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Surface-Independent and Oriented Immobilization of Antibody via One-Step Polydopamine/Protein G Coating: Application to Influenza Virus Immunoassay

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For the construction of high-performance biosensor, it is important to interface bioreceptors with the sensor surface densely and in the optimal orientation. Herein, a simple surface modification method that can optimally immobilize antibodies onto various kinds of surfaces is reported. For the surface modification, a mixture of polydopamine (PDA) and protein G was employed. PDA is a representative mussel-inspired polymer, and protein G is an immunoglobulin-binding protein that enables an antibody to have an optimal orientation. The surface characteristics of PDA/Protein G mixture-coated substrates are analyzed and the PDA/protein G ratio is optimized to maximize the antibody binding efficiency. Moreover, the antibody-immobilized substrates are applied to the detection of influenza viruses with the naked eye, providing a detection limit of 2.9×10^3 pfu mL^{-1}. Importantly, the several substrates (glass, SiO_2, Si, Al_2O_3, polyethylene terephthalate, polyethylene, polypropylene, and paper) can be modified by simple incubation with the mixture of PDA/protein G, and then the anti-influenza A H1N1 antibodies can be immobilized on the substrates successfully. Regardless of the substrate, the influenza viruses are detectable after the sandwich immunoreaction and silver enhancement procedure. It is anticipated that the developed PDA/protein G coating method will extend the range of applicable materials for biosensing.

1. Introduction

Immunoassays are one of the most widely used detection methods for biochemical analytes.[1] The fine accuracy and simple operation of immunoassays support their use in disease diagnosis, food safety, environmental protection, etc.[2] Recently, immunoassays have evolved into sophisticated analyte-sensing methods by employing autonomous platforms, emerging nanomaterials, and various kinds of readout signals (e.g., colorimetry, electrochemistry, surface plasmon resonance, fluorescence, and surface-enhanced Raman scattering).[3,4]

Since immunoassays are based on the antibody–antigen interaction, antibody immobilization on the sensor surface is an essential process for the development of most immunoassays.[5] In recent years, numerous strategies have been reported to control antibody immobilization, mainly by directing the orientation, stability, and density of bound antibodies on different sensor platforms.[6] Based on advances in material science and nanotechnology, recent immunosensors have been constructed with various kinds of new materials and nanostructures.[7] The method of antibody coupling, therefore, should vary and be optimized for each sensor. Because the choice of antibody immobilization method greatly affects antibody–antigen interactions on the sensor surface, the adoption of an improper surface modification procedure can affect the performance of the immunosensors.[8] If simple, substrate-independent, and properly oriented immobilization of bioreceptors is possible, it can be applied universally to various sensing platforms and further leads to the development of high-performance immunosensors. This possibility prompted

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us to develop a simple surface modification method that can optimally immobilize antibodies onto several kinds of sensor surfaces.

For the oriented antibody immobilization, the antibody-binding proteins such as protein G, A, and L have been widely used.[9] Since the antibody-binding proteins can target the Fc region of an antibody, they allow the antibody-binding sites to be exposed to antigen effectively.[10] A previous study showed that well-oriented antibodies could improve the antigen-binding capacity by up to eightfold compared to that of randomly oriented antibodies.[5] Meanwhile, dopamine is a well-known mussel-inspired adhesion molecule.[11] It was previously reported that a polydopamine (PDA) film could be formed on organic/inorganic surfaces simply by the self-polymerization of dopamine under alkaline conditions.[12] In addition, it was reported that molecules co-dissolved with dopamine could be immobilized onto surfaces during PDA formation.[13] This strategy enabled the effective modification of various kinds of surfaces and has thus been used for a variety of applications, such as tissue regeneration,[14] cell adhesion,[15] antibacterial surface development,[16] biosensing,[17] and so on.

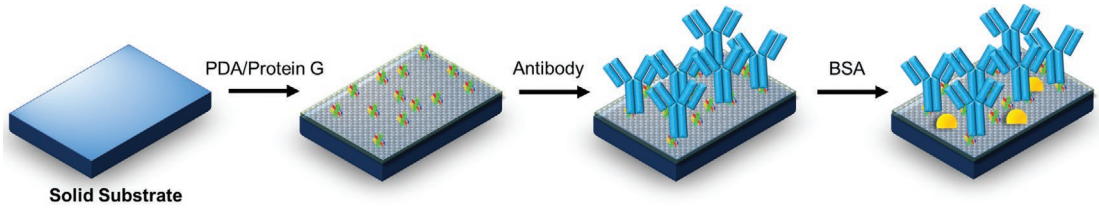
Herein, we hypothesized that simple, substrate-independent, and oriented immobilization of antibody is feasible by combining the surface-coating property of PDA and the antibody binding ability of protein G. As a proof-of-concept, we coated the glass substrates with the mixture of PDA and protein G, and then immobilized antibodies on the PDA/protein G mixture–coated substrates through the binding with the protein G. The surface characteristics of PDA/protein G mixture–coated substrates were analyzed by X-ray photoelectron spectroscopy (XPS), atomic force microscope (AFM), and ellipsometry. The PDA/protein G ratio was optimized through the fluorescence monitoring. Moreover, the optimally antibody-immobilized substrates were applied to the influenza virus immunoassays. After the sandwich immunoreaction and silver enhancement step, influenza virus could be detected on the antibody-immobilized surfaces with the naked eye with the detection limit of 2.9×10^3 pfu mL^{-1}. More importantly, the surfaces of several substrates (glass, SiO_2, Si, Al_2O_3, polyethylene terephthalate [PET], polyethylene [PE], polypropylene [PP], and paper) were successfully coated by the PDA/protein G mixture and the anti-influenza A H1N1 antibody sequentially. These substrates also could be employed for the detection of influenza viruses, suggesting the wide applicability of the present antibody immobilization method. We anticipate that the present PDA/protein G coating method will be useful not only in reducing inconvenient surface modification procedures for antibody immobilization but also in expanding the types of materials used for immunosensors.

2. Result and Discussion

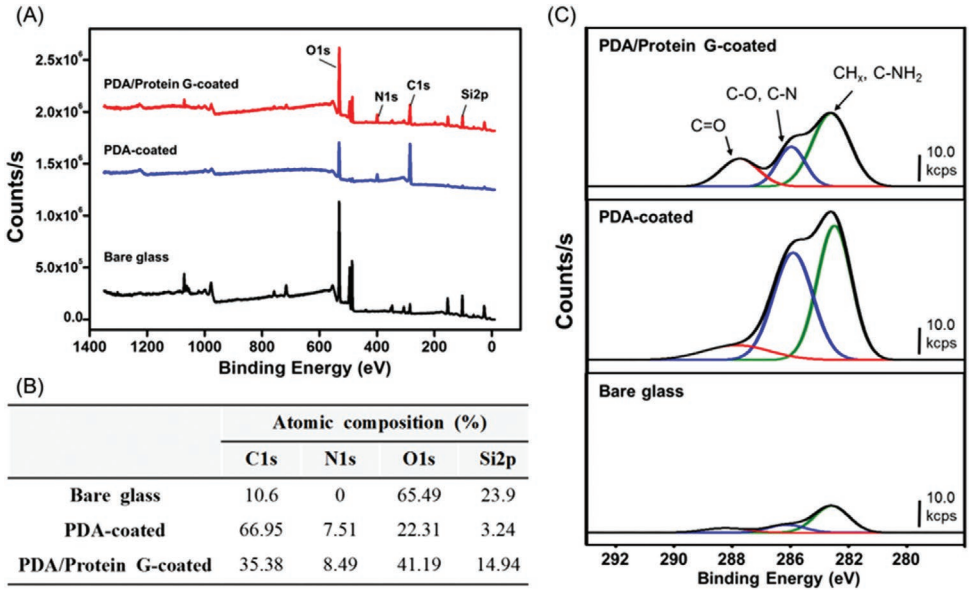
2.1. Surface Modification by Using a Mixture of PDA and Protein G

PDA coating methods were inspired by the amino acid composition of mussel adhesive proteins, and these methods have been widely demonstrated on numerous surfaces.[12] In addition, it was proven that one-step surface functionalization is possible by using a mixture of dopamine and other molecules.[13] Protein G can bind specifically to the Fc region of an antibody, allowing the binding site of the antibody to be exposed to antigen effectively.[5] Therefore, we hypothesized that protein G could be immobilized on the surface by treating a mixed solution of dopamine and protein G. **Scheme 1** shows the procedure for well-oriented antibody immobilization on a solid substrate by using the PDA/protein G mixture. First, we prepared the surface-coating solution by mixing protein G in pure water (30 mg mL^{-1}) and dopamine hydrochloride in a weak alkaline solution (2 mg mL^{-1}) at a 1:1 volume ratio. Using 2 mg mL^{-1} of dopamine solution is the most common method to form a thin PDA film.[12,18] Next, the solid substrate was immersed in the prepared solution for 2 h and washed. Then, the antibody was applied to the PDA/protein G-coated substrate for 1 h. Finally, bovine serum albumin (BSA) was treated on the substrate for the prevention of non-specific binding. Though the PDA film is known to have an antifouling effect,[19] the protein G exposed on the surface can cause nonspecific adsorption. This simple procedure enables the immobilization of all immunoglobulin antibodies optimally on various surfaces because of the synergistic contributions of PDA and protein G. We expect that the proposed method may be applied to a variety of immunoassays by choosing the appropriate antibody and sensor surface.

To verify the coating of PDA and protein G on the substrate, we analyzed the bare glass surface, PDA-coated surface, and PDA/protein G–coated surface by XPS. As shown in **Figure 1**a, N1s spectra were observed on the PDA- and PDA/protein G mixture–coated surfaces, but no N1s peak was observed on bare glass. In addition, relatively stronger C1s spectra were observed on PDA- and PDA/protein G mixture–coated surfaces than on the unmodified surface. The Si2p peak was measured most strongly on the bare glass surface. Since PDA and protein G are composed of chemical elements C, N, and O, the



Scheme 1. Schematic illustration of the surface modification step for the immunoassay using a mixture of PDA and protein G.



(B)

	Atomic composition (%)			
	C1s	N1s	O1s	Si2p
Bare glass	10.6	0	65.49	23.9
PDA-coated	66.95	7.51	22.31	3.24
PDA/Protein G-coated	35.38	8.49	41.19	14.94

Figure 1. A) XPS spectra and B) surface chemical composition of bare glass, PDA-coated, and PDA/protein G-coated surfaces. C) High-resolution XPS C1s spectra of bare glass, PDA coated-, and PDA/ protein G-coated surfaces.

XPS result indicates that the glass surfaces were coated with PDA or the PDA/protein G mixture successfully. The elemental percentages of the glass, PDA-coated, and PDA/protein G–coated surfaces also confirm the surface modification of glass by PDA or the PDA/protein G mixture (Figure 1b). Figure 1c shows the high-resolution XPS C1s spectra of the glass, PDA-coated, and PDA/protein G–coated surfaces. Interestingly, the carbonyl carbon peak (C=O) at 287.8 eV was increased only when the substrate was coated with the PDA/protein G mixture. On the glass surface and the PDA-coated surface, the carbonyl peak was negligible. The C=O peak intensity has been used to measure the extent of polypeptide coating on the surface because XPS C1s spectra of polypeptides typically show a strong C=O peak.[20] This result suggests that the protein G was successfully immobilized on the surface during the dopamine polymerization process.

Moreover, the topographies of the modified glass surfaces were analyzed by an AFM (Figure S1, Supporting Information). The Ra value which is used to evaluate the surface roughness on the basis of scan area was slightly increased after the PDA (Ra = 1.347) and PDA/protein G (Ra = 1.300) coatings. The thickness of the PDA and PDA/protein G layer on the modified surfaces was further investigated by ellipsometry. For the measurement of thickness, a Si wafer was employed after cleaning with methanol, acetone, and deionized water. The thickness of PDA film was measured as 22.45 ± 1.20 nm, and the thickness of PDA/protein G film was measured as 3.89 ± 0.08, 2.72 ± 0.12, 2.34 ± 0.11, 2.34 ± 0.06, and 1.68 ± 0.11 depending on the protein G concentration of 10 to 50 mg mL^{-1} (Figure S2, Supporting Information). The thickness of PDA film is higher than that of PDA/protein G film because the diluted amount of dopamine is included in the PDA/protein G mixture, which affects the rate of polymerization. These results showed that PDA and PDA/protein G film can be covered on the substrate during 2 h reaction time.

2.2. Optimized Antibody Immobilization on the Surface

As it was confirmed that protein G could be coated on the substrate by simply mixing it with a dopamine solution, we then investigated whether the antibody could be bound to the protein G on the substrate. To investigate the antibody-binding efficiency on the PDA/protein G-coated surface, we employed an antibody conjugated to the Alexa Fluor 647 dye. When the antibody bound to the PDA/protein G mixture–coated surface successfully, the fluorescence of the dye-conjugated antibody might be observed. Moreover, the fluorescence intensity could be related to the antibody-binding efficiency. To examine antibody immobilization on the substrates, we compared the fluorescence signals of the bare glass, PDA-coated, and PDA/protein G–coated surfaces after dye-conjugated antibody immobilization. In this experiment, the concentration of dopamine was fixed at 2 mg mL^{-1}, and the concentration of protein G was varied from 10 to 50 mg mL^{-1}. The fluorescent antibody was prepared after dilution to 1:10 (8.0 μg mL^{-1}) and 1:50 (1.6 μg mL^{-1}) by using a dilution buffer containing 0.05% Tween 20 and 1% nonfat milk. The fluorescent antibodies were incubated on each substrate for 1 h at room temperature. After washing and drying with nitrogen gas, fluorescence images were obtained with a scanner. **Figure 2**a shows fluorescence images of the bare glass, PDA-coated, and PDA/protein G–coated surfaces after antibody immobilization. Green fluorescence was observed on the PDA/protein G mixture–coated surfaces, whereas no fluorescence was observed on the bare glass and PDA-coated surfaces. Only after PDA/protein G coating could the antibody be immobilized on the surface. This finding indicates that the coated protein G retains the ability to bind to the antibody and acts as a critical factor for antibody immobilization. Because the PDA film is coated on the surface in a thin layer, the protein G can be exposed to the antibody, inducing the efficient interaction between protein G and the antibody. The previous study

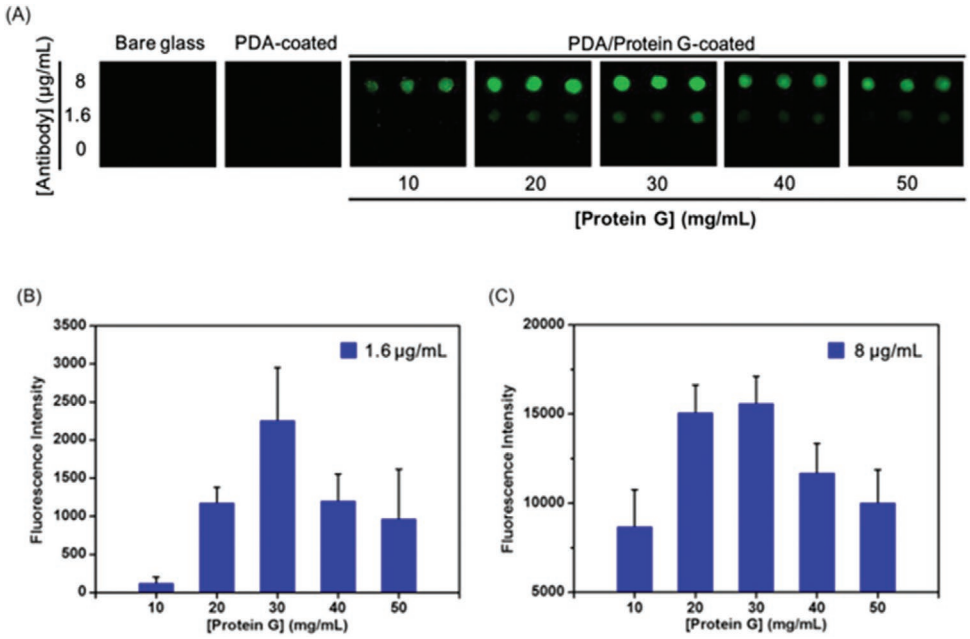


Figure 2. A) Fluorescence images of bare glass, PDA-coated, and PDA/protein G-coated surfaces after dye-conjugated antibody immobilization. B,C) Plots of fluorescence intensity depending on protein G concentration (from 10 to 50 mg mL^{-1}) with 1.6 and 8 μg mL^{-1} concentrations of dye-conjugated antibody. The data represent the mean plus standard deviation from three measurements.

suggested that PDA can be coated with a thickness of a few nanometers at this concentration[12] and it was consistent with the results from the ellipsometry. Furthermore, we obtained the fluorescence images of the bare glass and PDA-coated surfaces after the coating of protein G and dye-conjugated antibody sequentially (Figure S3, Supporting Information). The faint fluorescence signals were observed on the bare glass, suggesting that the small amounts of protein G and antibody were attached on the glass substrates by physical adsorption. The PDA-coated surface showed neglectable fluorescence due to the antifouling effect. These results proved that the protein G and corresponding antibody can be efficiently immobilized on the glass substrates by the surface-modification method employing the PDA/protein G mixture.

To optimize the antibody immobilization conditions, we measured the fluorescence intensity after changing the concentration of protein G (Figure 2b,c). The fluorescence intensity increased as the protein G concentration increased from 10 to 30 mg mL^{-1} and decreased at protein G concentrations over 30 mg mL^{-1}. Excessive amounts of protein G over 30 mg mL^{-1} might interfere with efficient binding to the antibody because of steric hindrance effects. The previous report also supports that the increase of the Fc region–binding protein density on the substrates can lead to the decrease of antibody-binding capacity due to the steric hindrance.[21] It is noteworthy that the fluorescence intensity was most strongly measured at a protein G concentration of 30 mg mL^{-1}, even after reacting with both low (1.6 μg mL^{-1}) and high (8.0 μg mL^{-1}) antibody concentrations. Based on this result, we estimated the optimal mixture of PDA and protein G to be a 1:1 volume mixture of 2 mg mL^{-1} dopamine and 30 mg mL^{-1} protein G. This optimized surface-coating mixture was used for the following experiments.

2.3. Naked-Eye Detection of Influenza Virus Using the PDA/Protein G Mixture–Coated Substrate

After optimal antibody immobilization on the PDA/protein G mixture–coated substrate, we applied this substrate to an immunoassay for pH1N1 influenza virus. Influenza viruses cause acute respiratory diseases, and influenza epidemics generate enormous costs to society each year.[22] Hence, a number of virus detection techniques have been developed to prevent the spread of diseases and to support the initiation of rapid antiviral therapy.[23] In general, immune reaction–based diagnosis methods, such as enzyme-linked immunosorbent assay and the rapid influenza diagnostic test, have been commercially used for the detection of viruses.[24] These methods typically show a sensitivity around 10^3–10^4 $TCID_{50}$ mL^{-1} of virus concentration.[25] Recently, innovative sensing strategies have been developed by using nano- and microstructures, contributing to the sensitive and accurate diagnosis of influenza viruses.[26] However, many immunosensing approaches still require specific antibody immobilization steps, depending on the types of materials and structures. If an antibody immobilization method can be established with a single optimal protocol, it may reduce the inconvenient steps needed to find the antibody immobilization method for each substrate and improve the reproducibility of immunosensors.

Figure 3a is a schematic illustration of the pH1N1 detection procedure with naked eye. To investigate the applicability of the PDA/protein G–coated substrate, we adopted the simple naked-eye immunoreaction protocol.[27] For the detection of pH1N1 influenza virus, the glass substrate was coated by using the optimized PDA/protein G mixture (1:1 volume mixture of 2 mg mL^{-1} dopamine and 30 mg mL^{-1} protein G). Subsequently,

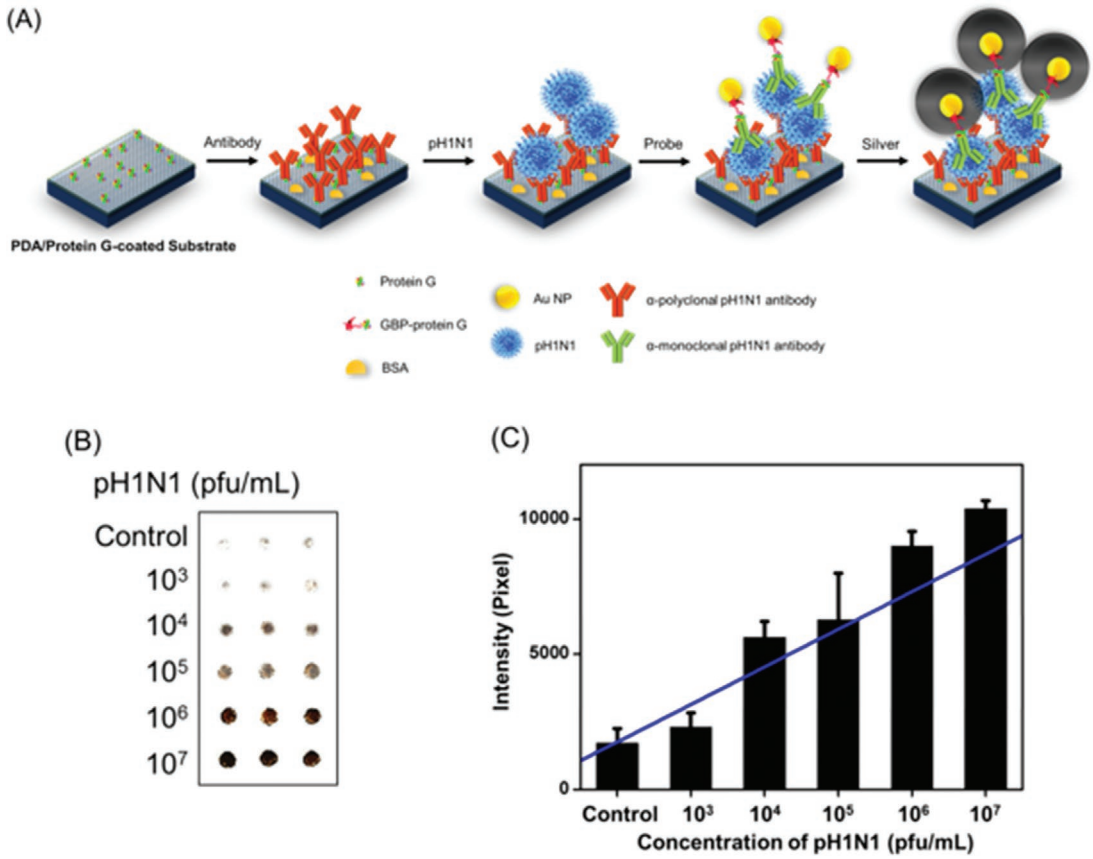


Figure 3. A) Schematic illustration of pH1N1 detection by the naked eye by using PDA/protein G mixture-coated substrates. B) Grayscale image of the Ag-enhanced substrate after pH1N1 virus detection (from 0 to 10^7 pfu mL^{-1}) on PDA/protein G mixture-coated surfaces. C) Plot of 8-bit grayscale values depending on the pH1N1 concentration. Blue line is the linearly fitted line. The data represent the mean plus standard deviation from three measurements.

a polyclonal antibody against pH1N1 was treated on the PDA/protein G–coated substrate, and BSA was added to prevent non-specific binding. Next, a solution containing pH1N1 was dropped onto the immune substrate, and the immunoprobes were applied. The immunoprobes were prepared by mixing Au nanoparticles (NPs), gold-binding peptide (GBP)-protein G, and an anti-pH1N1 monoclonal antibody. After the binding of the immunoprobes to the immune substrate, the immunoprobes were enhanced using an Ag enhancer solution, and the enlarged immunoprobes were observed by the naked eye. When the PDA is coated on the surface, the color of the surface can change to dark gray.[12] This dark surfaces could have a negative effect in distinguishing between the control and pH1N1 samples with naked eye. Thus, we tried to minimize the color changes during the PDA/protein G coating step. By using 2 mg mL^{-1} of dopamine solution, we were able to modify the surface with protein G in a very thin film state, providing less color change of the substrate.

Figure 3b shows a grayscale image of the naked-eye detection of the pH1N1 influenza virus (0, 10^3, 10^4, 10^5, 10^6, and 10^7 pfu mL^{-1}). In the blank sample, the spots are very faint. In the 10^3 pfu mL^{-1} pH1N1 sample, the spots appear slightly dark. At pH1N1 concentrations above 10^4 pfu mL^{-1}, dark spots are clearly observable. This result verifies that the PDA/protein G mixture–based antibody immobilization method can be applied to the detection of pH1N1. Figure 3c shows a plot of the 8-bit grayscale values as a function of the pH1N1 concentration. The intensity of the grayscale level is proportional to the pH1N1 concentration within the range of 10^3 to 10^7 pfu mL^{-1}, demonstrating the feasibility of quantitative influenza virus detection. We estimated the limit of detection (3.3σ per slope) to be 2.9×10^3 pfu mL^{-1}. σ is the standard deviation of the blank sample measured thrice. This detection limit is comparable to those of the previously reported influenza virus immunoassays which provided the detection limit of 10^3–10^4 pfu mL^{-1} level.[28–30] The sensitivity test was also performed using sandwich ELISA, and the result of the pH1N1 virus concentration-dependent absorbance value showed low detection efficiency compared to this study (Figure S4, Supporting Information).

To further examine the selectivity of this immunoassay, four types of viruses (pH1N1, H3N2, H5N2, and influenza B virus) were tested. The concentration of each virus was 10^6 pfu mL^{-1}. **Figure 4**a shows a grayscale image of naked-eye detection in the presence of four kinds of viruses. Strong dark spots are clearly observed only in the presence of the pH1N1 influenza virus, whereas weak spots are noted in the presence of the H3N2, H5N2, and influenza B viruses. A plot of grayscale values versus the type of influenza virus also confirmed the specificity

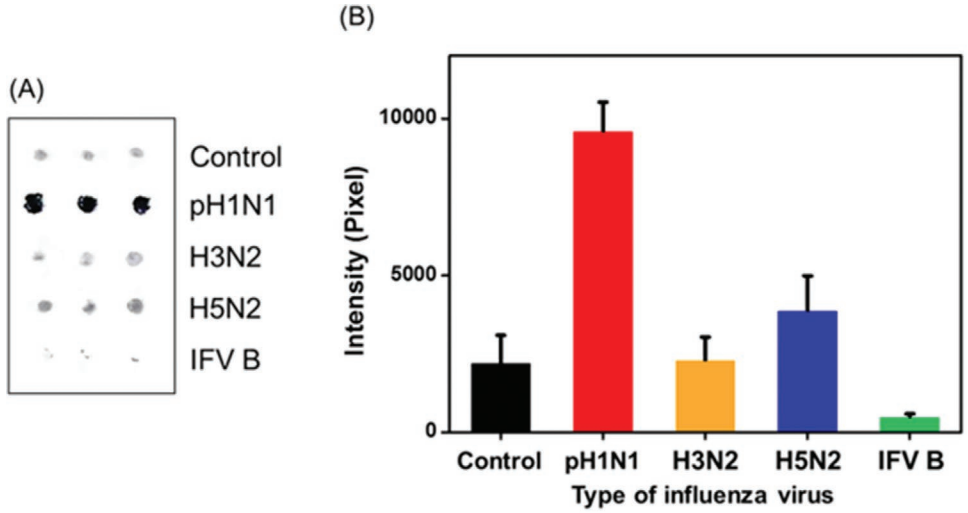


Figure 4. A) Grayscale image and B) plot of 8-bit grayscale values depending on the type of influenza virus (pH1N1, H3N2, H5N2, and influenza B virus). The concentration of each virus was 10^6 pfu mL^{-1}. The data represent the mean plus standard deviation from three measurements.

of this immunoassay for pH1N1 (Figure 4b). The specificity of used antibodies (anti-pH1N1 polyclonal and monoclonal antibody) was further confirmed using a conventional sandwich ELISA method, and the result provided a reliable result of the specificity test (Figure S5, Supporting Information). Taken together, these results suggest that the present antibody immobilization method could be employed for the development of immunosensors.

2.4. Surface-Independent Immobilization of Antibody and Detection of Influenza Virus

Dopamine has the property that it can be polymerized on any kind of material surface;[12] therefore, we expected that protein G mixed with dopamine solution could be coated on the surfaces regardless of the type of material. When the PDA/protein G mixture was successfully coated on the surfaces, the antibody could also be immobilized on the surfaces, as depicted in Scheme 1. We tried to modify several kinds of substrates, such as glass, SiO_2, Si, Al_2O_3, PET, PE, PP, and paper, by using the PDA/protein G mixture. Each substrate was incubated with the PDA/protein G mixture for 2 h at room temperature. Next, a polyclonal anti-pH1N1 antibody was treated on the PDA/protein G–coated surfaces for 1 h at room temperature, followed by BSA treatment. The resultant substrates were employed for the naked-eye sensing of pH1N1. **Figure 5** shows grayscale images of the naked-eye detection of pH1N1 influenza virus on the various kinds of substrates. Only in the presence of the pH1N1 influenza virus were dark spots observed on the substrates. In the blank sample, the spots were very weak. The images show that pH1N1 was successfully detected by the naked eye regardless of the surface. Even on the dark-colored Si and SiO_2 substrates, the influenza viruses could be detected by the naked eye. This result demonstrated that the proposed PDA/protein G mixture is able to modify a variety of substrates and further enables optimal antibody immobilization. This finding suggests that this simple and easy surface modification method can be adopted to develop various types of immunosensors. Although the previous surface modification methods for antibody

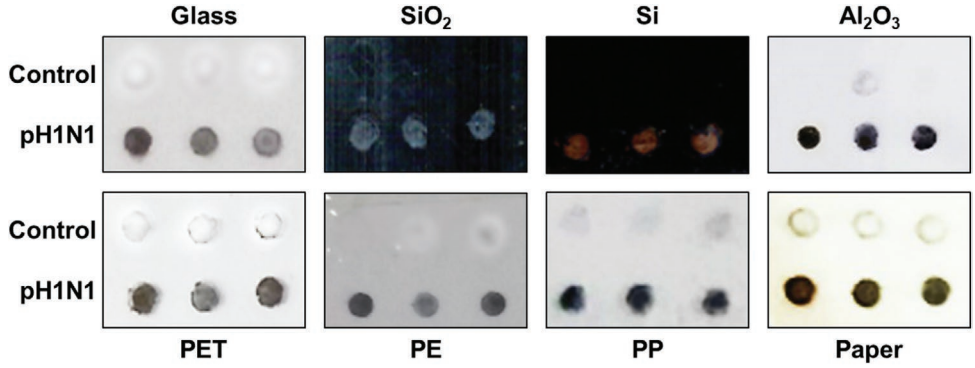


Figure 5. pH1N1 detection on various types of substrates (glass, SiO_2, Si, Al_2O_3, PET, PE, PP, and paper). All substrates were prepared in the same manner, with PDA/protein G mixture coating and immobilization of an antibody against pH1N1.

immobilization are complicated and depend on the type of substrate, the present PDA/protein G–based coating technique is expected to overcome these obstacles.

3. Conclusion

We developed a simple and useful surface modification method based on a PDA/protein G mixture, which allows optimal antibody immobilization on a variety of substrates. PDA/protein G coating and antibody immobilization on the substrate were successfully demonstrated by XPS and fluorescence. Moreover, the proposed surface modification method was applied to the detection of the influenza pH1N1 virus with naked eye. The influenza virus was detected with proper sensitivity and selectivity on the PDA/protein G/antibody-modified surface. Furthermore, the surface-coating method enabled the detection of influenza virus regardless of the type of substrate. We anticipate that the present approach will be applicable to a wide range of substrates and provide a convenient procedure for the development of biosensors.

4. Experimental Section

Materials: Dopamine hydrochloride (H8502), Tween 20 (P9416), Au NPs in phosphate-buffered saline (PBS) (753 610), BSA (A7906), and Ag enhancer solution A and B (S5020, S5145) were purchased from Sigma-Aldrich (St. Louis, USA). 1 M Tris-HCl, pH 8.5 (T2016-8.5) was purchased from Biosesang (Daejeon, Korea). GBP-protein G was purchased from Bioprogen (Daejeon, Korea). Protein G (77 676) was purchased from Thermo Fisher Scientific. Influenza A/CA/07/2009 (pH1N1) and influenza B/Victoria/Brisbane/60/2008 (IBV) suspended in cell culture medium (MEM medium, MDCK cells) were provided by the Korea Centers for Disease Control and Prevention, Korea. Influenza A/Brisbane/10/2007 (H3N2) and influenza A/aquatic bird/Korea/w351/2008 (H5N2) suspended in cell culture medium (MEM, MDCK cells) were obtained from Korea Research Institute of Bioscience and Biotechnology, Korea. Each virus titer of the stock solutions ranged between 5×10^6 and 5×10^7 pfu mL^{-1} and was determined by real-time reverse transcription polymerase chain reaction, according to a previous report.[31] The monoclonal anti-influenza A H1N1 antibody (ab128412) and goat anti-rabbit IgG H&L (HRP) (ab6721) were purchased from Abcam. The polyclonal anti-influenza A H1N1 (Swine Flu 2009) antibody (11 055-RP02) was purchased from Sino Biological. Alexa Fluor 647-FluoroNanogold rabbit anti-goat IgG (H+L) (7505) was purchased from Nanoprobes (New York, USA). TMB substrate reagent set was purchased from BD Biosciences. Microscopy slides (1 000 412) were purchased from Marienfeld (Germany). The Si, SiO_2, and Al_2O_3 substrates were purchased from Hi-Solar Co., Ltd. PE, PET, PP, and paper were purchased from a local market.

Instrumentation: XPS spectra were obtained by using a Thermo K-Alpha XPS (Thermo Fisher Scientific, USA). The XPS peak was analyzed by using a Thermo Scientific Advantage Data system. The AFM images were obtained in a non-contact mode by using a Scanning Probe Microscope (Nanoman, VEECO, New York, USA). The scan range was 5.0×5.0 μm, and the scan rate was 0.9 Hz. The film thickness of surfaces was measured by an ellipsometer (Elli-SE, Ellipso Technology Co., Suwon, South Korea). The incident angle was 70° and the refractive index was 1.46. More than five different points on each sample were measured and the average values were obtained. Absorbance was measured by using a BioTek Cytation 5 (Thermo Fisher Scientific, USA). Fluorescence images were obtained with a scanner (GenePix 4200A, USA), and the data were analyzed with ImageJ software (NIH; Bethesda, Maryland, USA). Grayscale images were obtained by using an optical flatbed scanner (SCX-4210) with a resolution of 600 dpi, and the data were analyzed with an 8-bit grayscale histogram using ImageJ software (NIH; Bethesda, Maryland, USA).

Surface Modification: For PDA coating, a dopamine solution (2 mg mL^{-1}) in Tris-HCl buffer (10 mM, pH 8.5) was applied to the surface for 2 h at room temperature. For coating with PDA/protein G, 2 mg mL^{-1} dopamine hydrochloride in Tris buffer solution (10 mM, pH 8.5) and various concentrations of protein G (10–50 mg mL^{-1}) in pure water were mixed at a 1:1 volume ratio. Each type of substrate was immersed in this solution for 2 h at room temperature.

Immobilization of a Fluorescent Antibody: A fluorescent antibody was prepared at concentrations of 8.0 μg mL^{-1} and 1.6 μg mL^{-1} by using dilution buffer containing 0.05% Tween 20 and 1% nonfat milk. The fluorescent antibodies were incubated with the PDA/protein G–coated surface for 1 h at room temperature. The surface was washed with 100 mM PBS buffer containing 0.1% Tween 20.

Sandwich-ELISA: Anti-pH1N1 monoclonal antibody, 1 μg mL^{-1}, diluted in 5% skim milk, was coated on a 96-well plate overnight at 4 °C. The plate was washed thrice with PBS buffer containing 0.05% Tween 20 (washing buffer). Skim milk, 5%, was incubated for 1 h at 37 °C, and the plate was washed thrice using washing buffer. Virus samples diluted with PBS buffer were incubated for 2 h at 37 °C and then the plate was washed thrice using washing buffer. Anti-pH1N1 polyclonal antibody, 1 μg mL^{-1}, diluted in 5% skim milk, was added on the plate for 2 h at 37 °C. After washing the plate thrice with washing buffer, 2 μg mL^{-1} of goat anti-rabbit IgG conjugated to HRP antibody, diluted in 5% skim milk, was treated on the wells for 1 h at 37 °C. The plate was washed thrice with washing buffer. TMB substrate reagent, prepared by mixing reagent A and B, was incubated for 20 min at room temperature. Absorbance was measured after addition of 2 M of sulfuric acid.

Preparation of the Immune Substrate: The bare substrate was modified with a dopamine (2 mg mL^{-1}) and protein G (30 mg mL^{-1}) mixture solution for 2 h at room temperature. The modified surface was washed with 100 mM PBS buffer containing 0.1% Tween 20. A polyclonal antibody (0.1 mg mL^{-1}) specific for the pH1N1 influenza virus was applied to the substrate for 1 h at room temperature and washed with 100 mM PBS buffer containing 0.1% Tween 20. BSA (0.1 mg mL^{-1}) was treated to the antibody-attached surface for 30 min to prevent non-specific binding.

Preparation of the Immunoprobe: GBP-protein G (0.1 mg mL^{-1}) was applied to 20 nm Au NPs in PBS (0.1 mM, pH 7.0) for 16 h at 4 °C. Unbound GBP protein G was removed by centrifugation at 12 000 rpm for 10 min. Au NP-GBP-protein G probes were resuspended in 10 mM PBS containing 0.05% Tween 20. A monoclonal antibody (10 μg mL^{-1}) against pH1N1 was added to the Au NP-GBP-protein G probes in 10 mM PBS containing 0.05% Tween 20 for 2 h at room temperature. After separation of the unbound antibody by centrifugation at 12 000 rpm for 10 min, Au NP-GBP-protein G-antibody probes were obtained.

Detection of Influenza Virus: A pH1N1 solution (3.5 μL) was dropped onto the capture substrate for 1 h at room temperature. The substrate was washed with 100 mM PBS containing 0.1% Tween 20. The pH1N1-captured substrate was exposed to the antibody-Au NP probes for 1 h at room temperature and washed with 100 mM PBS containing 0.1% Tween 20. Ag enhancer solutions A (Ag salt) and B (Initiator) were then mixed at a 1:1 volume ratio and applied to the substrate for 10 min. The resultant Ag-enhanced substrate was washed with ultrapure water and dried under nitrogen gas.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

antibody immobilization, immunoassay, polydopamine, protein G, surface modification

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[1] I. A. Darwish, *Int. J. Biomed. Sci.* **2006**, *2*, 217.
[2] N. B. Ramírez, A. M. Salgado, B. Valdman, *Braz. J. Chem. Eng.* **2009**, *26*, 227.
[3] B. R. Li, C. C. Chen, U. R. Kumar, Y. T. Chen, *Analyst* **2014**, *139*, 1589.
[4] Z. Wang, S. Zong, L. Wu, D. Zhu, Y. Cui, *Chem. Rev.* **2017**, *117*, 7910.
[5] A. K. Trilling, J. Beekwilder, H. Zuilhof, *Analyst* **2013**, *138*, 1619.
[6] M. D. Sonawane, S. B. Nimse, *J. Chem.* **2016**, *2016*, 1.
[7] W. Hu, C. M. Li, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.* **2011**, *3*, 119.
[8] C. Menti, M. Beltrami, M. D. Pozza, S. T. Martins, J. A. P. Henriques, A. D. Santos, F. P. Missell, M. Roesch-Ely, *Mater. Sci. Eng., C* **2017**, *76*, 1232.
[9] J. Seo, S. Lee, C. D. Poulter, *J. Am. Chem. Soc.* **2013**, *135*, 8973.
[10] E. S. Kim, C. K. Shim, J. W. Lee, J. W. Park, K. Y. Choi, *Analyst* **2012**, *137*, 2421.
[11] Y. Liu, K. Ai, L. Lu, *Chem. Rev.* **2014**, *114*, 5057.
[12] H. Lee, S. M. Dellatore, W. M. Miller, P. B. Messersmith, *Science* **2007**, *318*, 426.
[13] S. M. Kang, N. S. Hwang, J. Yeom, S. Y. Park, P. B. Messersmith, I. S. Choi, R. Langer, D. G. Anderson, H. Lee, *Adv. Funct. Mater.* **2012**, *22*, 2949.
[14] Y. J. Chuah, Y. T. Koh, K. Lim, N. V. Menon, Y. Wu, Y. Kang, *Sci. Rep.* **2016**, *5*, 18162.
[15] Y. Zhang, M. E. Lynge, B. M. Teo, R. Ogaki, B. Stadler, *Biomater. Sci.* **2015**, *3*, 1188.
[16] L. Su, Y. Yu, Y. Zhao, F. Liang, X. Zhang, *Sci. Rep.* **2016**, *6*, 24420.
[17] M. Toma, K. Tawa, *ACS Appl. Mater. Interfaces* **2016**, *8*, 22032.
[18] V. Ball, DF. Doriane, T. Valrie, R. David, *J. Colloid Interface Sci.* **2012**, *386*, 366.
[19] H. Guo, Z. Yao, J. Wang, Z. Yang, X. Ma, C. Y. Tang, *J. Membr. Sci.* **2018**, *551*, 234.
[20] H. Lee, J. Rho, P. B. Messersmith, *Adv. Mater.* **2009**, *21*, 431.
[21] S. Ghose, B. Hubbard, SM. Cramer, *Biotechnol. Bioeng.* **2007**, *96*, 768.
[22] M. E. Klepser, *Drugs* **2014**, *74*, 1467.
[23] S. V. Vemula, J. Zhao, J. Liu, X. Wang, S. Biswas, I. Hewlett, *Viruses* **2016**, *8*, 96.
[24] D. K. Kim, B. Poudel, *Yonsei Med. J.* **2013**, *54*, 560.
[25] J. Moon, S. Y. Yi, A. Hwang, G. Eom, J. Sim, J. Jeong, E. -K. Lim, B. H. Chung, B. Kim, J. Jung, T. Kang, *Rsc Adv.* **2016**, *6*, 88415.
[26] W. S. Lee, T. Kang, S. H. Kim, J. Jeong, *Sensors* **2018**, *18*, 307.
[27] S. Y. Yi, U. Lee, B. H. Chung, J. Jung, *Chem. Commun.* **2015**, *51*, 8865.
[28] S. G. Hwang, K. Ha, K. Guk, D. K. Lee, G. Eom, S. Song, T. Kang, H. Park, J. Jung, E. K. Lim, *Sci. Rep.* **2018**, *8*, 12999.
[29] H. J. Park, S. C. Yang, J. Choo, *Bull. Korean Chem. Soc.* **2016**, *37*, 2019.
[30] K. Kang, L. Chen, X. Zhao, C. Qin, Z. Zhan, J. Wang, W. Li, E. E. Dzakah, W. Huang, Y. Shu, T. Jiang, W. Cao, M. Xie, X. Luo, S. Tang, *PLoS One* **2014**, *9*, e92306.
[31] Y. M. Deng, N. Spirason, P. Iannello, L. Jelley, H. Lau, I. G. Barr, *J. Clin. Virol.* **2015**, *68*, 43.