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RESEARCH ARTICLE

Surface-Enhanced Raman Scattering of Dopamine on Self-Assembled Gold Nanoparticles

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Dopamine, a potent neurotransmitter in the brain, influences a variety of motivated behaviors and plays a major role in Parkinson's disease. In this study, the Raman signal of dopamine was detected on a fabricated nanoparticle-immobilized glass surface by surface-enhanced raman spectroscopy (SERS). Amine-modified glass was prepared by the self-assembly of amine-terminated silane on substrate, followed by the deposition of gold nanoparticles. The gold nanoparticles deposited on the glass surface were functionalized by anti-dopamine or dopamine. The antigen-dopamine was captured by antibody-assembled gold substrate and detected by SERS. The optical properties and morpology of the glass substrate with immobilized gold nanoparticles were analyzed by scanning electron microscopy and UV-VIS absorption spectroscopy. The Raman spectrum of dopamine displayed broad bands at 1267, 1331, 1158, 1478, 1578 and 1584 cm^{-1}. The strongest peaks in the spectra (at 1267 and 1478 cm^{-1}) were identified as phenolic carbon-oxygen and phenyl C=C stretches, respectively. A working curve of the SERS signal constructed from cathecol ring vibration versus antigen-dopamine concentration was obtained at 1478 cm^{-1}, and the non-optimized detection limit for anti-dopamine surface antigen was as low as 1 ng/ml. These results suggest that SERS-based immunosensor can be a promising tool for the detection and screening of neurotransmitters.

Keywords: Surface-Enhanced Raman Spectroscopy, Dopamine, Gold Nanoparticles, Anti-Dopamine, Immunosensor.

1. INTRODUCTION

Dopamine (DA), a potent neuromodulator in the brain, plays an important role in controlling movement and motivational/cognitive functions such that an imbalance in dopaminergic activity is thought to underlie several neuropsychiatric disorders.[1,2] The dopamine transporter is a membrane-bound protein expressed exclusively within dopaminergic neurons that recaptures dopamine released into presynaptic terminals, thereby limiting any spatial and temporal action.[3,4] The dopamine transporter is a major regulator of extracellular dopamine, and therefore understanding its mechanism of regulation may provide insights into the pathophysiology of many dopamine-related diseases.[5] Significant clinical knowledge would thus be obtained by physiologically evaluating dopamine with high sensitivity and selectivity. Detection of dopamine has been reported previously by various methods, including as fluorometry, chromatography and electrochemical technology.[6–8] The physiological concentrations of dopamine throughout the body are very low (from 0.01 to 1 μM in the extracellular fluid of the central nervous system), requiring a highly selective and sensitive detection method.[9–10]

Raman spectroscopy is a non-invasive technique that provides detailed molecular information.[11] However, normal Raman scattering is an extremely inefficient process characterized by a lack of intensity and sensitivity.[11] Generally, Raman spectroscopy results in low scattering cross-sections approximately 14 orders of magnitude smaller than the absorption cross-sections of florescent dye molecules and is limited as a readout method for immunoassays.[12] In order to achieve high sensitivity for a given sample, the scattering intensity should be greatly increased over currently available levels.[13] Surface-enhanced Raman spectroscopy (SERS) using metal nanoprobes has shown promise in increasing the low sensitivity inherent in Raman spectroscopy.[14] SERS enhances Raman signal intensity by 10^6–10^{14} fold and thus is sufficient to detect pico- to femtomolar amounts of biomolecules.[15] Functionalized SERS

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nanoparticles in the presence of Raman-active molecules are typically used for the detection, sensing or imaging of biological samples such as DNA, proteins, cells and tissues.[16] Especially, SERS has confirmed the viability of substrates such as microlithographically-prepared Ag posts, electron-beam lithographically-produced arrays of elongated Au nanoparticles, nanosphere lithographically-produced triangular nanoparticle arrays, gold-nanostructed films deposited on polystryrene colloidal crystal templates, and Au template particles grafted on silanized glass.[17–18] Additionally, the chemical synthesis of the Ag or Au SERS substrates has rapidly progressed in recent years due to the special optical properties of the substrates and their potential application to plasmonics and sensors.[12, 19]

In this study, we examined the functionalization of gold nanoparticles immobilized on a glass substrate by anti-dopamine and dopamine. Amine-modified glass was prepared by self-assembly of amine-terminated silane on substrate, and gold nanoparticles were deposited on the amine-modified glass substrate followed by functionalization by anti-dopamine and dopamine. We investigated the function and conformation of the fabricated nanoparticle-immobilized glass chip and verified the dopamine immobilization procedure by SERS and UV-VIS spectrophotometry. The binding activity of antigen-dopamine to antibody immobilized onto the glass surface was also examined. Elucidating the mechanism by which representative vibration bands are completely enhanced would therefore provide new insights into the SERS of dopamine-conjugated gold nanoparticles.

2. EXPERIMENTAL DETAILS

2.1. Materials and Synthesis of Dopamine-Bovine Serum Albumin (BSA) Conjugate

3,4-Dihydroxyphenethylamine (dopamine) was obtained from Sigma (USA). Au sol was purchased from Polysciences (60 nm particles, Washington, PA). Anti-dopamine was obtained from Abcam (UK). All other reagents used to prepare the and solutions were of analytical grade. Assembly of gold nanoparticles required a few steps. BSA was used as the antigen in preparation of dopamine-BSA conjugate.[20] Equimolar quantities (100 μM) of dopamine (18.9 mg), N-Hydroxysuccinimide (NHS, 11.5 mg) and N,N′-dicyclohexylcarbodiimide (DCC, 20.6 mg) were dissolved in 2 ml of dioxane. The mixture was then sonicated for 5 min, followed by incubation for 24 h at room temperature (24 ± 1 °C) and evaporation of the solvent under a gentle stream of nitrogen gas. The resulting residue was mixed with a solution of 280 mg (ca, 42 μM) of BSA in 3 ml of PBS (pH 7.2), which was sonicated for 2 min. After incubation at room temperature for 3 h, the conjugated mixture was dialyzed in PBS buffer at low temperature (3–5 °C) for 3 days and in distilled water for 1 day. Finally, the dopamine-BSA conjugate was lyophilized and stored at −20 °C.

2.2. Deposition of Gold Nanoparticles on Glass Substrate

The substrate upon which the colloidal gold layer was assembled was a commercially available glass slide (Matsunami, 20 × 20 mm) cut into four pieces. Glass substrates were cleaned overnight in diluted alkaline detergent, then washed extensively with distilled water and dried before use. The cleaned glass substrate was immersed in a 5% (v/v) solution of 3-aminopropyltrimethoxysilane (APTMS, Tokyo Kasei Kogyo) in toluene for 5 min, followed by rinsing five times in ethanol with sonication for 5 min and two times in water for 5 min. The silanized glass substrates were rinsed with distilled water and immersed in colloidal gold suspension for 3 h to fabricate a gold nanoparticle layer on both sides of the substrate. A conventional UV/VIS spectrophotometer (v630, JASCO, Japan) was used to measure the transmission spectra of Au nanoparticles deposited on the glass slide.

2.3. Functionalization of Immobilized Gold Nanoparticles on Glass Substrate

Gold nanoparticles deposited on glass were chemically modified by the formation of a self-assembled monolayer of 11-mercaptoundecanoic acid (Sigma Aldrich). The gold nanoparticle-immobilized substrate was immersed in ethanol and then incubated in a 10 mM solution of MUA in ethanol for 10 min. The substrate was then rinsed with ethanol and distilled water in successive fashion. This procedure requires very careful handling of the Au nanoparticle-deposited substrate since rinsing with or immersion in ethanol may cause mixing of water and ethanol on the substrate surface and therefore aggregation of Au nanoparticles. The MUA-attached, gold nanoparticle-deposited substrate was then immersed in an aqueous solution containing 0.1 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (WSC, Dojindo) and 0.1 M N-hydroxysuccinimide (NHS, Sigma) for 2 h. Following this, the COOH terminus of MUA was activated as a succinimide ester. For functionalization of dopamine (Sigma), a glass chip activated by NHS was incubated in 10 mM sodium acetate aqueous solution containing 10 mM dopamine for 3 h. The functionalized glass chips were stored at 4 °C until further use.

2.4. Characterization of the Fabricated Biosurface of Au Substrate

Proteins immobilized on the nanoparticles were determined by UV-VIS spectrophotometry.[21] The surface morphologies were analyzed by scanning electron microscopy (SEM) (ISI DS-130C, Akashi Co., Tokyo, Japan).

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2.5. Raman Spectrophotometry

Raman spectra were equipped with an infrared laser, a spectrometer and a liquid nitrogen-cooled CCD detector (NTEGRA spectra, NTMDT, Russia). A laser (785 nm) was used to measure excitation spectra, which were recorded between 400–2000 cm^{-1}.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Fabricated Biosurface on Dopamine-Conjugated Au Substrate

A schematic drawing of the Au nanoparticles and dopamine is shown in Figure 1. The glass surface was functionalized by amine using APTMS, followed by the adsorption of Au nanoparticles onto the amine-modified glass surface by electrostatic interaction.[22] Following this, a self-assembled monolayer of mercaptoundecanoic acid was fabricated on the deposited Au nanoparticles. Dopamine was then covalently immobilized to the monolayer following activation of the COOH terminus of mercaptoundecanoic acid by NHS.

The SAM image of the immobilized gold colloidal nanoparticles is shown in Figure 2. The average particle diameter was about 60 nm. Obviously, most gold nanoparticles self-assembled in close proximity to each other by forming randomly distributed one-dimensional patterns, whereas only a few isolated gold nanoparticles assembled on the glass substrate.[23–26]

The UV-VIS absorption spectrum of the immobilized gold colloidal nanoparticles after dopamine addition is illustrated in Figure 3 along with the spectrum of the gold colloidal suspension and dopamine solution. The existence of these particles in the UV spectral region ensures that resonant Raman scattering makes no contribution to SERS when visible and near-infrared excitations are employed. The plasmon resonance absorption of the gold colloidal nanoparticle suspension was observed at 532 nm. The absorption spectrum of the dopamine-linked substrate displays a sharp and symmetric peak centered around 523 nm. The molecular deposition of Au nanoparticles increases the absorbance and blue shift of the peak spectra. Due to a high affinity to gold, dopamine molecules are expected to be bound not only to the open surface of nanoparticles but also to sites in the gaps and junctions created between neighboring nanoparticles. Since the environmental dielectric constant is altered by the molecular layer, molecules inside interstitial regions are more effective for interparticle coupling.

3.2. SERS of Dopamine

The immobilization of dopamine on a self-assembled gold substrate was examined by Raman spectroscopy. SERS phenomena using Au nanoparticles have been previously well studied based on the fact their signals can be

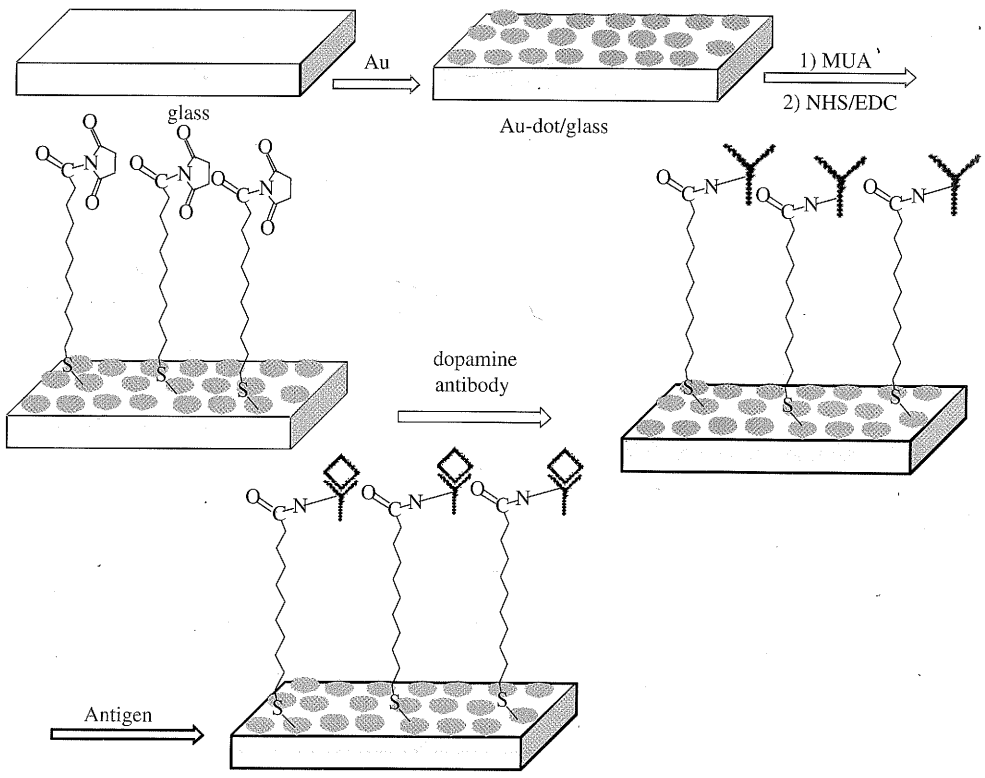

Fig. 1. Schematic drawing of the process of assembling antibodies onto the gold nanoparticle surface.

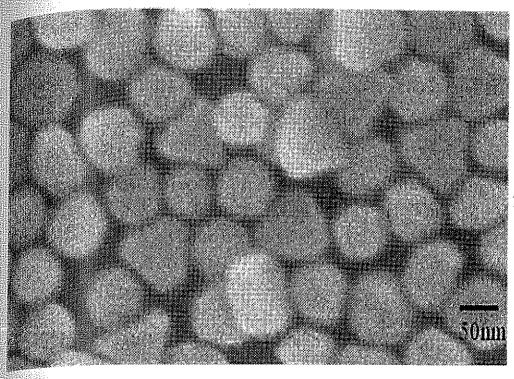

Fig. 2. Scanning electrode microscopic image of the SERS substrate through immobilized gold nanoparticles.

identified at the single-molecule level by association with the resonance effect of the adsorbed molecule.[26] Au nanoparticles also revealed the SERS effect, showing that molecules adsorbed to nanoparticles can be detected at the monolayer level even in off-resonance mode.[27] The Raman spectrum of the control substrate showed broad bands at 563, 627, 705, 857, 895, 1262, 1327, 1388 and 1693 cm^{-1}. The 1464 cm^{-1} band was the most intense and as such was subsequently used for recording of SERS spectra; it also deviates from first-order resonance of the gold particles. Figure 4(A) illustrates the wave number range of the obtained SERS spectra (500–2000 cm^{-1}). Specifically, peaks that appear are those most affected by adsorption, along with the conventional Raman spectrum of the test molecule excited by a 785 nm laser. It should be noted that the conventional Raman spectra were all recorded using excitation lines, but only one was selected due to similarities. It should be also noted that SERS spectra recorded from different spots on the surface are identical, which demonstrates reproducibility of the SERS-substrate. Increasing the dopamine concentration also increased the spectra patterns of SERS (Fig. 4(B)). As revealed in Figure 4(B), striking differences exist between the Raman

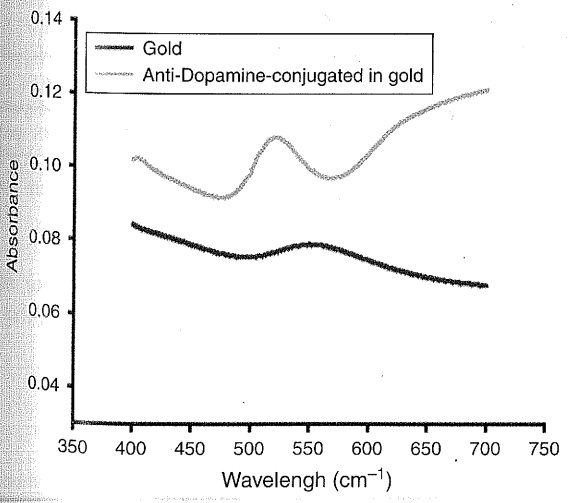

Fig. 3. UV-VIS absorption spectra of the gold substrate and anti-dopamine conjugated onto self-assembled gold substrate.

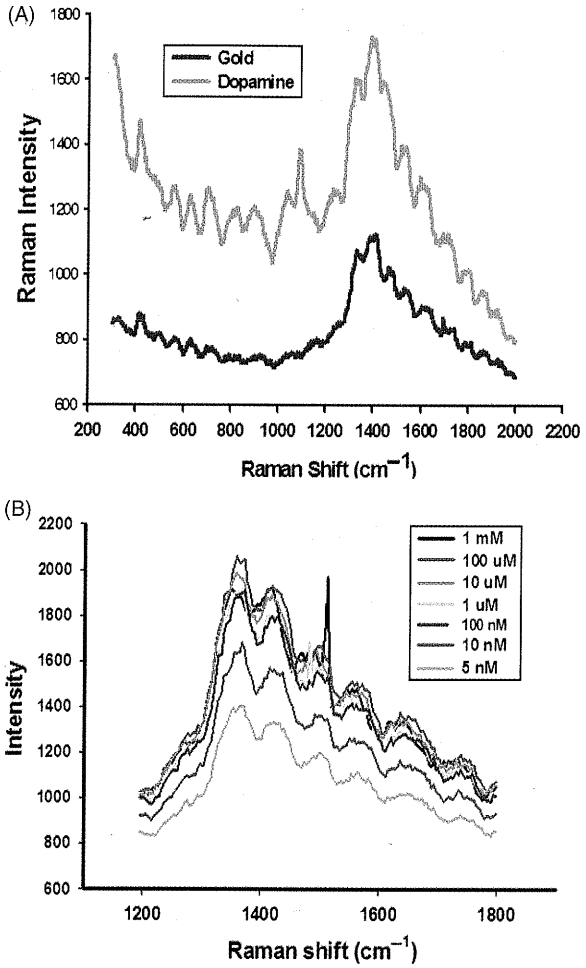

Fig. 4. The SERS spectra of different concentrations of dopamine adsorbed onto self-assembled gold nanoparticles at 785 nm. (A) The SERS spectra of dopamine was detected in the wavenumber range from 300 to 2000 cm^{-1}. (B) The concentration of dopamine was detected by SERS. Limited detection was observed at a dopamine concentration of 1 nM.

and SERS spectra. The Raman spectrum of a dopamine-adsorbed substrate showed broad bands at 1267, 1331, 1158, 1478, 1578 and 1584 cm^{-1}. These are also similar to the SERS spectra of metal catecholates and can be ascribed to catechol ring vibration and carbon–oxygen stretches. The 1478 cm^{-1} band indicates a catechol ring while the 1267 cm^{-1} wavelength is identified as the C–O stretch. The Raman scattering of adsorbed dopamine showed a υ_3 band at 1331 cm^{-1} and a υ_{8b} band at 1584 cm^{-1}, both of which are in good agreement with the spectra reported in the literature.[28–29] The results presented here suggest that a dopamine-linked Au nanoparticle substrate is an effective tool for studying enhancement mechanisms and for performing other SERS-based measurements. Thus, the similarities found between the SERS spectra of dopamine adsorbed on silver island films and the immobilized gold colloidal nanoparticles support the conclusion that charge-transfer is effective in enhancing Raman spectra recorded with a 785 nm laser line.[28–29]

3.3. Raman Spectrum of Antigen-Dopamine and Anti-Dopamine

Currently, there is no reliable method for conjugating dopamine for use in immunology or biosensor design in so far existing functional groups are not compromised.[30] We therefore employed a SERS-based immunosensor for the detection of low molecular weight molecules of biomedical interest with antibodies as the recognition molecules. Since dopamine is a small molecule, we detected it by investigating the principle of antibody-antigen binding. In the present study, dopamine was immobilized on SERS substrate using technology that employs the *in situ* addition of linkers followed by attachment. Monoclonal anti-dopamine antibody was applied to the surface to assess binding. Figure 4 depicts the SERS spectra of Au nanoparticle-deposited glass before and after dopamine binding in the spectra range of 400–2000 cm^{-1}. In Figure 5(A), the Raman spectrum of anti-dopamine showed strong peaks at 1047, 1151 and 1318 cm^{-1}. At 1047 cm^{-1}, the spectrum peaks belong to a C–C stretch of the Y conformer.[28–29] In Figure 5(B), the spectra of antigen dopamine appeared at 1235, 1323, 1388, 1473, 1478, 1553, 1586 and 1631 cm^{-1}. The antigen concentration-dependent SERS spectra changes in Figure 5(C). The results illustrate a change in intensity at 1478 cm^{-1} that illustrates cathechol ring vibration versus the concentration of antigen-dopamine. When the concentration of the antigen-dopamine is above 20 μg/ml, the intensity of the SERS signal at 1478 cm^{-1} increases rapidly. On the other hand, when the concentration of antigen-dopamine is 1 ng/ml, it is difficult to detect the dopamine signal from the SERS spectrum. Finally, we developed a calibration model that predicts the concentration of antigen in the range of 10 ng/ml–20 μg/ml. This may be explained as follows: for a antigen higher concentration, there are more immunogold nanoparticles on the chips. Thus, many gold aggregates are deposited onto the immunogold nanoparticles, leading to a higher gold background in the Raman spectra. These peaks showed different patterns than those of the dopamine-linked Au substrate. The Raman spectra of antigen-dopamine and anti-dopamine immobilized on gold substrate showed peaks at 1267, 1478 and 1578 cm^{-1}.[28–29] However, the peaks at 1267, 1331, 1158, 1478, 1578 or 1584 cm^{-1} [28–29] were not detected in the Raman spectra of antigen-dopamine. The immunoassay was performed with antibody consisting of gold layer. Thus, the results presented here suggest that the anti-dopamine assembled substrate is an effective tool for studying enhancement mechanisms as well as for performing other SERS-based measurements in the visible and near-infrared spectral regions. As a novelty, we obtained reproducible wavelength-scanned SERS measurements in a wide spectral range using the same substrate. In contrast to previous studies which present SERS spectra recorded with

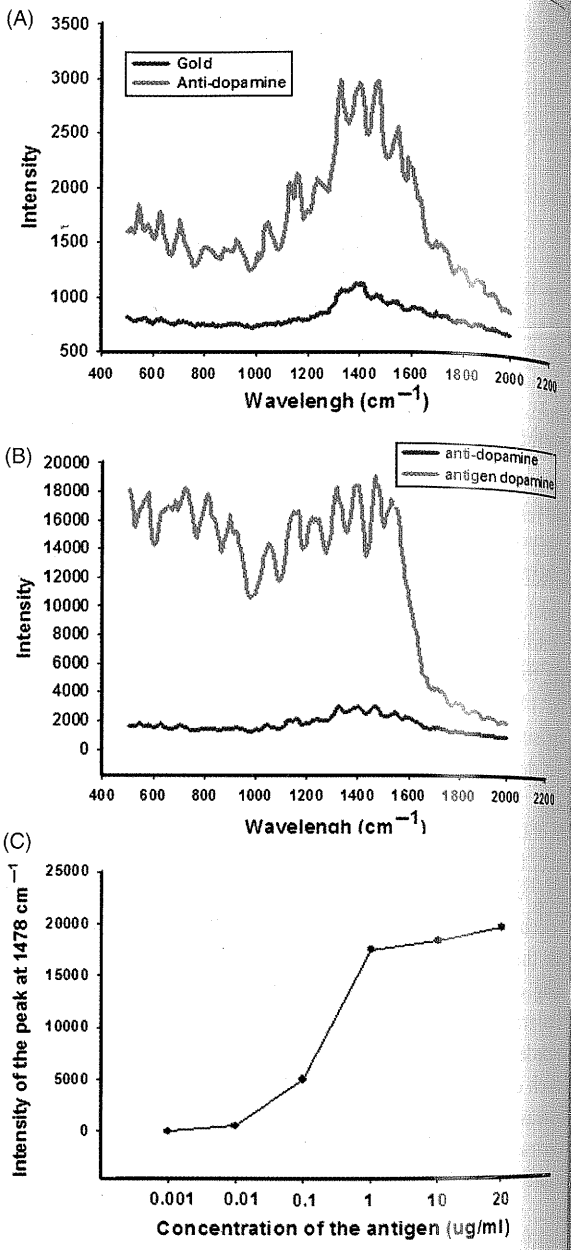

Fig. 5. The SERS spectra of antigen-dopamine and anti-dopamine assembled on a chip or antibody-dopamine assembled on a gold nanoparticle-deposited glass slide. (A) SERS spectra of anti-dopamine adsorbed on an Au nanoparticle-deposited glass slide. (B) SERS spectra of antigen-dopamine and anti-dopamine immobilized on a chip. (C) The relationship between the intensity of SERS signals at 1478 cm^{-1} and the concentration of antigen-dopamine.

different laser lines and on different substrates, our substrate represents a simple and low cost alternative that is easily implemented in the laboratory.

4. CONCLUSION

We used SERS spectroscopy to examine the association of antibodies to dopamine that were both covalently bound and adsorbed to Au nanoparticles deposited on a glass

substrate surface. By recording SERS spectra of dopamine molecules adsorbed to immobilized Au nanoparticles, we demonstrated the suitability of dopamine substrate for SERS detection. Also, antigen-dopamine was captured by the antibody-assembled gold substrate and detected by SERS. The SERS signal at 1478 cm^{-1} based on cathecol ring vibration versus concentration of antigen-dopamine was obtained and the non-optimized detection limit for anti-dopamine surface antigen was found to be as low as 1 ng/ml. The dopamine concentration in which detection was possible ranged from 1 mM to 1 nM as detected by SERS. The strongest peaks in the spectra are phenolic carbon–oxygen stretches as well as phenyl C=C stretches around 1267 and 1478 cm^{-1}, respectively. Our results illustrate that nanoparticle-based SERS is a promising tool for the detection and screening of neurotransmitters and antibodies. Therefore, our study presents an encouraging outlook for the development of portable detection systems capable of measuring dopamine in clinical and medical diagnostics.

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