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Clean Substrates Prepared by Chemical Adsorption of Iodide Followed by Electrochemical Oxidation for Surface-Enhanced Raman Spectroscopic Study of Cell Membrane

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Surface-enhanced Raman spectroscopy (SERS) has received renewed interest in recent years in fields such as trace analysis, biorelated diagnosis, and living cell study. However, the interference of impurities left on the surface from the preparation process of substrates or adsorbed from the ambient environment limits to some extent the application of SERS for analysis of trace or unknown samples. In the present paper, we propose a method to prepare clean SERS substrates by a combined method of chemical adsorption of iodide on the Au surface to remove the surface impurities and electrochemical oxidation of the adsorbed iodide to obtain a clean and impurity-free surface for SERS measurement. Time-dependent control experiment of untreated and treated substrate reveals that this method is very effective in obtaining substrates free of impurities. SERS mapping demonstrates the SERS activity of the substrate is homogeneous over the whole surface. The obtained clean substrate enables us to study the structures of the cell membrane with SERS and even to perform SERS mapping to visualize the distribution of amino acids over the membrane of a living cell.

The 21st century witnesses the wide and rapid application of surface-enhanced Raman spectroscopy (SERS) in various fields, including chemistry (single-molecule detection and trace analysis of metal cations, such as Cu^{2+}, Hg^{2+}, and Cd^{2+} and anions, such as ClO_4^-), physics (finding SERS hot spots and exploration of SERS mechanism), and life sciences (early detection and monitoring diseases and cell process).[1–12] From the application viewpoint, it is necessary to have SERS substrates with a high enhancement, clean surface, and high reproducibility. Intrinsically, SERS can be a robust and attractive spectroscopic technique for the detection of complex systems (i.e., proteins, viruses, or even whole cells) by developing reproducible highly sensitive and selective SERS-active substrates.[12–14] However, since the discovery of SERS, its availability as a routine analytical technique has not been fully explored and recognized by analytical scientists, which may largely be due to lack of effective methods to obtain clean and reproducible SERS-active substrates.

Among the wide variety of methods developed for preparing SERS-active substrates, electrochemical roughening, vacuum deposition, and chemical preparation of metal sols have been the most widely used methods.[15–17] The former two methods played the most important roles at the early stage of SERS. However, in recent years, the use of these two types of SERS substrates is shrinking due to the lack of surface uniformity and convenience in handling. By combining polystyrene template and vacuum deposition methods, the Van Duyne group has obtained highly reproducible and SERS-active substrates with an ease to tune the surface plasmon resonance (SPR) of the substrate so as to maximize the enhancement.[18] On the other hand, preparing Ag or Au SERS substrates using various chemical synthesis methods is receiving rapid progress in recent years due to their special optical properties and potential application in plasmonics and sensors, thanks to the development of nanoscience and nanotech-

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nology.[19] As a consequence, it is now possible to obtain Au or Ag nanoparticles of various shape and size and even different composition to tune their SPR to maximize the SERS enhancement. The obtained metal nanoparticles can be used for SERS measurement in two ways: (1) the synthesized, dispersed metal sols can be directly used for SERS measurement, giving a relative low signal, especially before aggregation; or (2) the metal nanoparticles in the sols can be assembled onto a solid surface to obtain a SERS substrate with a high SERS activity.

The common practice of preparing metal sols is to reduce metal salts with some reductants, such as citrate. Therefore, the surface of the nanoparticles will be inevitably covered by reductants. Furthermore, to prevent the aggregation of the sols or to obtain nanoparticles with desired shape and size, the metal nanoparticles have to be protected with some surfactants during the synthesizing process, such as CTAB or PVP.[20,21] The existence of these species on the metal nanoparticle surfaces will lead to at least two results: (1) the nanoparticle surface covered with these species will provide less active sites for interacting with the target molecule, especially for molecules with a weak adsorpability; (2) the SERS signal generated by these species may severely interfere with the SERS signals of target molecules, especially when the concentration of the target molecules is very low or the adsorpability is weak. For example, in single-molecule experiments, SERS signals of the impurities may appear at about the same level as that of the target molecules, showing Raman bands over the spectral range of 900−1700 cm^{-1}.[22,23] When SERS is applied to the biological systems, this problem will become even severe, because most biomolecules have very complex structure with lots of weak and broad bands over the spectral range from 600 to 1700 cm^{-1} attributed to C−C, C−H, C−N, and C−O vibrations.[12] Therefore, the SERS of impurities will severely interfere with the SERS measurement of biomolecules and cells, especially when the targets are unknown systems. To solve the problem, there have been many attempts to obtain clean SERS substrates under ambient conditions.[24] For instance, CN^- has been used to chemically remove layers of Ag to obtain a clean and fresh surface.[25] However, after this chemical treatment, CN^- will be left on the Ag surface due to the strong interaction of CN^- with Ag, which will occupy the surface adsorption sites and interfere with the measurement.[26] There were also attempts to remove the impurities by plasma cleaning or to directly obtain clean surfaces by either sputtering or plasma deposition under the vacuum condition.[24] These methods have been shown very effective in removing impurities in vacuum. However, the substrate will be quickly contaminated upon exposure to ambient air. Norrod et al. used alkanethiols to remove carbonaceous species on the surfaces of SERS substrates followed by ozone removal of alkanethiols.[27,28] This method may lead to the oxidation of the SERS substrate, which may eventually result in a decrease in the SERS enhancement, especially for those relatively active metal (e.g., Ag or Cu) surfaces. Chloride ion was also used to remove the carbonaceous contaminants on the SERS substrate of silver mirror. But it was not stated how to remove the specifically adsorbed chloride ion from the surface for a better interaction of the target molecules with the surface or to eliminate the interference of Cl^- to the adsorption of target molecules.[29] UV/ozonolysis as a simply method to remove impurities has been widely used for semiconductor substrate and indium tin oxide (ITO) substrate cleaning,[30] but for metals such as Au, the oxidation of Au[31] will also lead to a decrease of the SERS activity if the surface is not further reduced by hydrogen. Apart from these attempts, there are still other alternative but more commonly used methods to get rid of the contaminants: (1) to remove the adsorbed contaminants by applying a proper potential to desorb or oxidize the contaminants.[32] However, one has to be very careful about the readsorption of contaminants; (2) to remove the surfactants or reactants in the colloidal solution of metal nanoparticles by several cycles of centrifugation and rinsing with ultrapure water. However, one has to very cautious about the aggregation and thereafter the precipitation of metal nanoparticles after the removal of surfactants. Therefore, developing a method to prepare, preserve, and regenerate clean substrates is virtually necessary to the analytical and biological application of SERS.

Plasma membranes and the proteins therein provide a physical boundary between the cell and its environment, playing important roles in many fundamental biological processes such as signal transduction, cell−cell contact, selective transport of molecules, and other essential functions.[33] Now, the prevailing method for studying the plasma membrane protein is mass spectrometry, and it has identified a few thousand of the most abundant proteins in cells.[34] To be detected with mass spectrometry, the cell has to be crushed and it is necessary to prepare a clean plasma membrane fraction. Therefore, the investigation of the protein structure is not under the physiological condition of a living cell. On the other hand, SERS can be conveniently used for studying biological samples and even under living conditions. Not surprisingly, its first application in biology and life sciences dates back to the early stage of SERS.[35] Its application is booming in recent years with the fast development of bionanotechnology. The investigation includes the use of nanoparticles modified with SERS reporter molecules and monoclonal antibodies to identify the overexpressed plasma membrane proteins in disease cells and tissue for cancer diagnosis purpose.[36–39] However, this method can only be used for identifying the expression level of the known membrane proteins and cannot be used to identify the possible

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structural difference of the normal cells and cancer cells. SERS were also used to study the species released from the cell. For this purpose, a layer of silver nanoparticles was deposited on the cell plasma membrane to detect the released neurotransmitters.[40] One has to realize the potential disadvantages: first, the deposited nanoparticles may lead to a change of the cell membrane structure and thereafter influence the behavior of the living cell; second, some nanoparticles may enter the living cells by an endocytosis process; and last and most importantly, as the nanopartciles are distributed very unevenly on the surface, the obtained signal distribution over the cell membrane is a combination of concentration difference and the aggregation state of nanoparticles (different enhancement effect). Therefore, this technique is not suitable for quantitative analysis. SERS substrate consisting of silver nanorod array was used to directly detect spectral differences between viruses. However, the interference of carbonaceous species to the obtained SERS spectra is not negligible.[41] Direct detection of cell membrane is an important and very challenging issue in SERS and cell study. The key point is whether it is possible to obtain a clean SERS substrate with very homogeneous enhancement over the surface.

In the present paper, we make full use of the unique chemical properties of iodide, which can be adsorbed strongly on the Au surface and can be easily oxidized by electrochemical methods, to remove the impurities existing on the SERS substrates prepared by assembly of Au nanoparticles on the ITO surface. The clean substrate will be used to study the structures of the cell plasma membrane to understand the membrane protein.

EXPERIMENTAL SECTION

Preparation of SERS-Active Substrates. SERS-active substrates were prepared by chemical assembly of Au nanoparticles on the 3-aminopropyltrimethoxysilane (APTMS)-functionalized ITO surfaces. Au nanoparticles were synthesized according to the typical protocol: 200 mL of $HAuCl_4$ aqueous solution (2.91×10^{-4} M) was heated to boiling under vigorous stirring, and 1.3 mL of 1% trisodium citrate aqueous solution was added. The color of the solution changed notably within the first several minutes. A ruby-colored solution was obtained finally and was ready for chemical assembly.

Figure 1A shows the detail process to prepare the assembled substrates following the literature process with a slight modification.[17] ITO slides were ultrasonically cleaned in deionized water, isopropyl alcohol, acetone, and ultrapure water for 15 min, respectively, followed by cleaning in H_2O/H_2O_2 (30%)/NH_4OH (5:1:1) for 30 min. The slides were further cleaned by sonication in ultrapure water (Milli-Q) for 20 min and immersed into a 2% (volume concentration) APTMS aqueous solution for 12 h at room temperature for functionalization. Afterward, the substrates were rinsed with ultrapure water and annealed at 110 °C in an oven to remove the loosely bound or physisorbed APTMS molecules to avoid the aggregation of the nanoparticles due to the existence of excess amount of APTMS when the functionalized ITO substrate is immersed in the Au colloid solution.[42] After that, the substrates were immersed into the 50-nm Au colloid solution for 24 h to obtain a SERS substrate of assembled Au nanoparticle film on the ITO surface by APTMS.

Cleaning Step. The assembled Au/ITO substrates were immersed in 1 mM KI solution for 10 min. Then the electrode was electrochemically oxidized at ~0.8−1.0 V (Pt wire was used as quasi-reference electrode) for several seconds to remove the adsorbed iodide ion in 0.1 M $NaClO_4$ solution. Detailed procedure will be discussed in the result section.

Cell Culture and Sample Preparation. Chinese hamster lung fibroblasts cells (CHL cell line) were cultured in Dulbecco's modified Eagle's medium (Hyclone Co.) supplemented with 10% fetal bovine serum (Hyclone Co.) and 1% penicillin/streptomycin (Hyclone Co.) and incubated at 37 °C and 5% CO_2 atmosphere. After 72 h, the cells were collected and separated from the medium by centrifugation at 1000*g* for 10 min and then washed three times with sterile PBS (pH 7.4). The sediment was resuspended in PBS to obtain a homogeneous suspended cell solution with a final concentration of 3×10^6 cells/mL. The cells were dropped onto a clean substrate that had been rinsed with PBS solution for the immediate Raman measurement.

Apparatus. Raman spectra and images were obtained using a confocal microprobe Raman system (LabRam I, Jobin Yvon).[43] It is a single spectrograph instrument equipped with a holographic notch filter and a CCD detector. A He−Ne laser (632.8-nm wavelength) was used for the Raman measurement, and the laser power at the sample was ~0.4 mW. A long working distance 50× objective (NA = 0.55) was used both to deliver the laser on the sample and to collect the Raman scattering signal. The laser spot was ~2 μm in diameter. An acquisition time of 5 s was used to obtain SERS spectra with a high signal-to-noise ratio.

The electrochemical measurements were performed on a CHI631A electrochemical workstation (CH Instruments, Shanghai, China) in a conventional three-electrode system with two platinum wires as the auxiliary and quasi-reference electrode, respectively, and the assembled SERS substrate as the working electrode. The electrode potential during the Raman measurements was controlled by a potentiostat (XD-II, Xiamen University).

Scanning electron microscope (SEM) images were taken with a field emission microscope (Leo1530) operated at an accelerating voltage of 20 kV.

RESULTS AND DISCUSSION

Characterization of SERS Substrates. The morphology of the substrates with assembled Au nanoparticles on the ITO surface was characterized by SEM, and typical images are shown in Figure 1B. The inset shows the substrate at a higher magnification. It can be seen from the images that Au nanoparticles of ~50 nm are distributed fairly uniformly on the ITO surface. In order to characterize the uniformity of the surface SERS signal, a Raman mapping experiment was carried out after the surface was

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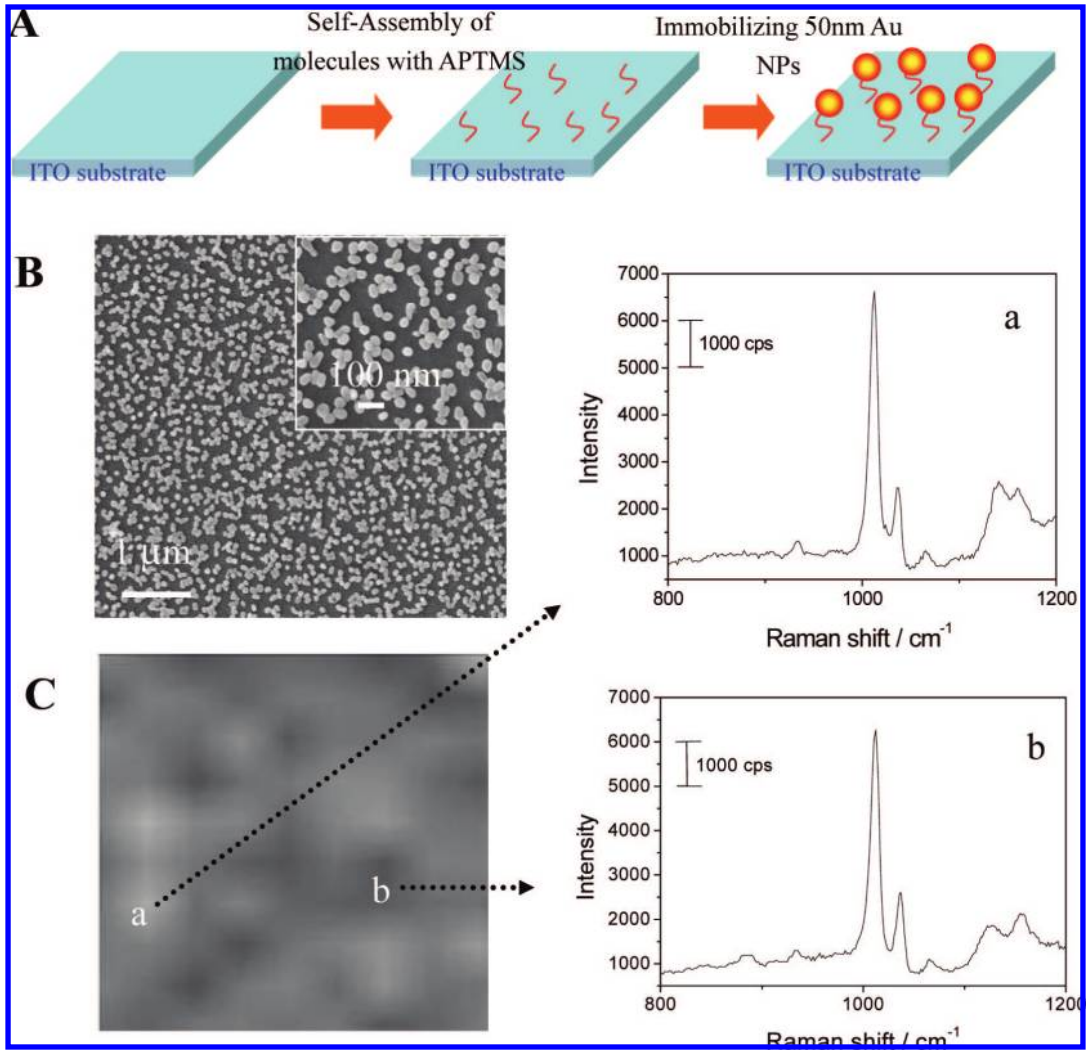

Figure 1. (A) Schematic diagram to show the self-assembly processes of Au nanoparticles on a ITO glass substrate. (B) SEM images of the assembled Au nanoparticles on the ITO; a higher magnification image is shown in the inset. (C) SERS mapping of the substrate using pyridine as the probe molecule: curve a shows the spectrum obtained at the strongest signal point and curve b shows the weakest signal point. The collection time was 1 s, and the laser power at the sample was 0.4 mW. Solution, 10 mM pyridine.

adsorbed with pyridine. Figure 1C shows the Raman mapping of the substrates, using the most intense band of pyridine at 1008 cm^{-1} as the mapping signal. The scan area was $20 \times 20\ \mu m^2$, the step size was 2 μm, and the acquisition time at each point was 1 s. The deviation of SERS intensities at the strongest point (Figure 1C-a) to the weakest point (Figure 1C-b) is less than 10%, indicating that such a substrate gives a very uniform SERS enhancement over the whole surface, although some aggregates can still be found at some spots. Generally, the SERS enhancement factor is $\sim 10^6$. However, if one carefully examines the spectral feature, one will find some peaks appearing in the spectral range of 1100−1200 cm^{-1}. There is no characteristic band related to the adsorbed pyridine in this spectral range, and they are believed to be related to the carbonaceous species or citrate adsorbed on the substrate during the preparation of the substrate and storage under ambient conditions.[24,26,44] In fact, there have been a lot of attempts, as described in the introduction, to eliminate the interference of these species to the SERS measurement with limited success. In the following sections, we will show how to eliminate the interference of these species to the SERS measurement by a combined chemical and electrochemical method.

Cleaning Protocols. Iodide is an ion that has a very strong interaction (specific adsorption) with most transition metal surfaces. At the early stage of electrochemical scanning tunneling microscopy, the strong adsorption character of iodide ion has been used to remove the adventitious contaminants on the metal surface due to the stronger interaction of iodide with the Au surface and, therefore, to preserve the cleanliness of the prepared Au single-crystal surface.[45]

To demonstrate the effect of iodide on removing the surface impurities, we did a control experiment. We first acquired a SERS spectrum directly on the assembled substrate before iodide treatment without any probe molecule, and the spectrum is shown in Figure 2A-a. The spectrum exhibits some broad bands characteristic of carbonaceous species. With a short acquisition time,

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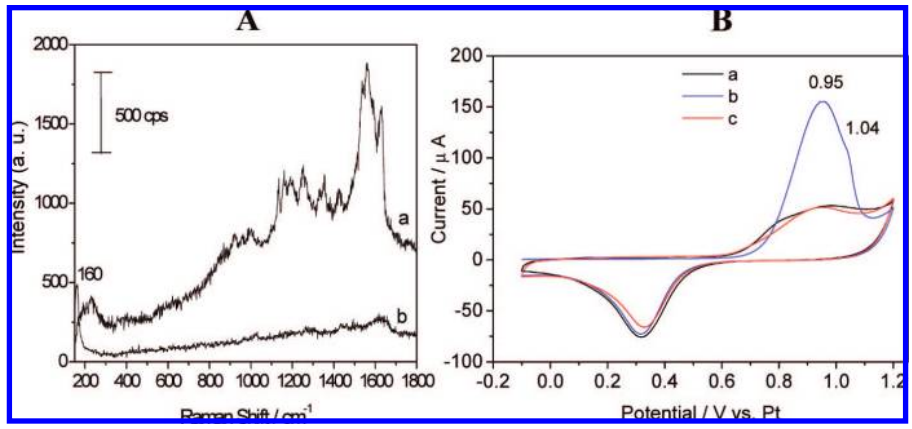

Figure 2. (A) SERS spectra obtained from the Au/ITO substrates without adding any adsorbate for the substrates (a) without any treatment and (b) after being treated with 1 mM iodide for 10 min. The collection time was 1 s, and the laser power was 0.4 mW. (B) Cyclic voltammograms obtained with the Au/ ITO substrates (a) and the Au/ITO substrate modified with iodide ions at the first (b) and the second (c) cycle in a 0.1 M $NaClO_4$ solution. Scan rate, 0.1 V/s.

such as 1 s, the spectral feature changed with time, indicating that these species were very unstable on the surface and can be easily decomposed under laser illumination. The intensity of impurities is ~1/3−1/4 of the signal of pyridine at a high pyridine concentration (0.01 M, as shown in Figure 1C-a, -b), and the signal will interfere with the pyridine spectra. We found that, at a lower pyridine concentration (10^{-5} M), the signal of pyridine will be overwhelmed by the signal of impurities. However, after the electrode was treated with iodide, a relatively clean SERS spectrum (Figure 2A-b) was obtained, with a strong band at 160 cm^{-1} characteristic of an Au−I band, indicating the formation of an adsorbed iodide layer on the substrate.[46] The result clearly indicates that iodide ion can significantly replace the surface species, leading to a relatively clean surface.

It should be pointed out that iodide is not only a strong adsorbate but also a strong reductant, which can be easily oxidized under a mild condition by simple chemical methods. For example, the adsorbed iodide could be easily removed by reacting with H_2O_2.[47] However, the further reaction of excess H_2O_2 with the metal surface will produce oxygen and oxidize the Au surface, leading to lowering of the SERS activity of the electrode surface.[48] In fact, at the early stage of electrochemical scanning tunneling microscopy, iodide was used to protect the surface from contamination and removed simply by applying a negative potential as iodide is an anionic species.[49] Therefore, we also applied a negative potential to our SERS substrate to check whether this method can be used to remove the iodide on our substrate. Indeed, iodide can be desorbed from the substrate at ~−1.2 V. However, we found that the iodide was adsorbed back to the substrate when the electrode was disconnected and kept at the open circuit potential (data not shown). If we can hold the potential while exchanging the solution to remove the desorbed iodide, it may be possible to obtain an iodide-free surface. However, this will complicate the experiment. Alternatively, if iodide can be converted into a species with a weak adsorpability, the cleaning procedure will become much easier. It has been demonstrated that interaction of iodide with the Au surface depends strongly on the electrode potential, and the adsorbed iodide can be oxidized to produce a weakly adsorbed species, IO_3^-, iodate, which can be easily rinsed from the surface.[47,50] Therefore, we carried out an electrochemical study of the relevant systems to find a suitable electrochemical condition for obtaining a clean surface.

Figure 2B shows the cyclic voltammograms of a blank Au/ITO substrate (Figure 2B-a) and Au/ITO substrate modified with iodide ions of first (Figure 2B-b) and second (Figure 2B-c) cycles in a 0.1 M $NaClO_4$ solution. On the bare Au surface, only peaks related to the oxidation of Au and the reduction of Au surface oxides were observed. However, on the substrate treated with I^-, a main peak at ~0.95 V and a shoulder peak at ~1.04 V were observed, which were attributed to the oxidation of the specifically adsorbed iodide to oxoanions of iodine, such as iodates, together with the oxidation of Au.[47,50] The two anodic peaks disappeared at the second cycle, indicating that the chemisorbed iodide had been completely oxidized to IO_3^- and that IO_3^- cannot be reduced to iodide (which may be adsorbed on the Au nanoparticles again). It should be pointed out that we chose $NaClO_4$ as the support electrolyte in this experiment because the adsorbed iodide is unstable in the acidic medium and can be oxidized to iodine or iodate.[51] This fact also suggests that it is chemisorbed iodide but not I_2 that can effectively replace the contaminants on the Au nanoparticles surface.

To monitor the electrooxidation process of iodide to IO_3^-, we chose pyridine again as the probe molecule. Figure 3A shows the SERS spectra acquired under the electrochemical condition at different potentials in 10 mM pyridine + 0.1 M $NaClO_4$ solution. The initial potential is the open circuit potential (OCP), and then the potential was gradually increased to 1.2 V until the complete oxidation of iodides. It is evidenced from the figure that the intensity of the band at ~160 cm^{-1} related to Au−I bond gradually decreases with the positive change of the potential and disappears at 1.1 V. A new peak at ~330 cm^{-1} launched at ~0.85 V, which

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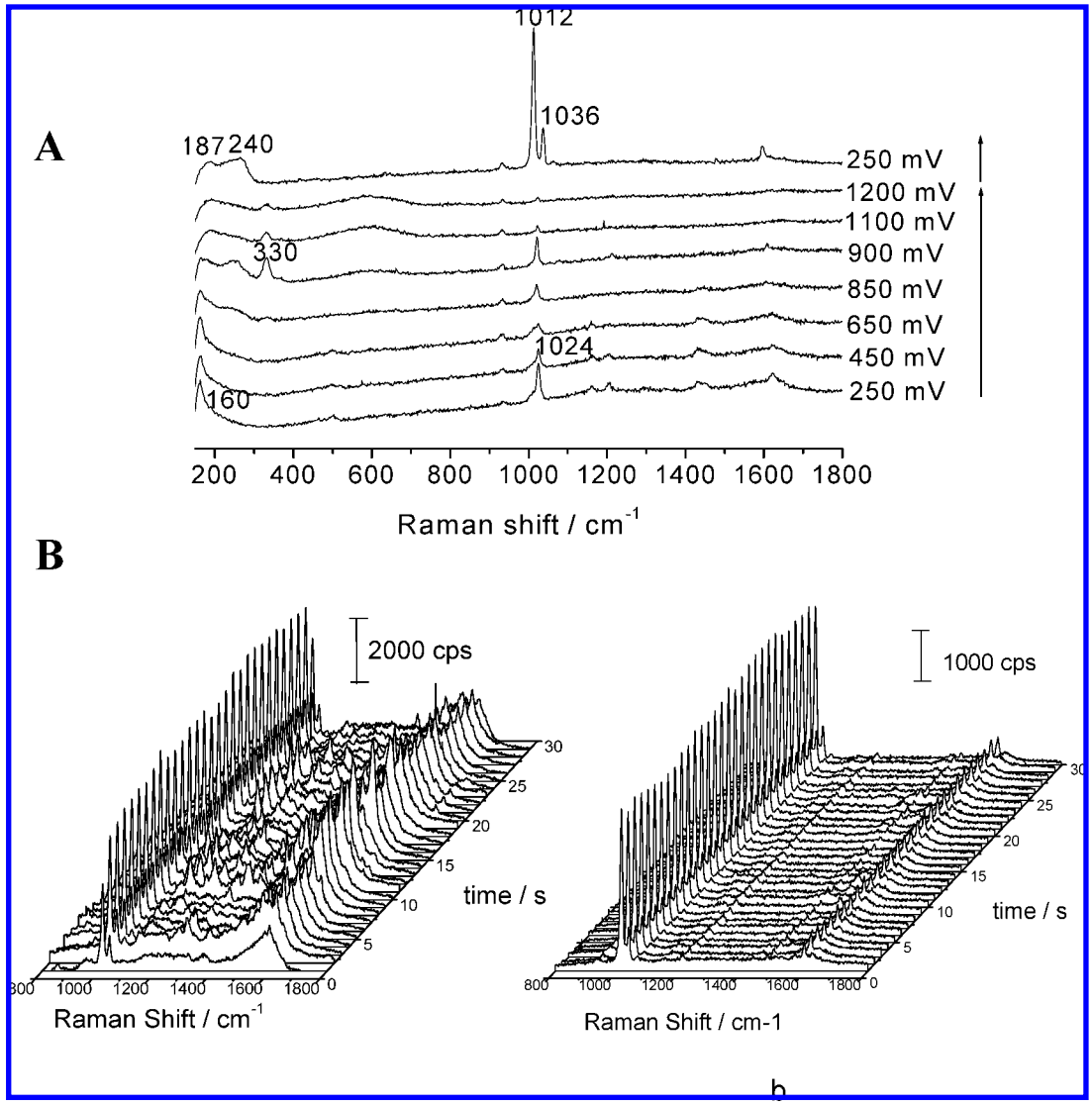

Figure 3. (A) Potential-dependent SERS spectra of pyridine adsorbed on the SERS substrate after being modified in 1 mM KI solution. The solution used for SERS measurement is 0.1 M $NaClO_4$ + 10 mM pyridine. (B) Time-dependent SERS signals of pyridine obtained at the same point of the substrate before treated with iodide (a) and after treated with iodide followed by the electrochemical oxidation (b). Solution, 10 mM pyridine.

has been attributed to the iodate.[49] When the potential was returned to the OCP, the 160-cm^{-1} band did not recover, indicating that I^- ions had been completely oxidized to iodate and iodate cannot be reduced again and adsorbed back to the Au nanoparticles, which is in good agreement with the electrochemical result.

Surprisingly, the SERS signal of pyridine adsorbed on the iodide-adsorbed nanoparticle surface is very different from that obtained from the bare Au surface. A band at 1025 cm^{-1} was observed, very close to the frequency of the pyridinium cation adsorbed on Ag or Au surfaces.[52,53] In our system, when iodide was adsorbed on Au nanoparticles, iodide might be partially or totally discharged to $I^{\delta-}(0 \leq \delta < 1)$ because of the reductive chemisorption of iodide.[54] Therefore, a monolayer of iodine will form on the Au nanoparticle surface. Then, pyridine may be adsorbed on the Au nanoparticles via the adsorbed iodine to form a metal−iodine−pyridine sandwich structure. The adsorption configuration is similar to that of cationic pyridinium, and one would anticipate a band at 1025 cm^{-1}. When the potential was increased, the intensity of 1025-cm^{-1} band will gradually decrease and finally disappear at 1.1 V, which is in accord with the decrease of the 160-cm^{-1} band, indicative of removal of adsorbed iodine. The 1025-cm^{-1} band did not recover when the potential was returned from 1.2 V to the open circuit potential. Instead, the characteristic SERS feature of pyridine adsorbed on the pure Au surface was observed with two strong peaks at 1008 and 1036 cm^{-1} and a broadband at 240 cm^{-1} attributed to the Au−N vibration.[55] As expected, no signals related to the impurities was observed when the potential was returned to the OCP (see the top spectrum), which suggests that the substrates was very clean after the cleaning procedure.

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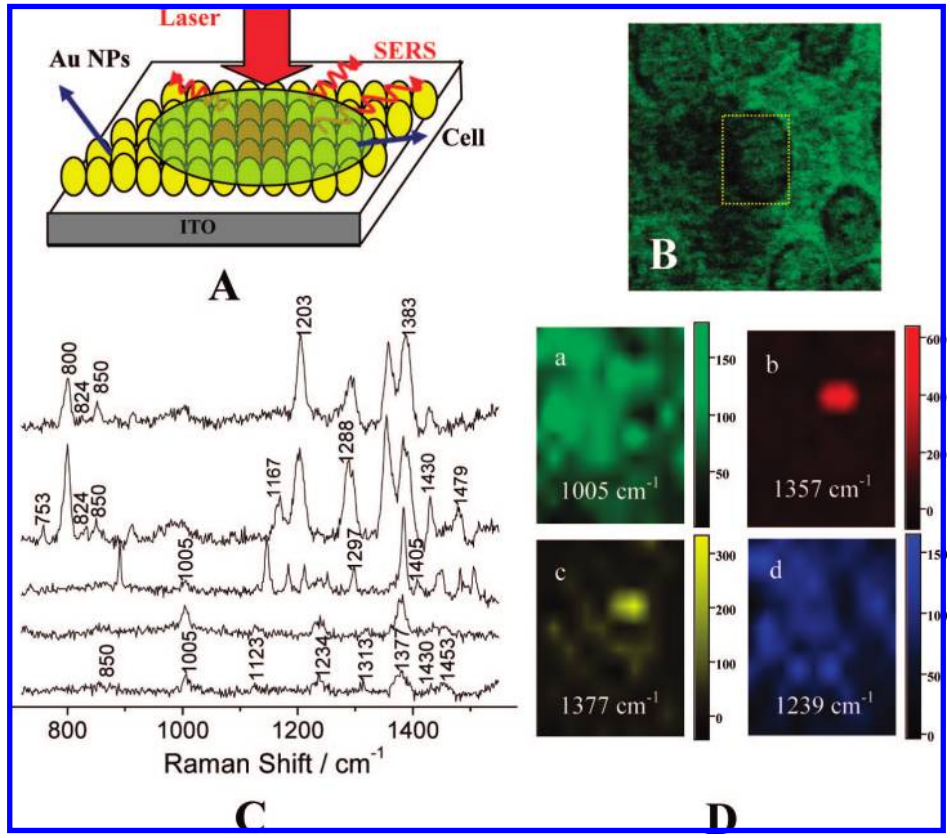

Figure 4. (A) Schematic diagram showing the experimental configuration for the SERS measurement of the cell membrane. (B) Video image of a CHL cell obtained in the transmission mode on a confocal microprobe Raman instrument. (C) SERS spectra obtained at different spots of a living cell. (D) SERS images obtained in the rectangular region of (B) using the SERS intensities of different amino acids as the imaging signal: (a) Phe (1005 cm^{-1}); (b) Cys (1357 cm^{-1}); (c) Pro (1377 cm^{-1}); and (d) Met (1239 cm^{-1}). The acquisition time for each point was 5 s, and the laser power at the sample was 0.4 mW.

To confirm the effect of the above cleaning procedure, we did a control time-dependent experiment still using pyridine as the probe molecule before and after the cleaning procedure in 10 mM pyridine solution. Different from the above experiment, the control experiment was performed without potential control. The SERS spectra (Figure 3B-a) obtained on the former substrate shows strong SERS signal of pyridine, with some fluctuating background signal from impurities. This background signal may severely interfere with the analysis of the SERS signal in the spectral range of 1200−1650 cm^{-1}. Whereas, with the latter substrate, the SERS spectra (Figure 3B-b) are very clean, without any signal from the impurities. This study further demonstrates that iodide can effectively replace the contaminants on the surface to produce a clean surface after the chemisorbed I^- has been removed by the electrochemical oxidation method. We should note that the SERS enhancement will decrease by ~50% after the cleaning treatment. The main reason may be due to the decrease in the effective electromagnetic coupling between Au nanoparticles as a result of the dissolution of surface atoms of Au nanoparticles under the existence of the adsorbed iodide. Both parallel UV−visible absorption and SEM measurements only reveal a slight change. However, it should be especially pointed out that an intensity decrease on this scale will be compensated by the much cleaner background that allows trace or unknown sample analysis.

The advantage of this cleaning method is that it can conveniently treat over the stored SERS substrate. Before use, the substrate need only be treated with iodide followed by electrochemical oxidation to produce a fresh surface and to provide more active sites for the target molecules. More importantly, the SERS-active substrate can be regenerated after SERS measurement and be used for other measurement, except for those molecules having a stronger interaction with Au than I^-. For example, in our SERS practice, we found that once 4,4′-bipyridine is adsorbed on the surface, it is really difficult to get rid of either by chemical oxidation (with even piranha solution) or electrochemical method, which is evidenced by the strong characteristic bands appearing in the SERS spectra. However, the signal of 4,4′-bipyridine disappears if the contaminated Au electrode is immersed in 1 mM KI solution for 10 min.

Such a clean substrate is very helpful for investigating or detecting unknown systems or systems with a very weak signal. To demonstrate this advantage, we study the plasma membrane of a living cell using this clean substrate.

SERS Study of the Plasma Membrane of a Living Cell. Figure 4A shows the experimental scheme for detecting the cell plasma membrane by SERS. The cell suspension was harvested by a trypsin solution, dropped on a clean SERS substrate, and incubated at room temperature for 30 min. The cell is transparent, and the lower part of the membrane is in contact with the SERS substrate to produce a SERS signal. A typical optical image of living CHL cells dispersed on the assembled Au substrate was obtained by using a LabRam I system using a 50 × long working distance objective in a transmission mode and shown in Figure

4B. Figure 4C shows SERS spectra obtained from the plasma membrane of living cells, which is dominated by protein features. As amino acids are the basic constituents of protein, we may use the well-recognized assignment of amino acids to assign the observed bands.[12,14,41,56–59] The band at 753 cm^{-1} may be assigned either to the ν(C−S) of P_C-G form, to the COO^- wagging mode, or to the CH_2 bending and CSH deformation modes of cysteine (Cys), 800 cm^{-1} to the vibrations of the carboxylate of Cys, 1357 cm^{-1} to ν(C-NH_3^+), or ω($C_{\alpha 1}H_2$) of Cys. The band at 1005 cm^{-1} can be attributed to the ring breathing vibration of phenylalanine (Phe), 1239 cm^{-1} to the δ(C−C_α-H) vibration of methionine (Met), and 1377 cm^{-1} to the ν_s(COOH) vibration of proline (Pro). The characteristic doublet at 824 and 850 cm^{-1} is assigned to the tyrosine (Try). The peak at 1453 cm^{-1} is due to the CH_2 deformation mode of proteins, and 1405 cm^{-1} to the COO^- symmetric stretching band. The bands at around 1288 and 1203 cm^{-1} may be attributed to the amide band. However, due to the complicated nature of the SERS spectra, some bands can only be left unassigned.

It should be pointed out that the SERS substrate prepared by the above method is very clean and the enhancement is reasonably homogeneous over the whole surface. Benefiting from this feature, we can perform SERS mapping over the whole living cell to obtain the species distribution in the region of interest. For this purpose, we perform a SERS mapping on a living cell over an area of 20 × 15 μm^2 with 1-μm step size to ensure a proper resolution. Figure 4D-a−d shows the SERS images using the SERS intensity of Phe (based on 1005 cm^{-1}), Cys (1357 cm^{-1}), Pro (1377 cm^{-1}), and Met (1239 cm^{-1}), respectively. It can be seen from the images that the distribution of different amino acids on the cell membrane is very different. We could further find that the intensity of Cys at 1357 cm^{-1} is higher than any other amino acid. It may be attributed to a stronger interaction of the thiol group of Cys with gold surface than other groups.

CONCLUSIONS

A method has been developed to obtain clean and homogeneous SERS substrates by chemical adsorption of iodide on the Au nanoparticles self-assembled on ITO substrates to remove the surface impurities. The subsequent electrochemical oxidation of the adsorbed iodide results in a fresh SERS substrate. The SERS study on the blank sample demonstrates that iodide can effectively remove surface impurities transferred from the preparation process. After the electrochemical oxidation of the adsorbed iodide and thereafter adsorption of probe molecule pyridine on the fresh surface, the SERS spectra of pyridine shows a very clean background that will not change with time. Such a homogeneous and clean substrate was used to study the plasma membrane of a living CHL cell by direct SERS study and SERS mapping, both of which reveal that different amino acids distribute very differently on the membrane.

This simple yet important approach will also find application in fields that require extremely clean background and low impurity level, including trace analysis, and the study of unknown sample and species with very weak adsorpability. The latter includes the study of interfacial water that has been suffering seriously from the impurities. The study along this direction is now being carried out in our laboratory.

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