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Articles

Fabrication of Chitosan-gold Nanocomposites Combined with Optical Fiber as SERS Substrates to Detect Dopamine Molecules

Jae-Wook Lim and Ik-Joong Kang*

Department of Chemical & Bio Engineering, Gachon University, Gyunggi-do 461-701, Korea
**E-mail: ijkang@gachon.ac.kr*
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This research was aimed to fabricate an optical fiber-based SERS substrate which can detect dopamine neurotransmitters. Chitosan nanoparticles (NPs) were firstly anchored on the surface of optical fiber, and then gold layer was subsequently deposited on the anchored chitosan NPs *via* electroless plating method. Finally, chitosan-gold nanocomposites combined with optical fiber reacted with dopamine molecules of 100-1500 mg/day which is a standard daily dose for Parkinson's disease patients. The amplified Raman signal at 1348 cm^{-1} obtained from optical fiber-based SERS substrate was plotted *versus* dopamine concentrations (1-10 mM), demonstrating an approximate linearity of $Y = 303.03X + 2385.8$ ($R^2 = 0.97$) with narrow margin errors. The optical fiber-based Raman system can be potentially applicable to *in-vitro* (or *in-vivo*) detection of probe molecules.

Key Words : Optical fiber, SERS, Chitosan-gold nanocomposites, Dopamine

Introduction

Parkinson's disease is a progressive neuro-degenerative disease whose effects include slow movement, body trembling, tetanus, and back curvature.[1-6] One of the causes of Parkinson's disease is closely related to levels of dopamine (DA) in the brain cortex. Lack of DA can lead to the development of Parkinson's disease, while excessive amounts of DA contribute to a type of schizophrenia called Bleuler disease.[7-9] Since the determination of DA in biological system is very informative in the diagnosis of many diseases, there have been considerable interests to develop detection methods of DA in the fields of biomedical chemistry and neurochemistry.

Selective and sensitive detection is critically needed due to the important role of DA in brain chemistry. Furthermore, the design of biosensor measuring at the site of dopamine action can provide the real-time information about effective treatment at proper time.[10,11] Many approaches have been conducted to detect the dopamine levels, such as HPLC, capillary electrophoresis,[12] electrochemical,[13] and optical (SPR, SERS) methods.[14,15] Among them, SERS has currently emerged as a promising technique for the rapid and direct detection of DA molecules which can minimize the difficulties attributed to many interfering compounds.[16,17] Besides, optical fiber-based SERS probe system can be potentially applied to *in-vivo* measurements of dopamine levels even though the probe system should confront with significant analytical challenges to be resolved.[11]

Surface-enhanced Raman scattering (SERS) refers to the amplification of scattered Raman signals of the probe molecules adsorbed on metallic substrates, which can provide the information of chemical structure and ultrasensitive trace detection of probe molecules.[18] The most prevalent types of SERS-active substrates are colloidal metal (Au, Ag) particles, solid types of metallic electrodes, and evaporated films of these metals.[19-21] Chitosan is a high-molecular biopolymer with many amine groups bearing high affinity to Au nanoparticles (NPs) with unique surface plasmon resonance.[22-25] Hence, chitosan nanoparticles can be employed as templates which can anchor Au NPs and grow into gold layer. The resultant chitosan-gold composites can be used as a biocompatible SERS substrate which can enhance the Raman signals of probe molecules.

In this research, chitosan nanoparticles were anchored on the surface of optical fiber by the functionalization of combined APTMS and glutaraldehyde ligands. After then, small gold seeds (3-5 nm) were deposited on the surface of chitosan NPs anchored on the optical fiber, followed by the subsequent growth of gold layer. Finally, chitosan-gold nanocomposites combined with the optical fiber was prepared and employed as a SERS substrate to detect dopamine levels in aqueous solution. Amplified Raman signals of DA were obtained from the optical fiber and calibrated over concentration ranges (1-10 mM) of DA molecules, demonstrating the linear sensitivity of Raman signals to DA levels.

Experimental

Reagents and Instruments. Tripolyphoshate (TPP, 95.0

%), potassium carbonate (K_2CO_3, 99.7%), gold chloride hydrate ($HAuCl_4 \cdot xH_20$, 99.999%), ascorbic acid ($C_6H_8O_6$, 99.0%), ammonium hydroxide (NH_4OH, 28% in water, 99.99%), 3-hydroxytyramine hydrochloride ($C_8H_{11}NO_2 \cdot HCl$, 99.9%), tetrakis-hydroxymethyl-phosphonium chloride (THPC, 80% in water), dopamine hydrochloride ($C_8H_{12}O_2Cl$, 99.99 %), glutaradehyde solution (50 wt % in H_2O, $C_5H_8O_2$), and (3-aminopropyl)trimethoxysilane ($C_6H_{17}O_3NSi$, 97%) were purchased from Sigma Aldrich (USA). HPLC water was bought from J. T. Baker. Acetic acid (CH_3COOH, 99.0%) and sodium hydroxide (NaOH, 96.0%) were used from Duksan Pure Chemical, Ltd (Korea). Optical fibers were purchased from Polyicro Technologie, Ltd (USA).

Surface Treatment of Optical Fiber. After the optical fiber was cut into a part of *ca.* 8 cm, it was soaked into H_2SO_4 solution at 120 °C with continuous stirring for one hour. When the clad part was completely removed, the exposed core part was cleaned by boiling distilled water, methanol, and then boiling distilled water. After then, the sample was reacted in Piranaha solution (1:3 = 30% H_2O_2: concentrated H_2SO_4) for 30 minutes, and cleansed in distilled water at 75 °C for 30 minutes, and sonicated in methanol for 15 minutes. The cleaned optical fiber was reacted with 2% (v/v) of APTMS for 2 h under inert N_2 purging. The resulting optical fiber was kept in a vacuum drier for 30 minutes at 120 °C and reacted with 10% (v/v) of glutaraldehyde for 2 h. The final sample was dried for 3 hours and kept in a desicator prior to further characterization.[26]

Chitosan-Gold Nanocomposites Combined with Optical Fiber. Chitosan powders of 0.06 g was put into a vial containing HPLC water of 29.94 mL and the mixture was stirred after adding 300 μL of acetic acid. After then, 20 mL of 0.1% (w/v) TPP was added into the solution and reacted for 1 h. Finally, the sample was centrifuged and purified to get chitosan nanoparticles (CNPs).[27] Optical fiber functionalized with glutaradehyde was put into 30 mL of 0.2 % CNPs and reacted with CNPs for 1 h under continuous N_2 purging.[26]

The optical fiber deposited by CNPs was reacted with 20 mL of gold colloids (2.1% (v/v)) for 1 h. After then, 10 mL of gold salts, 20 μL of ascorbic acid, and 60 μL of NH_4OH were added into sequentially to obtain chitosan-gold nanocomposites arrayed on optical fiber *via* electroless plating method. Finally, chitosan-gold nanocomposites combined with optical fiber reacted with 30 mL of dopamine solution (1-10 mM) for 5 minutes under constant stirring at 500 rpm. Raman Spectroscopy (LabRam HR, He-Ne laser 633 nm, Horiba company, France) was employed to detect dopamine adsorbed on optical fiber (SiO_2) samples.[28] Scheme 1 shows the whole fabrication process for optical fiber-based SERS substrates to detect dopamine molecules.[27-30]

Results and Discussion

According to the Scheme 1, chitosan nanoparticles were firstly anchored on the functionalized optical fiber by the combined treatment of APTMS and glutaraldehyde. After then, small gold seeds (3-5 nm) were attached on the chitosan NPs arrayed on the optical fiber, followed by the subsequent growth of gold layer in the presence of gold salts and ascorbic acids. The resulting chitosan-gold nanocomposites combined with optical fiber was prepared and used as a SERS substrate to detect dopamine levels.

For the sensitive analysis of surface treatments, contact angles of water on the modified glass (SiO_2) surface were

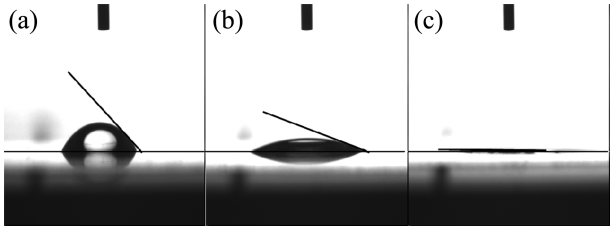
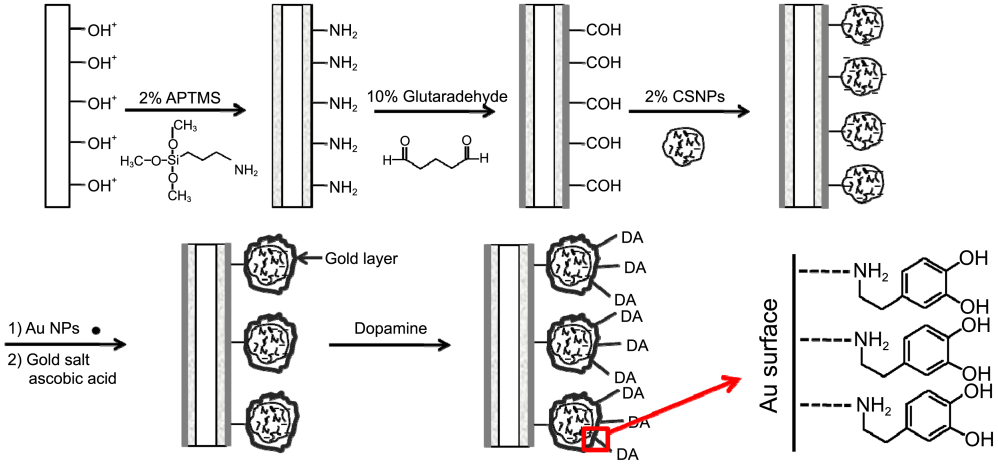


Figure 1. Air/H_2O contact angles on (a) pristine SiO_2 surface, (b) amine-terminated SiO_2 surface by APTMS functionalization, and (c) aldehyde-terminated SiO_2 surface by glutaraldehyde functionalization.



Scheme 1. Schematic fabrication steps to prepare chitosan-gold nanocomposites arrayed on optical fiber for Raman measurements of dopamine molecules.

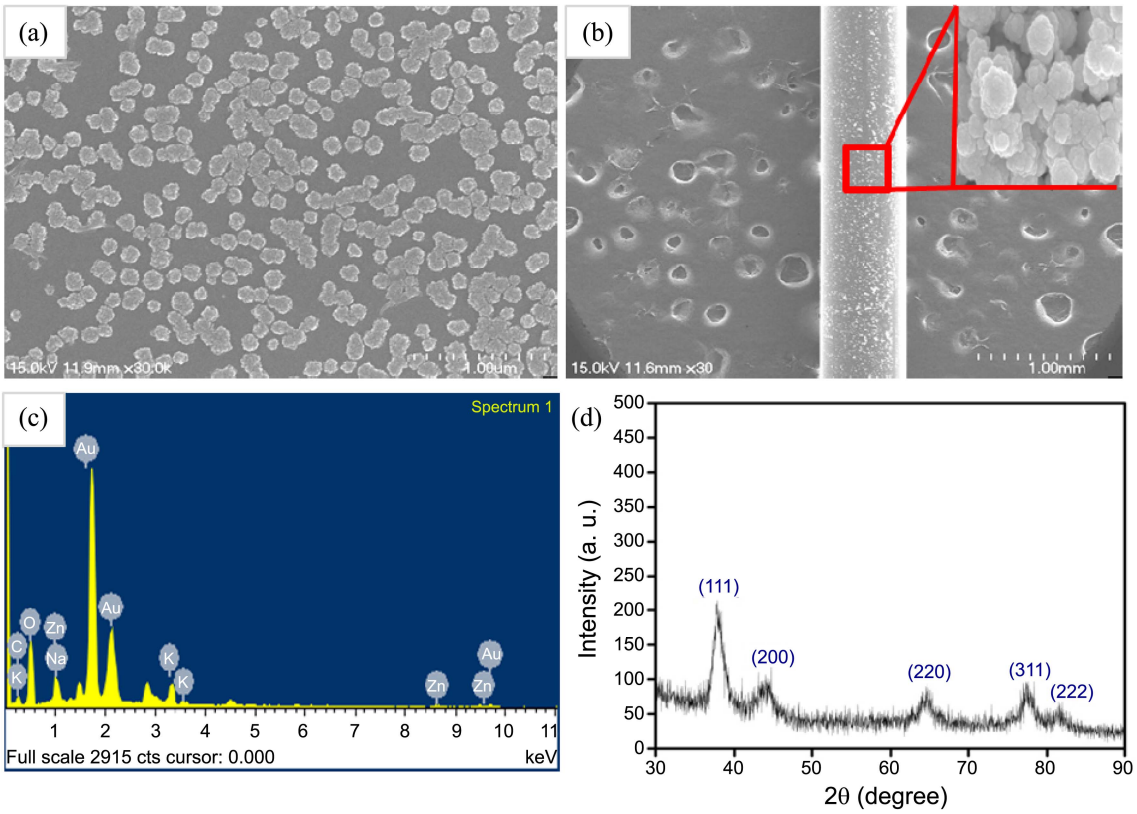


Figure 2. SEM images of (a) chitosan nanoparticles, (b) chitosan-gold nanocomposites on the optical fiber, (c) EDX analysis of chitosan-gold nanocomposites on optical fiber, (d) XRD analysis of chitosan-gold nanocomposites on the SiO_2 substrates.

measured using the contact angle meter (G10, KRUSS, Germany). Figure 1(a) shows the air/H_2O contact angle of 58.7° on pristine SiO_2 surface which mainly consisted of Si-O-Si groups with some OH groups. Figure 1(b) shows the air/H_2O contact angle of 16.5° on the SiO_2 surface functionalized by APTMS ligand. The contact angles were lowered due to the terminated amine groups with more hydrophilic characteristics of water-friendliness as compared to Si-O-Si groups of the pristine SiO_2 surface. Figure 1(c) shows the air/H_2O contact angles of 1.6° on the SiO_2 surface functionalized by Glutaraldehyde (*i.e.*, aldehyde-terminated SiO_2 surface), indicating that aldehyde groups exhibited more water-friendliness than amine groups. Contact angle measurements experimentally confirmed that the SiO_2 surface was successfully functionalized by APTMS and Glutaraldehyde, respectively, producing amine-terminated and aldehyde-terminated surfaces.[31]

The size and surface morphology of the samples were analyzed by scanning electron microscope (FE-SEM; S-4700, Hitachi's, Japan). Figure 2(a) shows the SEM image of chitosan nanoparticles which played as a template for gold attachments. The size was approximately ranged in ca. 90-120 nm and the shape was circular. Figure 2(b) shows the SEM images of chitosan-gold nanocomposites (CGNs) anchored on optical fiber. The CGNs exhibited the aggregated array on the surface of optical fiber. The size of chitosan-gold nanocomposite was estimated as *ca.* 100-150 nm, indicating the growth of gold layer over CNPs. As shown in Figure 2(c), EDX analysis showed the Au peak attributed to CGNs anchored on optical fiber, confirming the presence of gold layer over CNPs. Also, XRD analysis clearly showed the (111), (200), (220), (311), (222) facets corresponding to face centered cubic (fcc) crystal structure of metallic gold (JCPDS no. 04-0784).[32,33]

FT-IR spectroscopy (FT/IR-4100, Jasco, UK) was used to analyze the surface composition of as-prepared samples.

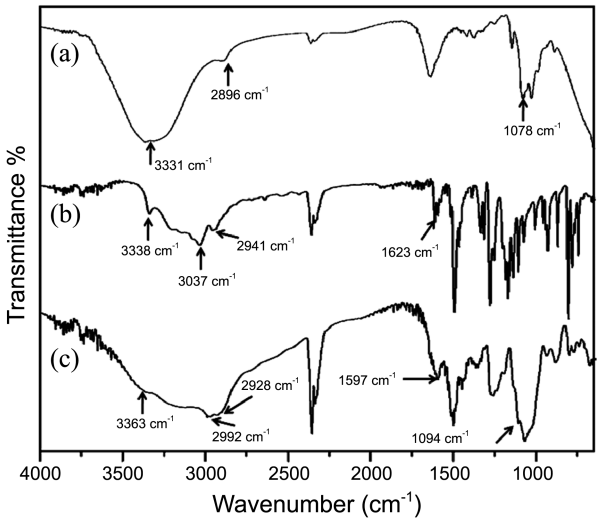


Figure 3. FT-IR spectra of (a) chitosan-gold nanocomposites, (b) Dopmaine, (c) dopamine-adsorbed chitosan-gold nanocomposites.

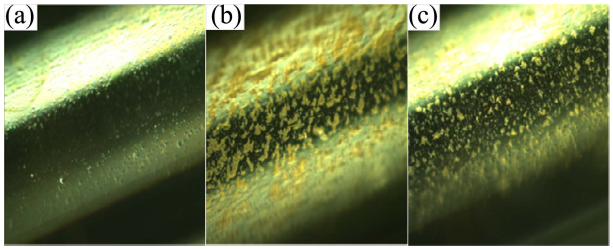


Figure 4. Microscopy images of (a) a optical fiber, (b) Chitosan-gold nanocomposites on the optical fiber, and (c) dopamine adsorbed chitosan-gold nanocomposites on the optical fiber.

Figure 3(a) shows the FT-IR peaks of CGNs attributed to broad stretching vibration of O-H and N-H at 3331 cm^{-1}, C-H stretching at 2896 cm^{-1}, and C-O stretching at 1078 cm^{-1}. Figure 3(b) shows the FT-IR peaks of dopamine (DA) attributed to stretching vibration of O-H and N-H at 3338 cm^{-1}, C-H stretching of aromatics at 3037 cm^{-1} and 2942 cm^{-1}, and N-H stretching at 1623 cm^{-1}. According to Figure 3(c), the noteworthy shift of absorption bands in dopamine-adsorbed CGNs were observed at 3363 cm^{-1} and 1094 cm^{-1} attributed to O-H stretching vibration and C-O stretching when compared to those of 3331 cm^{-1} and 1078 cm^{-1} in CGNs, respectively. The FT-IR peaks of 2942 cm^{-1} and 1623 cm^{-1} attributed to C-H stretching of aromatics and N-H stretching in dopamine molecules were also shifted to 2928 cm^{-1} and 1598 cm^{-1} in dopamine-adsorbed CGNs, due to the molecular interactions between amine groups of dopamine and gold surface of CGNs.[34,35]

Figure 4 shows the optical image of various optical fibers using microscopy (BX51, Jinoptic, Korea). Figure 4(a) and 4(b) shows the optical images of pristine optical fiber and optical fiber combined with CGNs, respectively. Figure 4(c) shows the optical image of dopamine-adsorbed CGNs combined with optical fiber. When CGNs was reacted with dopamine molecules, the golden color of microscopic image was distinctly lessened. Also, the optical images exhibited the segregated array of CGNs which was widely distributed over optical fiber.

Figure 5(a) is a schematic diagram of the Raman analysis system using optical fiber substrates which can detect the dopamine levels. Figure 5(b) shows the Raman shift of dopamine molecules at 1348 cm^{-1} and 1565 cm^{-1} according to Raman measurements. The Raman peak at 1565 cm^{-1} was assigned to cathechol ring moiety of dopamine molecules and the peak at 1348 cm^{-1} was assigned to the stretching of the catechol C-O band.[36] According to Figure 5(b), the intensity of Raman peak was increased in proportion to dopamine concentrations. Figure 5(c) is the calibration curve plotting the Raman intensity at 1348 cm^{-1} *versus* dopamine concentrations, showing an approximately linear curve of $Y = 303X + 2385$ ($R^2 = 0.9709$). The optical fiber-based system exhibited the potential application to *in-vitro* detection of dopamine molecules by showing the approximately linear dependence of Raman signal intensity on probe concent-

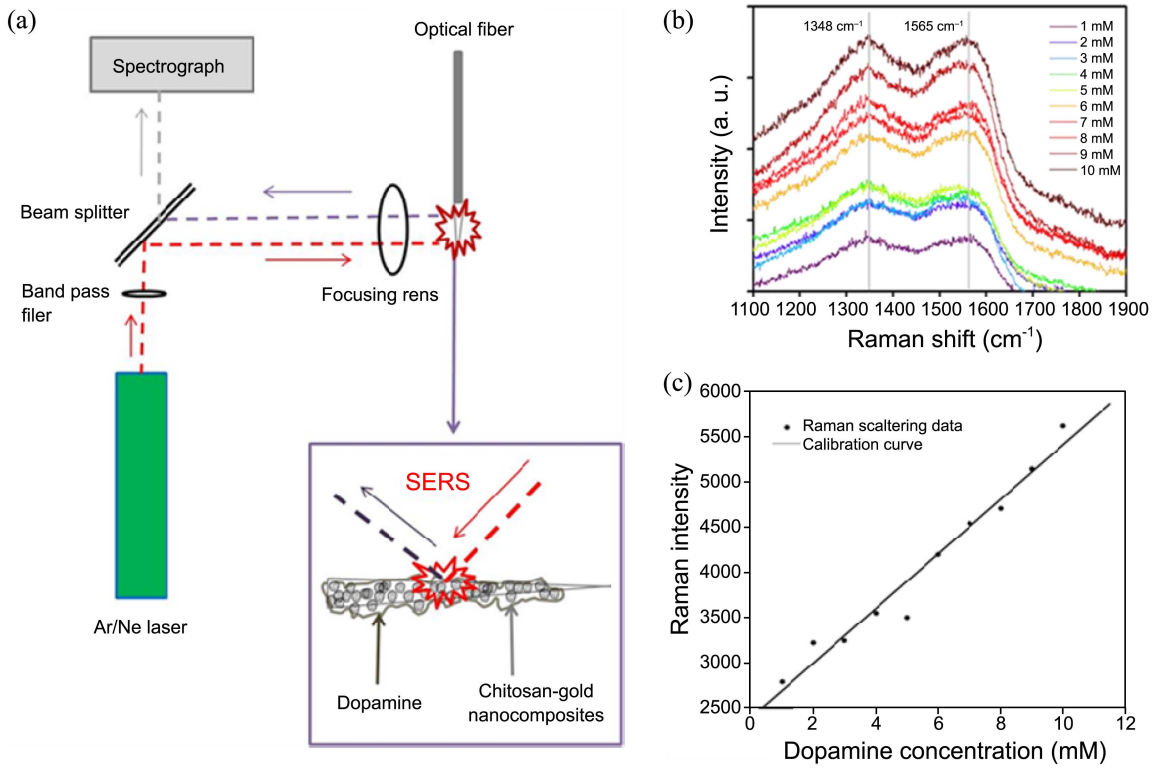


Figure 5. (a) Schematic diagram of SERS measurement system, (b) Raman spectra of dopamine molecules adsorbed on chitosan-gold nanocomposites on the optical fiber over dopamine concentration ranges, (c) Calibration curve of Raman intensity at 1348 cm^{-1} *vs.* dopamine concentration ranges (1-10 mM).

rations.

Conclusions

In this work, Raman scattering of dopamine molecules over optical fiber-based substrates were investigated. In order to analyze dopamine levels, the surface of optical fiber was combined with chitosan-gold nanocomposites. According to SEM analysis, chitosan-gold nanocomposites were well deposited on the surface of optical fiber. XRD and EDX analysis clearly demonstrated the Au peak originated from chitosan-gold nanocomposites which can sense dopamine levels. It was experimentally confirmed that Raman intensity of dopamine components at low concentrations were amplified on the optical fiber combined with chitosan-gold nanocomposites as SERS substrates. Also, the amplified Raman peaks were increased in proportion to the concentration of dopamine molecules. The linearly calibrated curve was obtained as a liner plot: $Y = 303X + 2385$ ($R^2 = 0.97$). The optical fiber-based Raman system can be potentially applicable to *in-vitro* (or *in-vivo*) detection of dopamine levels, if the performance of the optical fiber-based SERS substrate is enhanced enough to detect the probe molecules more sensitively.

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