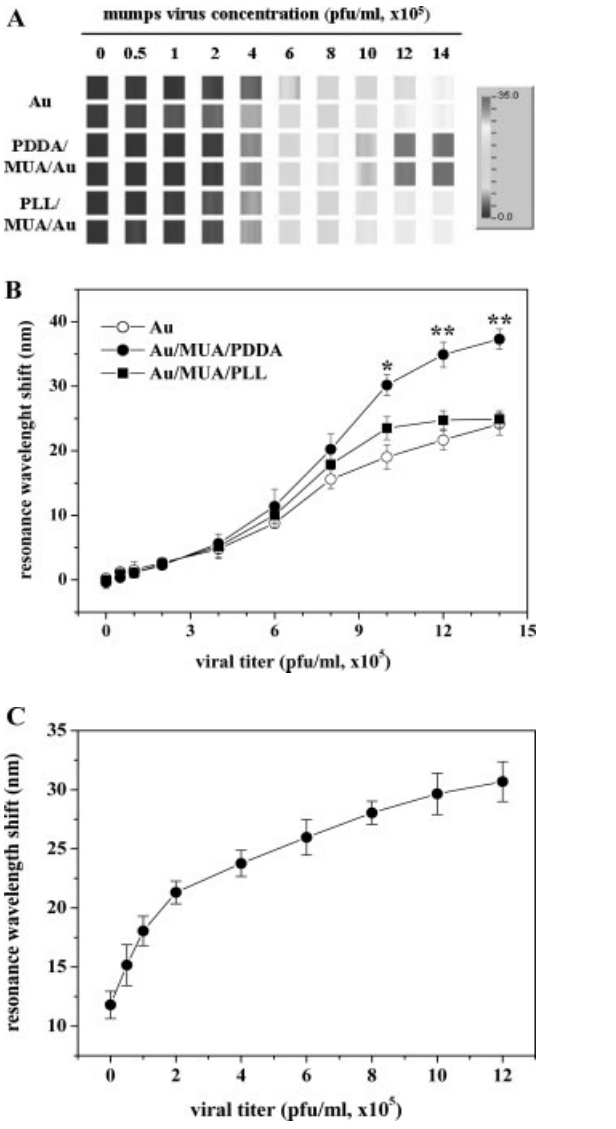

Figure 3. Analysis of mumps virus on arrays by the spectral SPR biosensor. Various concentrations of mumps virus in PBS (A, B) or culture medium (C) were applied to the surface of bare gold (Au), poly-L-lysine, or PDDA, and the virus bound onto the arrays were analyzed by the line-scanning mode of the spectral SPR biosensor as described in Section 2. The results are expressed as means ± SD from three separate determinations (*p<0.05; **p<0.005).

bound to the PDDA surface by color spectra, which were constructed by net shift of SPR wavelengths. The results showed a dose-dependent change of color spectra from blue to red. The net shift of SPR wavelengths obtained from the line-scanning analysis were used to calculate the average SPR wavelength shift of each array spot, whose results were shown in Fig. 3B.

Subsequently, we compared the PDDA surface with bare gold or the previously used poly-l-lysine surface [32] in the adsorption to the virus by the line-scanning mode of the SPR

biosensor. As shown in Figs. 3A and B, at the concentrations lower than 8×10^5 pfu/mL, three surfaces showed similar changes of SPR wavelength shift in a dose-dependent manner, indicating that no significant difference exists among the surfaces in the adsorption. However, at the concentrations higher than 10×10^5 pfu/mL, the PDDA surface showed a significantly higher adsorption than the bare gold or poly-L-lysine surface. In addition, a further increase of resonance wavelength shift was observed by higher concentrations of mumps virus until 28×10^5 pfu/mL on the PDDA surface (data not shown). These results suggested that the cationic polyelectrolyte proves to be a superior surface for a high-throughput analysis of mumps virus by the spectral SPR biosensor.

The scientific community would greatly benefit from the ability to simply and simultaneously determine the viral titer during cultivation with the protein arrays, because the plaque assay takes about a week. Thus, we applied the PDDA arrays to the determination of viral titers in culture media by using the spectral SPR biosensor. Serial samples with different number of mumps virus in culture media were prepared and introduced to each spot of the PDDA arrays; and the interaction of the virus with the PDDA surface was analyzed by the SPR biosensor. As shown in Fig. 3C, the shift of SPR wavelength increased according to the viral concentration in culture media, demonstrating that the viral titer was successfully determined by the PDDA array-spectral SPR biosensor system. A background signal observed from the virus-free sample indicated a possible interaction between the components of culture media with the PDDA surface. As a result, the spectral SPR biosensor based on the PDDA arrays is a useful system for a high-throughput analysis of mumps virus in culture.

3.3 High-throughput analysis of antimumps virus antibody on the virus antigen arrays with a spectral SPR biosensor

It is an important issue to analyze immunoglobulins of sera in a high-throughput format for rapid serodiagnosis of infectious diseases including mumps. However, there is no report on the array-based serodiagnosis with the SPR biosensors. With that motivation, we investigated a possible analysis of antimumps virus on protein arrays with the spectral SPR biosensor. To test this possibility, antigen arrays were prepared by immobilization of intact viruses on the PDDA surface and fixation with formaldehyde, and incubated with various concentrations of monoclonal antimumps virus or anti-GST antibodies prepared in PBS. Then, the amount of the antibodies bound to the antigen arrays was analyzed with the spectral SPR biosensor. As shown in Fig. 4, the increase of the SPR signal was proportional to the concentration of antimumps virus antibody. However, no significant interaction of the virus arrays with anti-GST antibody was observed at any concentration, indicating that the interaction of mumps virus with its antibody was specific. The

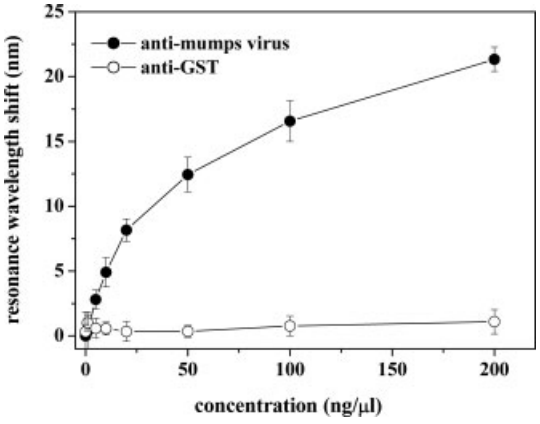

Figure 4. High-throughput analysis of antimumps virus antibody in PBS on the viral arrays by the spectral SPR biosensor. The indicated concentrations of antibodies against mumps virus and GST in PBS were applied onto mumps virus arrays, and the arrays were analyzed by the line-scanning mode of the spectral SPR biosensor as described in Section 2. The results are expressed as means ± SD from three separate determinations.

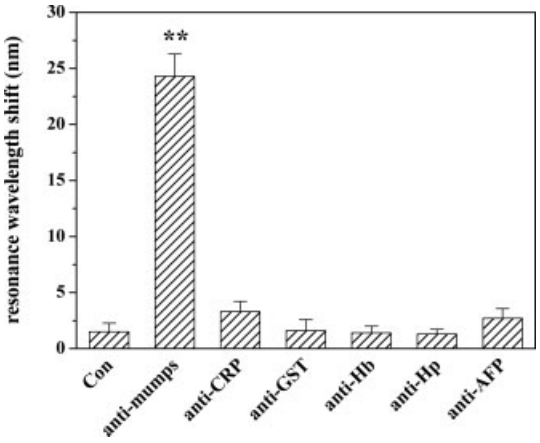

Figure 5. Specific interaction of mumps virus with antimumps virus antibody. Mumps virus was immobilized onto the PDDA surface of gold arrays, and incubated with antibodies against mumps virus (mumps), C-reactive protein (CRP), GST, hemoglobin (Hb), haptoglobin (Hp), and α-fetoprotein (AFP). Then, interaction of the antibodies with mumps arrays was analyzed by the line-scanning mode of the spectral SPR biosensor as described in Section 2. The results are expressed as means ± SD from three separate determinations (**p<0.005).

specific interaction of the virus with antimumps virus antibody was further studied by incubation of the antigen arrays with various antibodies against C-reactive protein, GST, hemoglobin, haptoglobin, and α-fetoprotein. As shown in Fig. 5, no antibody except antimumps virus antibody showed a significant interaction with the virus arrays. Thus, the shift of SPR wavelength on the antigen arrays has been caused by the specific interaction of the mumps virus with its antibody.

Then, we studied the possible application of the virus arrays based on the spectral SPR biosensor to the high-throughput detection of antibodies in human serum. mAb against mumps virus was serially diluted with normal human serum, and the interactions with the virus arrays were analyzed with the spectral SPR biosensor. As shown in Fig. 6, the SPR signal was dose-dependent with the maximal increase at 100 ng/μL, but no significant interaction was observed by incubation with anti-GST antibody in human serum. These results suggested that antimumps virus antibody in serum was successfully analyzed on the virus arrays with the SPR biosensor. In conclusion, it is likely that the spectral SPR biosensor based on virus arrays can be used as a model system for a rapid serodiagnosis of infectious diseases.

4 Discussion

A rapid detection of infectious diseases including mumps is critically important to minimize transfer of the diseases and also to evaluate their effective treatment strategies. Mumps is a highly contagious disease among school children. It is characterized by fever and parotitis, and less frequent but more serious acute inflammatory reactions [26]. However, it is difficult to accurately diagnose mumps virus infection only by clinical observations. The detection of antibody against mumps virus in serum plays an important role in immunity surveillance, monitoring the efficacy of vaccination programs, identifying susceptible cohorts in the population, and shaping future vaccination policies. Thus, in this report, we presented a new approach for a high-through analysis of mumps virus and the virus-specific mAb on a polyelectrolyte surface by the spectral SPR biosensor. Gold arrays were

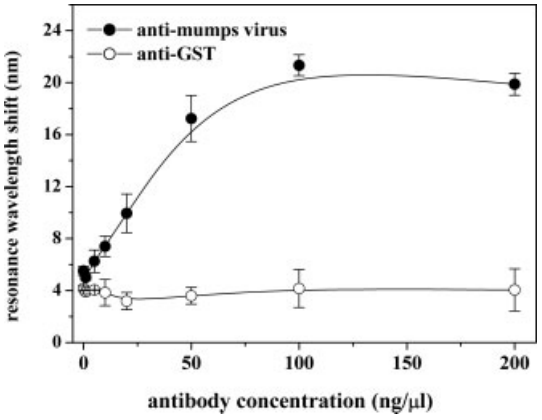

Figure 6. High-throughput analysis of antimumps virus antibody in human serum with the viral arrays by the spectral SPR biosensor. The indicated concentrations of mAb against mumps virus and GST in human serum were applied onto mumps virus arrays, and the arrays were analyzed by the line-scanning mode of the spectral SPR biosensor as described in Section 2. The results are expressed as means ± SD from three separate determinations.

modified by successive incubation with MUA and a polyelectrolyte, PDDA, with the self-assembled monolayer method, and the PDDA arrays were successfully used to analyze mumps virus in a high-through manner. The PDDA surface showed a superior adsorption to the virus than the previously used poly-L-lysine surface. Then, the mumps virus arrays prepared on the PDDA arrays were used to analyze monoclonal antimumps virus antibody in a parallel format. A dose-dependent increase of the SPR signal was observed when various concentrations of the mAb in buffer or serum were applied to the virus arrays. In addition, the antigen–antibody interaction was specific. Thus, the array-based spectral SPR biosensor was a useful system for a high-through analysis of mumps virus and antimumps virus antibody on the polyelectrolyte surface.

Positively charged polymers, such as poly-L-lysine and DEAE [24, 30–32], have been used to immobilize and analyze various viruses. Poly-L-lysine is a widely used polymer in the analysis of viruses [30–32]. An ELISA has been reported for the detection of human enteric viruses based on the poly-L-lysine [30]. Poly-L-lysine was also used to study the inhibitory effect of reverse transcriptase inhibitors on cell-free HIV in microwell plates [32]. The intrinsic adsorption rate of whole adenovirus onto a DEAE surface has been studied by an *in situ* SPR biosensor [24]. However, the surface charge of the polymers is affected by the pH environment surrounding the polymers [1]. It is very essential to uniformly immobilize viruses when virus arrays are prepared for the analysis of viral antibodies in buffer or serum. Thus, we have designed and constructed a new array surface for immobilizing virus using a cationic polyelectrolyte, PDDA, which is not affected by buffer pH. The PDDA surface was formed on the surface of gold arrays by using a monolayer of MUA as a linker layer. The PDDA provided an efficient surface to immobilize mumps virus in comparison with the widely-used poly-L-lysine. Two surfaces showed a similar adsorption to mumps virus at the concentrations lower than 8×10^5 pfu/mL. The binding capacity of poly-L-lysine surface to mumps virus was saturated at 10×10^5 pfu/mL, whereas the PDDA surface showed a continuous increase in the adsorption at the concentrations higher than 10×10^5 pfu/mL. However, beyond 28×10^5 pfu/mL, the virus sample was not detectable because of the detection limit of the spectrometer of the spectral SPR biosensor. Thus, the PDDA provided a much greater binding surface to mumps virus than poly-L-lysine. In addition, the PDDA arrays were successfully applied to the determination of the cultured mumps virus titer. By using the PDDA arrays based on the spectral SPR biosensor, we could determine the virus titer within 3 h during cultivation. On the contrary, it takes about a week to determine the virus titer by the frequently used plaque assay. Thus, it is likely that the PDDA is a useful surface for immobilizing viruses including mumps virus to prepare virus arrays and determine the virus titer during culture.

The spectral SPR biosensor based on the polyelectrolyte surface can be used as a useful system for a high-throughput serodiagnosis of infectious diseases. It has been reported that

viral infections were detected on arrays by the fluorescence labeling method [13, 14]. This method has been often used for serodiagnosis of infectious diseases due to its high sensitivity and compatibility with the DNA microarray technology. However, it is essential to label proteins or antibodies with fluorophores, which might cause the loss of their biological activities [1]. An *in situ* SPR biosensor was used to detect infection of human hepatitis B virus [19] or antiadenoviral antibodies among the patients dosed with an adenoviral-based gene therapy vector [17], but there is no report on the array-based analysis of viral infections by the SPR biosensor. In this report, we demonstrated a successful analysis of antimumps virus antibody spiked in human serum in an array format by the spectral SPR biosensor. In addition, we analyzed antimumps virus antibody in sera from five people, who were vaccinated with attenuated mumps virus in infancy, on mumps virus arrays with the spectral SPR biosensor. We were able to detect the viral antibody in five sera with a variation of 1.7-fold among the people (data not shown), indicating that our system can be applied to the serodiagnosis of mumps. Our analysis system is a simple and label-free method based on the array format, and thus disadvantages of the fluorescence labeling method can be overcome. To our understanding, this is the first report on the array-based analysis of antiviral antibodies in serum with an SPR biosensor.

In summary, we have coupled the protein array technology based on the PDDA surface with the spectral SPR biosensor to the analysis of the antibody in serum against mumps virus in a high-throughput manner. This novel approach is simple, label-free, and rapid, and thus this method can be applied to a rapid serodiagnosis of infectious diseases as well as the determination of virus titer during cultivation.

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