Fabrication of nanostructures for surface-enhanced Raman scattering

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**FABRICATION OF NANOSTRUCTURES FOR
SURFACE-ENHANCED RAMAN SCATTERING**

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FABRICATION OF NANOSTRUCTURES FOR SURFACE-ENHANCED RAMAN SCATTERING

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SUMMARY

Due to the wide application in the fields of physics, chemistry and biology, surface enhanced Raman scattering (SERS) attracted much attention in the past decades. Especially thanks to the development of nanoscience and nanotechnology, many SERS-active substrates have been fabricated using various techniques. In this thesis, several simple and convenient methods are developed to fabricate efficient SERS substrates. Moreover, a simple, convenient and flexible tool is developed for highly sensitive Raman detection.

First, a simple and convenient method is developed for fabrication of SERS substrate by electroless deposition of Ag nanoparticles (NPs) on to catalytic etching-induced Si nanowells. The obtained Ag NP-coated Si nanowells show a dramatic Raman enhancement of the adsorbed *p*-aminothiophenol (PATP, Raman probe) molecules. After formation of sandwich nanostructures by adsorption of Au NPs on PATP-coated Ag NPs, the Raman enhancement increases further. Furthermore, the effect of the pore size and depth of Si nanowells as well as the size of Ag NPs coated on Si nanowells on Raman enhancement are systematically studied.

Second, a simple, convenient and controllable method is developed to fabricate high-density Ag or Au nanogaps on Si wafer. These nanogaps can serve as Raman hot spots (an area in which extremely high Raman enhancement is attained), leading to a dramatic enhancement of Raman signals. The high-density nanogaps can be formed by repeating the electroless deposition of Ag NPs (or Au

NPs) and the PATP monolayer coating on Ag NPs (or Au NPs) surface. After removal of PATP by O₂ plasma, the as-fabricated SERS substrates are useful for detection of molecules.

Third, a method is developed for fabrication of an efficient SERS substrate by combination of metallic nanostructures and graphene, which shows dramatic Raman enhancement and efficient adsorption of aromatic molecules. The fabricated Ag or Au NP-decorated reduced graphene oxide (rGO) on Si substrate is used as an efficient SERS substrate to detect the adsorbed aromatic molecules with low detection limit at nM level. Systematic studies on the effects of NP size and substrate morphology on Raman enhancement are presented. This method might be useful for the future application in detection of biomolecules, such as DNA and protein.

Fourth, a simple but efficient method for highly sensitive Raman detection is proposed by covering a thin polydimethylsiloxane (PDMS) elastomer pre-coated with a layer of Au or Ag NPs onto the detected substrates, resulting in dramatic enhancement of Raman signals. Moreover, this NP-coated PDMS elastomer can be used for chemical imaging with high sensitivity. As a proof of concept, Raman mapping on the micropatterns of PATP and methylene blue are obtained.

In summary, in this thesis, several simple methods are developed for fabrication of efficient SERS substrates for detection of many molecules. More importantly, a simple, convenient but efficient tool is developed for highly sensitive Raman detection and chemical imaging on solid substrates. This might be useful for many applications, such as biomedical detection, in near future.

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CHAPTER 1

INTRODUCTION

1.1. Background

Since discovered by Chandrasekhara Venkata Raman in 1921 [1-2], Raman scattering (or Raman effect), the inelastic scattering of a photon, has been widely used to study vibration of molecules [3-5]. However, Raman signals are quite weak even though laser has been introduced as source light, which limits the applications of Raman spectroscopy.

In 1970s, Martin Fleischman and co-workers discovered that Raman scattering of pyridine adsorbed on electrochemically roughened silver was dramatically enhanced [6], which is called surface enhanced Raman scattering or surface enhanced Raman spectroscopy (SERS). Afterwards, various structures have been proven to be SERS-active [7-11]. Especially since 1990s, thanks to the development of nanoscience and nanotechnology, SERS have been widely studied and the research paper related to SERS boosted in the past several years. Many metallic nanostructures have been fabricated to serve as SERS materials by introducing Raman hot spots, where extremely high Raman enhancement was obtained [12-28]. Recently, the sensitivity of Raman detection has been improved to single-molecules level, so called single-molecule SERS (SM-SERS). Moreover, tip-enhanced Raman scattering (TERS) was invented in the past decade, opening

a gate for highly sensitive nanoscale chemical imaging [29-38]. Due to the development of various SERS substrates and techniques, SERS has been employed as an efficient tool for the detection of biomolecules, cell, virus, *etc.* [18-19,21-23].

Although many strategies have been employed for fabrication of SERS substrates [15,39-57], the normally used nanolithography methods are complicated and costly, especially when the precision control is in the scale of several nanometers. Therefore, it is of great interest and importance to develop simple, convenient and cheap ways for fabrication of SERS substrates with high enhancement factor for sensitive Raman detections.

Chemical imaging is an important advantage of Raman spectroscopy, which is limited by the weak Raman signal. This weak Raman scattering can be dramatically improved by using SERS. However, the detected molecules are normally pre-coated on the SERS substrates, so it is difficult to be used for chemical imaging. Although TERS can be used for nanoscale chemical imaging, but the special set up for TERS and tip fabrication are complicated [32-34,36-38]. Moreover, the total scattered Raman signals are quite weak, due to the limited tip area (typically tens of nanometers in diameter) [32,36-37]. Therefore, it is still required to develop a simple, convenient and flexible way for sensitive Raman detection and chemical imaging.

1.2. Literature Review

1.2.1. Raman scattering

When light is scattered from an atom or molecule, most photons are elastically scattered, called Rayleigh scattering [58]. These elastically scattered photons have the same wavelength and frequency with the incident photons. However, approximately 1 in 10 million photons is scattered inelastically, which is called Raman scattering (or Raman effect) [1-3]. The scattered photons are different with the incident photons in wavelength and frequency. There are two kinds of Raman scattering, as shown in Figure 1-1. For the scattered photons with larger wavelength after inelastic scattering, it is called Stokes scattering. The energy of photons was partly lost during this Stokes scattering. The lost energy is normally applied to activate the vibration of the molecules. Contradictory, that with smaller wavelength is called anti-Stokes scattering. The energy of the scattered photons increases during this scattering, which comes from the vibration of the molecules.

Figure 1-1. The energy transition during Rayleigh scattering, Stokes scattering and anti-Stokes scattering [3].

Raman scattering can only be activated in the presence of non-zero polarizability variation with respect to the normal coordinate, that is $\left| \frac{\partial \alpha}{\partial Q} \right| > 0$ [2-3]. Comparatively, infrared (IR) spectroscopy occurs only when there is a change in the dipole moment (μ) of the detected molecule, that is $\left| \frac{\partial \mu}{\partial Q} \right| > 0$ [2-3]. Unlike that in IR spectroscopy, the vibration of water molecules is inactive in Raman spectroscopy, avoiding the influence of water in detection [5]. This is an advantage of Raman spectroscopy, since water is hard to be eliminated in many cases.

As a technique studying molecular vibration, Raman spectroscopy can provide structural information of molecules, but the extremely small cross section (σ), typically 10^{-30} cm^2 to 10^{-25} cm^2 , limits the applications of Raman spectroscopy. Raman cross section is determined by the polarizability of molecules in an electric field, which influences the intensity of the Raman scattering signals. To improve the intensity of the Raman scattering, one way is the resonance Raman Scattering, by choosing suitable incident light to fit the energy of the photons to the excited energy level of the molecules. In this case, the electrons can be excited to the excited state, instead of the virtual state, resulting in the enhancement of Raman scattering. Another way is to introduce a more intense light source, such as a laser. However, even though laser can be used as light source to enhance the Raman signals, there are still some problems. First, the Raman signal is still weak in some cases. Second, the laser is too strong for some molecules, which can be destroyed by the laser. Therefore, it is necessary to develop a strategy to enhance the Raman signals for molecule detection.

1.2.2. Surface enhanced Raman scattering (SERS)

Due to the importance and weak signal of the normal Raman scattering, it is necessary and important to develop methods to enhance the Raman scattering. In the 1970s, the enhancement of Raman signal on roughened Ag surface was firstly discovered [39]. Afterwards, many strategies were developed to fabricate SERS-active substrates, and the application of Raman scattering was largely extended.

The SERS effect is used to amplify Raman signals by several orders of magnitude [7,10,12,23]. By constructing various metallic nanostructures, Raman signals of pre-coated molecules on the metallic nanostructures can be dramatically enhanced [10,12-28,59-61], thus enhance the sensitivity of Raman detection. Since its discovery, SERS has been intensively studied from both theoretical and experimental aspects. Many structures, fabricated by various methods, have been proven to be SERS-active. The fabricated SERS substrates have been widely used in Raman detection of many molecules with sensitivity down to the single-molecule level.

1.2.2.1. Mechanism of SERS

In order to understand the dramatic Raman enhancement on SERS substrate, it is necessary and important to investigate the mechanism. So far, many mechanisms have been proposed [10,14,62-63]. However, it is still not very clear and under debate. The proposed mechanisms have been classified as electromagnetic mechanism (EM) and chemical mechanism (CM).

a) Electromagnetic mechanism (EM)

When light shined onto metallic nanostructures with features much smaller than the wavelength of incident light, the plasmon oscillation happens due to the variation of the dipolar term (Figure 1-2) [64]. In this case, the incident light resonance with electron cloud of the metallic nanoparticles (NPs), resulting localized surface plasma confined around the metallic NPs [15-17,60]. Due to the localized surface plasma around the NPs, the electromagnetic field can be enhanced by several orders at a specific location, which can act as Raman hot spot, resulting in dramatically enhancement of Raman signals of adsorbed molecules [64]. The Raman enhancement from plasmon oscillation is proportional to E^4 , since the field enhancements occur twice.

Figure 1-2. Schematic illustration of the localized surface plasma resonance around spherical NPs [64].

b) Chemical mechanism (CM)

Although EM of Raman enhancement can be applied regardless of the studied molecules, the magnitude of observed Raman enhancement cannot be fully explained. In some cases, a different enhancement mechanism that does not involve surface plasma is proposed involving the charge transfer between the

chemisorbed species and metal surface. This is called chemical mechanism (CM) [62,65-66], which only applies in specific cases and occurs together with the EM in most cases.

Figure 1-3. Schematic representation of a charge-transfer mechanism in SERS cross-section [10].

In normal Raman scattering, the highest occupied molecular orbital (HOMO) to lowest unoccupied molecular orbital (LUMO) transition for many molecules requires much more energy than the light involved in Raman experiments [10], which typically sits in infra-red or visible range. When the molecule chemically adsorbed on metal surface, the Fermi level of the metal locates between the HOMO and LUMO of the adsorbed molecules, thus light with much lower energy, in which range the incident light may fall in, can be employed to make the transition (Figure 1-3). In this case, the metal acts as a charge-transfer intermediate, so the CM is also called charge transfer mechanism in some cases.

1.2.2.2. Important aspects of SERS

In order to evaluate and compare the fabricated SERS substrates, several factors have been employed to appraise the SERS substrates fabricated using various methods [10,12,14,16,21-22,61]. Among them, the following aspects are relatively more important.

a) SERS substrates and probes

First, in a SERS experiment, the characteristics of the fabricated SERS substrates are important for applications. The methods used for SERS substrate fabrication, distribution of Raman hot spots, uniformity and reproducibility of the fabricated SERS substrates play important roles on SERS detection. For practical application, the methods for SERS substrates should be simple and cheap. The Raman enhancement on the fabricated SERS substrates should be large and uniform.

Second, Raman probes should be carefully chosen in order to obtain good results. It is better to choose the Raman probes with electronic energies close to the exciting laser energy. If the normal Raman signal of studied probes is strong, the SERS signal will definitely be stronger than that with weaker normal Raman signals. Furthermore, the Raman probes should be efficiently adsorbed on the studied SERS substrate.

Third, the detected molecules should be adsorbed, chemically or physically, on the fabricated SERS substrate. Otherwise, it is difficult to detect molecules, especially quantitatively, using the SERS substrates. Many methods can be used

to increase the adsorption ability of SERS substrates, such as surface modification of the SERS substrates, composite with other materials with good adsorption ability.

b) Enhancement factor (EF)

EF is an important parameter to evaluate the fabricated SERS substrates [61]. Note that Raman enhancement on SERS substrate is quite different at different locations. In some area, the Raman enhancement is significant larger than other locations, which are called Raman hot spots [11,15-16,18,23,45,48,51,56,67]. Therefore, in order to investigate the Raman enhancement of SERS substrates, two kinds of SERS EFs are employed: maximum SERS EF, which stands for the Raman enhancement at Raman hot spots, and average SERS EF, which stands for the average Raman enhancement on the whole SERS substrates.

The maximum SERS EF typically occurs at specific location, which is called Raman hot spots. Only the molecules adsorbed in these locations can be enhanced dramatically. The maximum SERS EF may be order of $\sim 10^6$ on a spherical NPs, and can be as high as $10^{10} - 10^{11}$ on specific location of anisotropic NPs, such as apex of a metallic tip, or nanometer gaps between two NPs.

The average SERS EF stands for the average EF of the whole SERS substrates, including the specific Raman hot spots and other areas. Although the specific Raman hot spots have a very large EF up to $10^{10} - 10^{11}$, the portion of the Raman hot spots on SERS substrates is limited, leading to the much smaller average EF. The average EF of an efficient SERS substrate is normally around 10^6 .

The average EF can be calculated using this equation [68].

$$EF = (I_{\text{SERS}}/I_{\text{bulk}}) \cdot (N_{\text{bulk}}/N_{\text{SERS}})$$

Obviously, in order to obtain a huge maximum EF at some locations, the EFs will vary dramatically on the whole SERS substrates. In contrast, in order to get a uniform Raman enhancement on SERS substrates, we have to sacrifice the average EF.

c) Sample preparation

Normally, the detected molecules have to be transferred onto the 2D SERS substrate for detection. Therefore, the adsorption ability of the detected molecules and the interaction between the detected molecules and the SERS substrates are very important for Raman detection. In normal condition, the detected samples are prepared in solution and then transferred to SERS substrate, which is a critical step, since too many effects are involved. Therefore, in order to compare different SERS substrates, it is important to consider the effect of these sample preparation process.

d) Characteristics of SERS signals

The SERS signals detected on the SERS substrates are slightly different from that obtained in normal Raman spectrum [7,10]. First, the bands in the SERS spectroscopy shift a little bit and/or broaden, normally due to the covalent bonding between the Raman probes and the SERS substrates. Second, some Raman bands which cannot be observed due to the selectivity rules will show up due to the different polarization effects on the SERS substrates. Third, a strong

background can always be observed in SERS spectrum, the mechanism of which is still unclear now. One possible explanation is the enhanced fluorescence signals. Last but not least, the SERS signals normally fluctuate during detection, which may be due to photo-bleaching.

1.2.3. Development of SERS

Since its discovery in 1970s [6], the SERS have been intensively studied due to its high sensitivity for Raman detection and wide applications in many fields [18-19,21-23]. SERS-related research boosted in the past decade (Figure 1-4), thanks to the prompt development of nanoscience and nanotechnology. Many theoretical and experimental studies have been done in the past years, and many novel nanostructures and techniques have been developed for SERS applications.

Figure 1-4. Publications related to "surface enhanced Raman scattering" or "surface enhanced Raman spectroscopy" in the past years. (from Web of Knowledge^{SM})

*Analyzed on Jun 14, 2011.

1.2.3.1. Fundamental studies

Fundamentally, researchers have investigated the localized surface plasmon around target NPs and charge transfer process. As discussed above, EM contributes more to the Raman enhancement around metal NPs, so it is important to study the distribution of electromagnetic field around various NPs [15,42,44,50]. The distribution of electromagnetic field around various NPs has been summarised in Figure 1-5.

Figure 1-5. Localized surface plasma around (A) spherical NPs, (B) dimer, (C) triangle, (D) nanorod, (E) ellipse, (F) tip-to-tip triangles and (G) tip-to-end triangles [15,42,44,50].

CM was initially studied on atomic flat metal surface, to get rid of the influence EM effect from rough metal surface [7]. One recent paper has

investigated the chemical mechanism on graphene surface [69], which is atomic smooth.

1.2.3.2. Design and fabrication of SERS substrates

SERS was firstly discovered on roughened Ag surface, and was then found on many other substrates [15,39-57]. Thanks to the development of nanoscience and nanotechnology, many metallic nanostructures Up to now, lots of metallic structures, such as nanogaps [15,70-75], triangles [76], nanorods [50,77-78], nanorices [50], nanoshells [79-81], nanorings [82-85], nanocrescents [86-87], nanostars [53], nanoflowers [88-89] and nanodendrites [56], have been proven to be SERS-active, since the dramatic Raman enhancement can be generated through the Raman hot spots in these nanostructures. Au and Ag NPs with various shapes and dimensions have been synthesized using chemical method, generating efficient SERS substrates after being deposited on solid substrates. Many nanolithography methods, such as e-beam lithography, colloidal lithography, nanoimprint lithography, focus ion beam lithography, have been applied to fabricate metallic nanostructures, especially ordered nanostructures, on substrates, serving as efficient SERS substrates [15,20-21,25,27,40,48,53,55,64,90].

a) Chemical synthesis of metallic NPs

As shown in Figure 1-6, uniform Au and Ag NPs, such as nanorods, nanoplates, nanocubes, nanotriangle, nanotetrahedro, nanostars, nanoshells, nanocages, *etc.* [15,43,46,49-50,52-53,55-57], have been synthesized in the past decades, serving as efficient SERS materials by providing Raman hot spots at

specific positions of the synthesized NPs. The plasmon resonance range of the synthesized NPs can be easily tuned by changing the size and shape of the NPs [41], hence it is easy to optimise the optical property of the synthesized NPs.

Figure 1-6. Various shaped NPs for SERS applications [44,90].

The synthesized NPs can be used for SERS in solution as Raman hot spots, and also can be deposited on solid substrate, generating SERS substrates. Due to the convenience of NP synthesis and flexibility of NP shape and dimension tuning, it can work as a convenient and efficient way to fabricate SERS materials for many applications.

b) Self-organization of NPs

The synthesized NPs can also act as building blocks for ordered patterns of NPs, which have been proven to be efficient SERS substrates. As known, the localized surface plasmon resonance comes from the electron oscillation in metal NPs [63,91]. When two NPs are close to each other, the electron oscillation in one

NP will lead to the oscillation in another NP. Therefore, the plasmon resonance of the two NPs is coupled together. This coupling is dependent on many parameters, such as shape, spacing, orientation, surrounding environment, *etc.* [63,91]. Usually, the gap regions in NP array act as hot spots for Raman scattering. Therefore, it is of great interest to assemble the synthesized NPs into ordered arrays for SERS applications [13,20]. As shown in Figure 1-7, Langmuir-Blodgett (LB) method is an efficient one to fabricate self-assembled monolayer of various NPs, such as spherical NPs, nanocubes, nanorods, nanowires, *etc.*, generating efficient SERS substrates.

Figure 1-7. (A) Langmuir-Blodgett method used for fabrication of NP monolayers [92]. (B) Self-assembled monolayer of spherical Au NPs and (C) variation of EFs with the NP size and the wavelength of incident laser [91]. (D-I) Self-assembled monolayers of spherical NPs, nanocubes, nanorods, truncated nanotubes, nanowires and nanooctahedron, respectively [92-95].

Besides the NP monolayer fabricated using LB method, NPs can also be patterned using templated assembly method, obtaining patterned NP arrays for SERS applications [96-103].

c) Nanolithography methods

Conventional nanolithography methods, such as photo lithography, e-beam lithography [104-105], focus ion-beam lithography, nanoimprint lithography [106-107], have been employed to fabricate ordered SERS substrates, with the advantages of controllability, reproducibility and uniformity. However, these methods are highly costly, if the precision is down to the scale of tens of nanometer. Colloidal lithography method [83,87,108-109], which is much cheaper and convenient than conventional nanolithography methods, has been widely applied to fabricate ordered nanostructures for SERS application. Until now, arrays of triangles, rings, crescents, *etc.*, have been successfully fabricated using this method [83,87,108-109], proving that colloidal lithography is a convenient, flexible and cheap method for fabrication of SERS substrates.

Figure 1-8. (A) Colloidal lithography [83,87,108-109], (B) nanoimprint lithography [106-107] and (C) e-beam lithography [104-105] methods for fabrication of ordered patterns as SERS substrate.

Using lithography-based methods, arrays of different sized and shaped NPs have been fabricated for SERS applications, and many fundamental studies related to these fabricated ordered structures have been carried out, contributing to the development of SERS.

1.2.3.3. Single-molecule SERS (SM-SERS)

Recently, the sensitivity of Raman detection on SERS substrates has been improved to the single-molecule scale [9,22,51,110]. It has been reported that single-molecule Raman detection can be achieved with a minimum EF of 10^7 - 10^8 [111].

In SM-SERS, the most important task is to locate the molecules in Raman hot spots, hence producing sufficient strong Raman signal [9,22,51,110]. Therefore, the research focuses on developing efficient way to locate the molecules in Raman hot spots, such as molecularly-bridged dimers [112]. To achieve this goal, Langmuir-Blodgett films, bi-analyte method, tip-enhanced Raman scattering, *etc.* [9,22,51,110], have been employed in SM-SERS.

1.2.3.4. Tip-enhanced Raman scattering (TERS)

In the past decade, TERS has been developed for nanoscale chemical imaging [29,32-33,36-37,113], by measuring the properties of molecules at the single sharp tip. In TERS, an illuminated SPM or AFM tip is employed, around which the surface plasma is localized, resulting in dramatic Raman enhancement on solid substrates. Three kinds of illuminating methods have been developed: (1) side illumination, (2) in-line illumination (objective), (3) in-line illumination (parabolic mirror) (Figure 1-9). Characterization of molecules can be realized by constructing Raman hot spots *via* moving the tip. So far, TERS has been widely used in fields of chemistry, biology and materials science.

Figure 1-9. (A-B) Scheme showing tip-enhanced Raman scattering, which can dramatically enhance Raman signals of molecules around the illuminated tip [32]. Three employed TERS setup geometries: (C) side illumination, (D) in-line illumination (objective) and (E) in-line illumination (parabolic mirror) [37].

The major advantage of TERS is the nanometer lateral resolution and dramatic Raman enhancement around the tip, which opens a gate for nanoscale chemical imaging using Raman spectroscopy. Therefore, TERS has been used for detection of carbon nanotubes, biopolymers, viruses, biological tissues and other nanostructures [30,32-34,36-38].

1.2.3.5. Applications of SERS

Due to the high sensitivity and intrinsic SERS property compared to conventional Raman spectroscopy, SERS has been used for molecular and

cellular analysis in the fields of chemistry, biology and medicine, *etc.* [17-19,21,24-25,28,59,64].

a) Molecular sensing using SERS

In the past years, thanks to its high sensitivity, SERS has been intensively studied and applied to the detection of molecular vibration. As known, normal Raman spectroscopy can be used to study molecular vibration, but is limited by the weak Raman signal [3]. Compared with normal Raman spectroscopy, the SERS signal is much stronger, hence easier to be detected [7,10]. Moreover, it is possible to detect the Raman signals that cannot be obtained with conventional Raman spectroscopy.

b) Application in life science

The life science has attracted much more attention in recent years, due to the desire for longer and healthier life. Due to the high sensitivity and chemical bond sensitive Raman signal, SERS has been widely applied in life science in recent years, to detect DNA, RNA, protein, as well as cell imaging [18-19,25,59]. Due to the development of SM-SERS and TERS, these techniques have been widely applied in life science.

Another important tool is the newly developed shell-isolated nanoparticle-enhanced Raman scattering (SHINERS) [67], which employed thin silica or aluminum oxide Au NPs to precisely characterize various molecules, surfaces and biological samples. The use of the shell-isolated SERS-active NPs has the benefits of protecting the NPs from contact with the probed molecules and

allowing the NPs to conform to different contours of samples. This method can be developed to be a simple, flexible and fast characterization tool for basic and applied study.

1.2.4. Current research on SERS

Thanks to the development of nanoscience and nanotechnology, SERS has experienced great improvement in the past years, and widely applied in many fields. So far, SM-SERS, TERS and applications in biology have become hot topics [12-19,21-28,59-60,64,90].

Although SERS has been intensively studied in the past years, more studies should be carried out to investigate the mechanism, develop new and simple method for fabrication of SERS substrates, improve TERS and SM-SERS, and extend the applications of SERS, especially in detection of DNA, RNA, protein, virus and cell imaging [19,21,25-26,59].

CHAPTER 2

AIMS AND MOTIVATION

2.1. Objective and Scope

Due to the importance of the SERS substrate and limitation of the common methods to fabricate SERS-active substrates, this project aims to develop simple and convenient methods to fabricate SERS-active substrates. Another objective is to develop a convenient and flexible tool for highly sensitive Raman detection of many molecules.

To achieve our objective, the following work was carried out. First, a solution based methods was developed to fabricate Si nanowell-based SERS substrates and high-density nanogaps as SERS substrates. Second, Au or Ag NPs were combined with rGO sheets for SERS applications. Third, NP-coated PDMS elastomers were used for highly sensitive Raman detection and chemical imaging.

The fabricated SERS substrates were characterized by confocal Raman spectroscopy, field-emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), atomic force microscopy (AFM) and UV-Vis spectroscopy.

2.2. Hypothesis

Based on the work proposed above, the following hypotheses have been made.

First, the rough Ag or Au surface can enhance the Raman signal significantly. Efficient surface enhanced Raman scattering (SERS) substrate with a high enhancement factor (EF) might be produced on a highly roughened Ag or Au surface. Accordingly, the catalytic etching induced one-dimensional Si nanostructures might be an ideal platform for SERS application after they are decorated with Ag or Au nanoparticles (NPs).

Second, Ag or Au nanogaps serve as Raman hot spots due to the strong coupling between the approached NPs. The efficient SERS substrate with a high EF might be generated by fabrication of the high-density Ag or Au nanogaps on solid surface using a direct assembly method.

Third, one major drawback for conventional Ag or Au SERS substrates is their poor adsorption of non-thiolated molecules. The novel two-dimensional material, graphene, shows the efficient adsorption for various aromatic molecules thanks to their π - π interactions. An efficient SERS substrate for aromatic molecules might be fabricated by decoration of graphene with Ag or Au NPs.

Finally, in the conventional SERS system, detected molecules have to be coated on the SERS substrate, and it cannot be used for Raman mapping. It will be promising to propose a simple and convenient SERS method for sensitive Raman detection of any molecules or chemical patterns on surface. This might be realized by covering a metallic NP-coated PDMS elastomer on the detected substrate.

CHAPTER 3

EXPERIMENTAL DETAILS

3.1. Chemicals and Materials

Hydrogen peroxide (H_2O_2 , 30 %), concentrated sulfuric acid (H_2SO_4 , 98 %), hydrogen fluoride (HF, 40 %), potassium permanganate (KMnO_4), gold (III) chloride trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), silver nitrate (AgNO_3), sodium citrate (Na_3Ct), sodium borohydride (NaBH_4), cetyltrimethylammonium bromide (CTAB), 11-amino-undecane-1-thiol (AUT), 16-mercaptohexadecanoic acid (MHA), 1-octadecanethiol (ODT), *p*-aminothiophenol (PATP), 3-aminopropyltriethoxysilane (APTES), *n*-octadecyltrichlorosilane (OTS), methylene blue (MB), rhodamine 6G (R6G), rhodamine B (RB), methyl violet (MV), hydrazine, ethanol and acetone were purchased from Sigma-Aldrich Pte Ltd (Singapore). Natural graphite was purchased from Bay Carbon (Bay City, MI, USA) and used for synthesizing graphene oxide (GO). Silicone elastomer and curing agent (Sylgard® 184 silicone elastomer kit) were purchased from Dow Corning Corporation (Midland, MI, USA). Silicon (100) and silicon dioxide wafers (300 nm oxide layer) were purchased from Bonda Technology Pte Ltd (Singapore). All chemicals were used as received without further purification. Milli-Q water obtained from Milli-Q System (Millipore, Billerica, MA, USA) was used in all experiments.

3.2. Fabrication of Si Nanowell Structures for SERS

3.2.1. Fabrication of Si nanowells

Silicon (100) wafers were first sonicated in acetone for 10 min and rinsed with Milli-Q water, and then immersed into a freshly prepared Piranha solution ($V(H_2SO_4):V(H_2O_2) = 7:3$) at 100 °C for 30 min. After being rinsed with Milli-Q water and dried with N_2 , the Si wafers were immersed in a mixed aqueous solution of HF (1 wt%) and $AgNO_3$ (2 mM) [114-115] for 15 s - 5 min, to deposit Ag NPs. These deposited Ag NPs served as catalysts in the process of subsequent catalytic etching of Si in a mixed aqueous solution of H_2O_2 (0.6 wt%) and HF (5 wt%) [115-116], to form high-density nanowells on Si surface.

3.2.2. Fabrication of Si nanowell-based SERS substrates

The formed nanowells were coated with Ag by immersing in a mixed aqueous solution of HF (1 wt%) and $AgNO_3$ (2 mM) for 15 s - 3 min, generating Ag NP-coated Si nanowells as SERS substrate. A self-assembled monolayer (SAM) of PATP formed on the fabricated SERS substrate by immersing it into a PATP ethanolic solution (1 mM) for 10 min. After being thoroughly rinsed with ethanol and dried by N_2 , this substrate was immersed into a colloidal solution of 13 nm Au NPs for 10 min to adsorb a layer of Au NPs, resulting in Ag NP-PATP-Au NP sandwich structures for SERS application.

In some cases, before the electroless deposition of Ag NPs, the Si nanowells were treated with O₂ plasma (Harrick Plasma PDC-32G-2, Harrick Plasma, Ithaca, NY, USA) for 1 min at a pressure of 200 mTorr and a power of 6.8 W.

3.3. Fabrication of High-Density Ag or Au Nanogaps on Si

3.3.1. Fabrication of high-density nanogaps on Si

After Si wafers were immersed in Piranha solution at 120 °C for 30 min [117-119], they were washed thoroughly with Milli-Q water and dried with N₂, followed by heating at 150 °C in an oven for 8 h. The high-density nanogaps were fabricated on these Si wafers. Briefly, Ag nanoparticles (NPs) were deposited on Si by immersing it in a mixed aqueous solution of HF (2 %) and AgNO₃ (4 mM) [114-115] for 15 s - 5 min. Then the Ag NP-coated Si was immersed in ethanolic solution of PATP (100 µM) for 10 min, washed thoroughly with ethanol and dried with N₂ [119]. Afterwards, the above processes of NP deposition and PATP modification were repeated for several times. For multi rounds deposition of Ag NPs, the reaction time for each steps were 60, 60, 30, 30 and 30 s, respectively. The Raman spectra were measured after every round of NP deposition except the first round, which was measured after PATP coating.

High-density Au nanogaps were prepared by simply replacing the AgNO₃ with HAuCl₄ in above reactions. For multi-round deposition of Au NPs, the reaction time for each steps are 30, 30, 15, 15 and 15 s, respectively. In some cases,

ethanolic solutions of AUT or MHA with same concentration replaced PATP in above reactions.

3.3.2. Removal of thiol molecules from nanogaps for Raman detection

The O₂ plasma treatment was carried out in a Harrick Plasma PDC-32G-2 at a pressure of 200 mTorr and a power of 6.8 W for 1 min. 50 µL of respective ethanolic solutions (1 µM) were dropped on the O₂ plasma-treated high-density Au nanogaps (prepared by four rounds of Au deposition and cut to 8 mm× 8 mm) for detection.

3.4. Fabrication of Ag or Au NP-decorated Reduced Graphene Oxide (rGO) Monolayer for Aromatic Molecule Detection

3.4.1. Preparation of rGO monolayer on Si

Graphene oxide (GO) was synthesized from natural graphite by using the modified Hummers method [120-121]. The synthesized GO was re-dispersed in a mixed solution of water and methanol (1:5), and then assembled on flat Si substrates or Si nanowell substrates using Langmuir-Blodgett (LB) method [122-123]. Briefly, 8-12 mL GO solution was spread onto water surface in the LB trough at a drop rate of 100 µL/min. After compression of the GO film on water surface, a freshly Piranha solution-cleaned Si (or Si nanowell) substrate was vertically dipped into the solution and then slowly pulled up (2 mm/min) to get

single-layer GO film on Si. The GO sheets on Si surface were reduced to obtain reduced graphene oxide (rGO) sheets by hydrazine vapor [120,124-125].

3.4.2. Fabrication of Ag NP-decorated rGO monolayer on Si

The rGO-covered Si substrate was immersed in a mixed aqueous solution of HF (2 %) and AgNO₃ (4 mM) for 15 s - 5 min to synthesize a layer of Ag NPs onto rGO and exposed Si surface [114]. The size and distance of Ag NPs can be easily tuned by varying the reaction time. The Ag NPs can be replaced with Au NPs by simply replacing AgNO₃ with HAuCl₄ in the reaction mentioned above.

3.4.3. Adsorption of aromatic molecules on Ag NP-decorated rGO

The R6G, MV, RB and MB were adsorbed onto the as-fabricated SERS substrates by immersing the 10 mm × 6 mm SERS substrates in 1 mL of the respective aqueous solutions (400 μM) for 1 h. The substrates were then thoroughly washed with Milli-Q water and subsequently dried with N₂. The bulky samples of R6G, MV, RB and MB powder on Si were measured by Raman spectroscopy and used for control experiments.

3.5. Fabrication of NP-Coated PDMS Elastomers for Enhancement of Raman Scattering

3.5.1. *Synthesis of the Au and Ag NPs*

The Au NPs and Ag NPs used in this work were synthesized using a two-step method [126-127]. Briefly, 13 nm Au NPs was prepared by boiling of HAuCl₄ (0.5 mM) and Na₃Ct (1 wt%) for 20 min [128-129]. The as-prepared Au NP seeds (different amount) were added into 20 mL solution of HAuCl₄ (0.5 mM) and heated to boiling, followed by addition of 2 mL Na₃Ct (1 wt%), to obtain the bigger Au NPs.

The Ag seed was firstly synthesized by addition of 60 μ L of NaBH₄ (0.1 M) into 2 mL mixed solution of Ag NO₃ (0.5 mM) and Na₃Ct (1.5 mM), followed by aging for 2-5 h. Then the as-prepared Ag NP seeds (different amount) were added into 20 mL Ag NO₃ (0.5 mM) and heated to boiling, followed by addition of 2 mL Na₃Ct (1 wt%), to obtain bigger Ag NPs.

These synthesized Au or Ag NPs were used directly in the following experiments.

3.5.2. *Preparation of Ag or Au substrate*

The freshly washed Si substrates were coated with 3 nm Ti as an adhesion layer and subsequently with 30 nm Ag or Au using a magnetron sputtering system

(base pressure $< 1 \times 10^{-5}$ Torr). The surface roughness (root-mean-square, RMS) of a $1 \times 1 \mu\text{m}^2$ Ag surface is 6.2 nm, measured by tapping mode atomic force microscopy (TMAFM).

3.5.3. *Micropattern fabrication of PATP and MB for chemical imaging*

The micropatterns of PATP and MB were fabricated *via* microcontact printing (μCP) of PATP on Ag substrates followed by passivation with MB. A PDMS stamp was first inked with 2 mM PATP ethanolic solution for 10 s. After drying it with N_2 gas, the coated PDMS stamp was covered on a fresh Ag surface for 60 s. The PATP-patterned Ag substrate was then passivated with MB by immersing it into a 1 mM ethanol solution of MB for 10 s, followed by rinsing thoroughly with ethanol and drying with N_2 .

3.5.4. *Fabrication of NP-coated PDMS elastomers*

Thin PDMS elastomers were fabricated by pouring a mixture of Sylgard® 184 elastomer and curing agent (w/w = 10:1) on a flat Si substrate and heating at 70 °C for 12 h after degassing [130]. The as-prepared PDMS elastomers were treated with O_2 plasma (Harrick Plasma PDC-32G-2) at a pressure of 200 mTorr and a power of 6.8 W for 30 s, and then immersed in an aqueous solution of APTES (1 wt%) for 10 min [118], followed by thoroughly rinsing with Milli-Q water and drying with N_2 . The APTES-modified PDMS elastomer was immersed in the respective Au or Ag NP solutions for 10 min, followed by thoroughly rinsing with Milli-Q water and drying with N_2 . The Au or Ag NP-coated PDMS

elastomer was employed as an efficient and convenient tool for Raman detection by simply covering it onto the detected substrate.

3.6. Characterizations

3.6.1. Confocal Raman spectroscopy

The Raman detection was carried out on a WITec CRM200 confocal Raman microscopy system with the excitation line of 633 nm and an air cooling charge coupled device (CCD) as the detector (WITec Instruments Corp, Germany). The Raman band of a silicon wafer at 520 cm^{-1} was used as a reference to calibrate the spectrometer.

3.6.2. Ultraviolet-visible (UV-Vis) spectroscopy

UV-Vis absorption curves of the NPs were obtained on a UV-Vis 2501 Spectrometer.

3.6.3. Atomic force microscopy (AFM)

AFM images were obtained by using Dimension 3100 (Veeco, Santa Barbara, CA, USA) in tapping mode with a Si tip (Tap300-Al, BudgetSensor®, spring constant: 42 N m^{-1} , resonant frequency: 320 kHz) under ambient conditions.

3.6.4. *Field emission scanning electron microscopy (FE-SEM)*

FE-SEM was performed with a JEOL JSM-6340F or JSM-7600F field-emission scanning electron microanalyzer. All samples were deposited on Si substrates, dried naturally, and imaged with an accelerating voltage of 5 keV.

3.6.5. *Transmission electron microscopy (TEM)*

TEM was conducted at 200 keV with a JEOL JEM-2010 instrument. TEM samples were prepared by placing a drop of the sample solution on a holey carbon film supported by copper grids and dried naturally.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Ag-Coated Si Nanowells and Subsequent Sandwich Nanostructure for SERS Application

4.1.1. Background

In the past decades, surface enhanced Raman scattering (SERS) techniques have attracted increasing attention due to their applications in physics, chemistry and biology [18-19,21-23]. Up to now, lots of metallic structures, such as nanogaps [15,70-75], triangles [76], nanorods [50,77-78], nanorices [50], nanoshells [79-81], nanorings [82-85], nanocrescents [86-87], nanostars [53], nanoflowers [88-89] and nanodendrites [56], have been proven to be SERS-active, since the dramatic Raman enhancement can be generated through the Raman hot spots in these nanostructures. Comparing with the nanostructures mentioned above, the sandwich metallic nanostructure is simple but efficient one for highly sensitive Raman detection of molecules [13]. However, the high enhancement factor (EF) up to 10^{10} - 10^{11} can only be obtained at the specific locations of the aforementioned structures, and the average EF for the whole substrates is still quite low [14,23]. Moreover, the commonly used methods for fabrication of SERS substrates, such as colloidal lithography [82,131-132], e-beam lithography [68] and templated assembly of nanoparticles (NPs)

[50,81,133-135], are generally complicated and are highly costly with controlled precision of several nanometers. Therefore, it is necessary to develop a simple and convenient method to fabricate SERS substrate with high average EF over the whole substrate.

It has been reported that Si nanostructures can be easily metalized with Ag or Au by electroless deposition method [114-116,136]. Some of these metalized Si nanostructures have been utilized for SERS applications [115,137-139]. However, up to now, only limited types of Si nanostructures have been used for SERS applications. Moreover, the Raman enhancement on these metalized Si nanostructures is still not fully understood. Therefore, the detailed investigation on fabrication and Raman enhancement of the Si nanostructure-based SERS substrates is required.

In this section, we develop a simple and convenient method to fabricate the SERS substrate with high average EF. In a typical experiment, Ag NP-coated Si nanowells were fabricated through the electroless deposition of Ag NPs onto catalytic etching-induced Si nanowells. Significant Raman enhancement of adsorbed molecules was observed on these Ag NP-coated Si nanowells. Importantly, the Raman enhancement further increased by formation of the sandwich structures *via* adsorption of Au NPs on PATP-coated Ag NPs. The Raman enhancement on these SERS substrates can be tuned by controlling the pore size and depth of Si nanowells as well as the size of the Ag NPs coated on Si nanowells.

4.1.2. Results and Discussion

4.1.2.1. Fabrication of Ag-coated Si nanowells for SERS

The fabrication of Si nanowell-based SERS substrate and subsequent sandwich structures is shown in Figure 4-1. In a typical experiment, Si nanowells were fabricated by using the modified Peng's method [116], as shown in Figure 4-1A-B. Briefly, a Si (100) wafer was immersed in a mixed aqueous solution of HF and AgNO₃ [114-115] for electroless deposition of Ag NPs on Si (Figure 4-1A). These Ag NPs serve as catalyst in the subsequent catalytic etching process in a mixed aqueous solution of HF and H₂O₂, to obtain Si nanowells (Figure 4-1B). The fabricated Si nanowells were subsequently coated with Ag NPs by simply immersing them in a mixed solution of HF and AgNO₃ for a certain period [114-115], forming Ag NP-coated Si nanowells that serve as SERS substrates (Figure 4-1C). The fabrication of Si nanowells and subsequent Ag NP coating are performed in aqueous solution at ambient conditions, which are simple and convenient. In addition, this method is highly tunable, as the dimension of Si nanowells and size of coated Ag NPs can be easily adjusted by varying the reaction time. The Raman measurement was carried out after formation of self-assembled monolayer (SAM) of *p*-aminophenol (PATP, a Raman probe) on the coated AgNPs. In order to further increase the Raman enhancement, the sandwich structures, referred to as Ag NP-PATP-Au NP, were generated by adsorbing Au NPs onto the PATP-coated Ag NPs on Si nanowells (Figure 4-1D), through the electrostatic interaction between the negatively charged Au NPs and positively charged PATP.

Figure 4-1. Schematic illustration of fabrication of Si nanowell-based SERS substrate. (A) Ag NPs were deposited on Si (100) wafer. (B) Si nanowells formed by catalytic etching of Si wafer. (C) The formed Si nanowells in (B) were metalized with Ag NPs. (D) Au NPs were adsorbed on the PATP-coated substrate in (C) *via* the electrostatic interaction, to form sandwich structures.

4.1.2.2. Raman enhancements on the fabricated SERS substrate

The Raman enhancement on Ag NP-coated Si nanowells and the subsequent sandwich structures was investigated. As shown in Figure 4-2A, high-density Si nanowells were successfully fabricated using the previous mentioned modified Peng's method. These Si nanowells were then metalized with Ag (Figure 4-2B), forming Ag NP-coated Si nanowells, which were then coated with PATP for investigation of Raman enhancement. The intensities of Raman bands at 1076 and 1142 cm^{-1} (represent a_1 and b_2 vibration modes of PATP, which sit at 1089 and 1142 cm^{-1} respectively for bulk [140]) on the Ag NP-coated Si nanowells at reaction time of 30 s were 65 and 7.9×10^3 times larger than those of bulk, respectively (Figure 4-2C). The enhancement factor (EF) can be calculated by

using this equation, $EF = (I_{\text{SERS}}/I_{\text{bulk}}) \cdot (N_{\text{bulk}}/N_{\text{SERS}})$ [68]. The average EFs over whole surface for Raman bands at 1076 and 1142 cm^{-1} were 2.8×10^6 and 3.4×10^8 , respectively. After forming the Ag NP-PATP-Au NP sandwich structures by adsorption of 13 nm Au NPs, these EFs enhanced further to 8.1×10^6 and 7.0×10^8 for Raman bands at 1076 and 1142 cm^{-1} , respectively. These results demonstrate that high EF over the whole surface can be easily obtained using our methods.

Figure 4-2. SEM images of the Si nanowells (A) before and (B) after deposition of Ag NPs. Inset in (A): section view of Si nanowells. Scale bar = 200 nm. (C) Raman spectra of bulk PATP on flat Si substrate (a, 100-fold magnified) and PAPT SAM adsorbed on Ag NPs coated on Si nanowells before (b) and after (c) adsorption of Au NPs.

4.1.2.3. Role of the Si nanowells on Raman enhancement

In order to study the significant role of Si nanowells on the dramatic Raman enhancement, Raman spectra of PATP adsorbed on Ag NPs coated on flat Si surface and Si nanowells were compared in Figure 4-3. On the Ag NP-coated Si nanowells, Raman bands at 1076 and 1142 cm^{-1} are 4.2 and 7.4 times stronger (Figure 4-3A), respectively, than those on the Ag NP-coated flat Si surface. After formation of the sandwich structures by adsorption of 13 nm Au NPs on PATP-coated Ag NPs, the Raman bands at 1076 and 1142 cm^{-1} on Ag NP-coated Si nanowells were 5.0 and 7.6 times stronger (Figure 4-3B), respectively, than those on Ag NP-coated flat Si surface. These results demonstrate that the Si nanowells play a vital role on the dramatic Raman enhancement of PATP adsorbed on Ag NPs coated on Si nanowells, which is due to the enhanced light absorption arising from the Si nanowells [141].

Figure 4-3. Raman spectra of PATP adsorbed on Ag NPs coated on (a) flat Si wafer and (b) Si nanowells before (A) and after (B) adsorption of Au NPs.

4.1.2.4. Effect of pore size of Si nanowells on Raman enhancement

The results mentioned above proved that our method is a simple and convenient one to fabricate SERS-active substrate with dramatic Raman enhancement. Moreover, the Raman enhancement can be easily tuned by varying the pore size and depth of the Si nanowells as well as the size of Ag NPs coated on Si nanowells. Herein, the influences of these factors on the Raman enhancement were investigated.

First, the pore size of Si nanowells plays an important role on Raman enhancement of the fabricated SERS substrate. The pore size of Si nanowells can be easily tuned by controlling the size of the Ag NPs deposited on Si wafer (Figure 4-1A), the dimension of which was copied in the subsequent catalytic etching process (Figure 4-1B). Notwithstanding the size variation of the Ag NPs, the average diameter increased with reaction time (Figure 4-4A-C), and the Ag NPs fused together after long time reaction (Figure 4-4D). As prolonging the reaction time from 15, 30, 60 to 240 s, the Raman signals of adsorbed PATP on the Ag NP-coated Si nanowells varied slightly (Figure 4-4E), indicating the tiny effect of the pore size of Si nanowells on the Raman enhancement. However, the situation is quite different after adsorption of Au NPs (Figure 4-4F). The Raman signals of adsorbed PATP firstly enhanced with the pore size increase of the Si nanowells at reaction time (reaction in Figure 4-1A) from 15 to 60 s, and then decreased at 240 s reaction. We believe the reason is that the fabricated Si nanowells at 15 s reaction is too small for the 13 nm Au NPs to enter, limiting the quantity of the formed sandwich structures, especially on sidewalls of the Si nanowells. As prolonging the reaction time from 30 to 60s, the Si nanowells

became big enough for the 13 nm Au NPs to enter, resulting in a larger Raman enhancement due to the increased Ag NP-PATP-Au NP sandwich structures on both top and sidewalls of the Ag-coated Si nanowells.

Figure 4-4. (A-D) Si nanowells with different pore sizes generated at reaction time of 15, 30, 60 and 240 s, respectively (see Scheme 1A). Scale bars = 200 nm. (E-F) Raman spectra of PATP adsorbed on Ag NP-coated Si nanowells with different pore sizes (A-D) before (E) and after (F) adsorption of Au NPs.

4.1.2.5. Effect of depth of Si nanowells on Raman enhancement

Second, the depth of Si nanowells also plays an important role on Raman enhancement of the fabricated SERS substrate. As shown in Figure 4-5, Raman intensities of PATP, both before and after adsorption of Au NPs, firstly increased with the depth increase of Si nanowells (from 40 to 220 nm), indicating the significant role of the depth of Si nanowells on the dramatic Raman enhancement. However, the Raman signal dropped a lot for the Si nanowells with depth up to 900 nm, due to the enhanced light absorption [141] on these Si nanowells. The Si nanowells became dark black, confirming the strong absorption of incident light. This can also be confirmed by the decreased reflection of incident light with the increased depth of the Si nanowells (Figure 4-7). In this case, only scant light is scattered and arrives at the air-cooled charge coupled device (CCD). To confirm this, Si nanowells with different depths were prepared using catalytic etching (Figure 4-1A-B), generating Ag NPs sitting at bottom of Si nanowells

Figure 4-5. Raman spectra of PATP adsorbed on Ag NP-coated Si nanowells with different depths before (A) and after (B) adsorption of Au NPs.

(Figure 4-1B), which were then coated with PATP. As increasing the depth of formed Si nanowells, the Raman signals of adsorbed PATP kept stable firstly (depth less than 450 nm), and then decreased dramatically (depth up to 450 nm). When increasing the depth to 900 nm, the Raman signals can hardly be observed (Figure 4-6). This confirmed the limited amount of light arriving at CCD when scattered by the Si nanowells with large depth, which can also be confirmed by the little light reflection from the Si nanowells (Figure 4-7).

Figure 4-6. Raman spectra of PATP adsorbed on the Ag NPs sitting at bottom of Si nanowells with different depths.

Figure 4-7. Reflection spectra of Si, Ag NP-coated Si, and the Si nanowells with different depths.

4.1.2.6. Effect of the size of coated Ag NPs on Raman enhancement

Third, the Raman enhancement on fabricated SERS substrate can also be affected by the size of Ag NPs coated on Si nanowells. It was reported that Ag NPs with several tens of nanometers showed optimized plasmon resonance with the 633 nm laser [23]. In this thesis, the Ag NP-coated Si nanowells at 30 to 60 s reaction show higher Raman enhancement than those with shorter or longer reaction time (Figure 4-8), since these Ag NPs fall into the optimized range.

Figure 4-8. Raman spectra of PATP adsorbed on different sized Ag NP-coated Si nanowells before (A) and after (B) adsorption of Au NPs.

The results presented herein proved that our method is a simple, convenient and tuneable one for fabrication of SERS substrates with high average EF over whole surface. Compared with the commonly used lithography methods, which are complicated and become highly difficult and costly when the precision goes down to scale of several nanometers [68,82,131-132], our method is a highly simple and convenient one to fabricate SERS substrate, as all the processes are performed in aqueous solution at ambient condition. Moreover, high average EFs up to 10^8 are obtained on the fabricated SERS substrates, which is still difficult to be achieved using the commonly used methods. In addition, the EFs can be easily tuned by varying the reaction times in corresponding steps, indicating that our method is a flexible and tuneable one for fabrication of SERS substrates.

4.1.2.7. Effect of O₂ plasma treatment on Raman enhancement

The aforementioned results demonstrate that the Ag NP-coated Si nanowells can lead to dramatic Raman enhancement of the adsorbed PATP, and this

enhancement can increase further by introducing sandwich structures *via* adsorption of Au NPs. However, due to the hydrophobic nature of the Si nanowells after catalytic etching [114,116,136], the aqueous solution of HF and AgNO₃ was hindered from flowing into the pore of Si nanowells, resulting in few adsorption of Au NPs on sidewalls of Si nanowells (Inset b in Figure 4-9A). Obviously, in order to further enhance the Raman signal, it is feasible and necessary to increase the quantity of the formed Ag NP-PATP-Au NP sandwich structures, in other words, to increase the quantity of the adsorbed Au NPs. In this work, O₂ plasma treatment was carried out before the deposition of Ag NPs [142-143]. As reported, the water contact angle on Si-H surface is around 71°, which decreases to around 0° after O₂ plasma treatment. Hence, the mixed aqueous solution of HF and AgNO₃ can flow into the Si nanowells to increase the Ag coverage on sidewalls of the Si nanowells. Thus, more Ag NP-PATP-Au NP sandwich structures formed after adsorption of 13 nm Au NPs on the Ag NP-coated Si nanowells with pore size around 60 nm (Inset in Figure 4-9A), leading to three time enhancement of Raman signal (Figure 4-9). However, for the Si nanowells with pore size around 20 nm, the Raman signals hardly increased by introducing O₂ plasma treatment (Figure 4-9B), due to the limited pore size of Si nanowells for the 13 nm Au NPs. These results demonstrate that O₂ treatment on the Si nanowells affects the obtained Raman enhancements.

Figure 4-9. Raman spectra of PATP adsorbed on the Au NP-adsorbed Ag NP-coated Si nanowells with pore size around (A) 60 nm and (B) 20 nm that treated (b) or not-treated (a) with O₂ plasma. Insets in (A) show section views of the Au NP-adsorbed Ag NP-coated Si nanowells that treated (upper image) or not-treated (lower image) with O₂ plasma, scale bars = 100 nm.

4.1.3. Short summary

In conclusion, a simple, convenient and tunable method was developed to fabricate SERS substrate with high average EF up to 3.4×10^8 by the electroless deposition of Ag NPs on Si nanowells. An even higher average EF up to 7.0×10^8 was obtained by producing Ag NP-PATP-Au NP sandwich structures *via* adsorption of Au NPs onto as-fabricated Ag NP-coated Si nanowells. Moreover, the fabricated SERS substrate can be easily tuned for proposed applications by varying the pore size and depth of the Si nanowells as well as the size of the Ag NPs coated on Si nanowells. All the aforementioned advantages promote our method to be a highly simple and convenient one for highly sensitive Raman detection.

4.2. High-Density Nanogaps as SERS Substrates for Detection of Molecules

4.2.1. Background

Recently, the NP monolayer, fabricated by self-assembly of NPs using Langmuir-Blodgett method, has proven to be an efficient SERS substrate owing to the introduction of high-density metallic nanogaps down to the scale of several nanometers [93,140,144-145]. The Raman enhancement varies with the shape, size of the NPs as well as the distance among them, *i.e.* the formed nanogaps between NPs. However, the fabrication procedure of nanogaps is complicated and the density of the obtained nanogaps is still not high enough.

Herein, we report a simple, convenient and tunable method to fabricate efficient SERS substrates with high-density Ag or Au nanogaps on Si wafers by repeating the electroless deposition of Ag NPs (or Au NPs) and coating of PATP on the deposited Ag NPs or Au NPs through the self-assembly process. Thus the as-fabricated substrate show dramatic Raman enhancement of PATP. After removal of PATP with O₂ plasma, this SERS substrate can serve as an efficient tool for detection of other molecules.

4.2.2. Results and Discussion

4.2.2.1. Fabrication of high-density Ag or Au nanogaps

As shown in Figure 4-10A, Ag NPs (or Au NPs) can be deposited on Si using the electroless deposition method by simply immersing the Si wafer into a mixed

aqueous solution of HF and AgNO₃ (or HAuCl₄), referred as Ag (or Au) growth solution, for a certain period [114-115]. After a self-assembled monolayer (SAM) of *p*-aminothiophenol (PATP) [146] was formed on the Ag NPs (or Au NPs), this NP-coated Si substrate was rinsed with ethanol and dried with N₂. Then it was immersed in the Ag (or Au) growth solution again, more new Ag NPs (or Au NPs) were deposited on the exposed Si surface to form nanogaps between the new NPs and the PATP-coated old ones (Figure 4-10B). By repeating the experiments mentioned above, more NPs were deposited on Si surface to form more nanogaps (Figure 4-10C). The obtained high-density Ag (or Au) nanogaps can serve as Raman hot spots (Figure 4-10D), and an efficient SERS substrate was obtained.

Figure 4-10. Schematic illustration shows the fabrication of high-density Ag or Au nanogaps on Si wafer. (A) Ag or Au NPs deposited on Si wafer. (B) Second round of Ag or Au NPs deposition on the exposed Si region of PATP-coated substrate (A). (C) Third round of Ag or Au NPs deposition on the exposed Si region of PATP-coated substrate (B), obtaining high-density Ag or Au nanogaps. (D) Dramatic Raman enhancement of PATP on substrate (C).

4.2.2.2. Raman property on high-density Ag nanogaps

Figure 4-11A-C showed the formed Ag NPs on Si surface after immersing Si in the Ag growth solution for 30, 60 and 120 s, respectively. It can be found that the size of Ag NPs increased while the size of nanogaps decreased with increasing reaction time. Note that the Ag NPs fused together after long time deposition, *e.g.* 120 s (Figure 4-11C), which lead to the lower density of formed nanogaps. The formed Ag nanogaps can serve as Raman hot spots, resulting in Raman enhancement after PATP SAM formed on Ag NPs by immersing the substrate in PATP ethanolic solution, rinsing with ethanol and drying with N₂. However, the observed Raman signal only varied slightly with the reaction time used for deposition of Ag NPs increased from 15, 30, 60, 90 to 120 s (Figure 4-12A), indicating that the Raman enhancement is less sensitive to the size of the formed Ag NPs on Si surface, due to the limited density of nanogaps formed.

In order to further enhance the Raman signal, we developed a simple approach to increase the density of nanogaps. Note that there are empty areas on Si uncovered by Ag NPs, which were formed at reaction time of 60 s (Figure 4-11B). After it was coated with PATP, rinsed with ethanol, dried with N₂, and then immersed in the Ag growth solution for another 60 s, new Ag NPs formed and grew on the exposed Si areas, generating more new nanogaps (Figure 4-11D). Using this approach, the density of Ag nanogaps increased significantly, resulting in 4.5 - 15 time increase of Raman intensity of PATP at 1072 cm⁻¹ (represents *a*₁ vibration mode of v(CS), which sits at 1089 cm⁻¹ for bulk [146-147]), by varying the reaction time of second round deposition of Ag NPs from 20, 40 to 60 s (Figure 4-12B). However, the size of Ag nanogaps cannot be further reduced by

increasing the second-round reaction time of Ag deposition (Figure 4-11D), due to the coverage of PATP SAMs on the original Ag NPs in the sample of Figure 4-11B. Therefore, the Raman signal of PATP cannot be further increased (Figure 4-12B).

Figure 4-11. SEM images of Ag NPs deposited on Si at reaction time of (A) 30 s, (B) 60 s and (C) 120 s. (D) SEM image of second round deposition of Ag NPs on the PATP-coated substrate (B) at reaction time of 60 s. Scale bars = 200 nm.

After two rounds of 60 s Ag deposition, the formed Ag NPs covered most of Si surface (Figure 4-11D). However, there was still some empty area, where new Ag NPs can be deposited to increase the density of Ag nanogaps. By repeating the electroless deposition of Ag NPs, the density of Ag nanogaps kept increasing, and hence continuously enhanced the Raman signal of PATP (Figure 4-12C-D). However, after the fourth round of Ag deposition, only slight Raman enhancement can be observed (Figure 4-12C-D). At this stage, the Si surface was

almost covered by Ag NPs. Thus, the density of Ag NPs and nanogaps cannot be increased any more. The relative Raman intensities of PATP for band at 1072 cm^{-1} were 15, 27, 28 and 29 after 2, 3, 4 and 5 rounds of Ag deposition, as compared to the Raman intensity of first time deposition of Ag NPs on Si (*i.e.* the sample in Figure 4-11B), respectively (Figure 4-12D). The detailed Raman intensity variations in each step are shown in Figure 4-13, indicating that the nanogaps between Ag NPs deposited in different steps mainly contribute to the dramatic Raman enhancement of PATP. As shown in Figure 4-13, the Raman intensity of

Figure 4-12. (A) Raman spectra of PATP on different sized Ag NPs on Si formed at reaction time of 15, 30, 60, 90 and 120 s. (B) Raman spectra of PATP obtained after second round of Ag deposition on the sample in Figure 4-11B at reaction time of 0 (*i.e.* the sample of Figure 4-11B), 20, 40 and 60 s. (C) Raman spectra of PATP on 1-5 round of 60 s-deposition of Ag NPs on Si. (D) Raman intensity variation of band at 1072 cm^{-1} in (C).

Figure 4-13. Raman intensity variation of band at 1072 cm^{-1} after 1-5 rounds of Ag NP deposition. The 2(a), 3(a), 4(a) and 5(a) show the Raman intensity after 2nd, 3rd, 4th and 5th rounds of Ag NP deposition, respectively. The 2(b), 3(b), 4(b) and 5(b) show the Raman intensity after PATP SAMs coating on the samples of 2(a), 3(a), 4(a) and 5(a), respectively.

PATP increased dramatically after the second round of Ag deposition (2a), while no significant increase of Raman intensity can be observed after the substrate is coated with PATP SAMs (2b). Similarly, after the third, fourth and fifth round of Ag deposition (3a, 4a and 5a), the Raman intensity increase obviously. However, it is hard to observe the Raman intensity variation after the coating of PATP on each round of Ag deposition (3b, 4c and 5c). This indicates that the obtained Raman enhancement of PATP mainly originates from the molecules located in the Ag nanogaps among the deposited Ag NPs generated in different steps of Ag deposition, due to the dramatic EF at these hot spots. The average EF can be calculated using this equation, $EF = (I_{\text{SERS}}/I_{\text{bulk}}) \cdot (N_{\text{bulk}}/N_{\text{SERS}})$ [68]. The average

EFs of PATP coated on the fourth round-deposited Ag NPs were 2.0×10^6 and 1.2×10^8 for bands at 1072 and 1042 cm^{-1} , respectively.

4.2.2.3. Raman property on high-density Au nanogaps

Besides Ag, high-density Au nanogaps can also be prepared on Si using the aforementioned method, by simply replacing the AgNO_3 with HAuCl_4 in the growth solution [114-115]. Similar to the results obtained from Ag nanogaps, the

Figure 4-14. (A) Raman spectra of PATP on different sized Au NPs on Si formed at reaction time of 15, 30, 60, 90 and 120 s. (B) Raman spectra of PATP obtained after second round of Au deposition on the 30 s-deposited Au NPs on Si at reaction time of 0 (*i.e.* the first time Au NPs deposited on Si at reaction time of 30 s), 15, 30 and 45 s. (C) Raman spectra of PATP on 1-5 round deposition of Au NPs on Si at reaction time of 30, 30, 15, 15 and 15 s, respectively. (D) Raman intensity variation of band at 1072 cm^{-1} in (C).

Figure 4-15. Raman intensity PATP on the first circle deposition of Au NPs and LB fabricated Au NPs (20nm) monolayer.

Raman signal of PATP on Au NP-coated Si enhanced with increasing density of Au nanogaps. However, Au nanogaps exhibited a weaker Raman signal, due to the different plasmon resonance light scattering (633 nm laser) from Au NPs. As increasing the reaction time of the first time Au deposition from 15, 30, 60 to 120 s, the Raman signal of PATP on the Au NP-coated Si did not increase significantly (Figure 4-14A). In this case, the Raman enhancement is comparable to that obtained on the monolayer of 20 nm Au NPs fabricated by using Langmuir-Blodgett method (Figure 4-15). However, after the second round Au deposition on the 30 s-deposited Au NPs on Si at reaction time of 15 s, the Raman intensity of PATP increased 3.5 times for band at 1072 cm^{-1} (Figure 4-14B). As increasing the reaction time of second-round Au deposition on Si from 30 to 60 s, the Raman intensity of PATP cannot continuously increase, due to the PATP SAMs coated on the original Au NPs which stop the further reduction of the nanogap size. However, the Raman intensity of PATP further enhanced by

repeating the processes of PATP coating and Au deposition. Here, the reaction time for first two rounds of Au deposition is 30 s, while it is 15 s for the following rounds of Au deposition. The relative Raman intensities of PATP for band at 1072 cm^{-1} increased dramatically to 18.3 and 23.1 after third and fourth rounds of Au deposition, respectively (Figure 4-14C-D), as compared to the Raman intensity of first time deposition of Au NPs on Si at reaction time of 30 s. However, the Raman intensity cannot be continuously increased by repeating the above procedures, due to the almost full coverage of Si surface by Au NPs after several rounds of Au deposition. The detailed Raman intensity variations in each step are shown in Figure 4-16, indicating the significant role of formed nanogaps, among the Au NPs generated in different steps, on the dramatic Raman enhancement.

Figure 4-16. Raman intensity variation of band at 1072 cm^{-1} after 1-5 rounds of Au NP deposition. The 2(a), 3(a), 4(a) and 5(a) show the Raman intensity after 2nd, 3rd, 4th and 5th rounds of Au NP deposition, respectively. The 2(b), 3(b), 4(b) and 5(b) show the Raman intensity after PATP SAMs coating on the samples of 2(a), 3(a), 4(a) and 5(a), respectively.

Besides PATP, it was proved that SAMs of 11-amino-1-undecanethiol (AUT) and 16-mercaptohexadecanoic acid (MHA) [117,119] can also be used to stop the growth of NPs, hence to construct high-density Ag or Au nanogaps for SERS application.

4.2.2.4. High-density nanogaps on Si nanowells

By transferring the high-density nanogaps from flat Si surface onto Si nanowell structures, the Raman intensity of PATP can be further enhanced (Figure 4-17), due to the enhanced light absorption from the 1D Si nanowells, which is consistent with previous report [141].

Figure 4-17. Raman spectra of PATP after four rounds of Ag deposition on Si nanowells.

4.2.2.5. Removal of PATP molecules from high-density nanogaps for sensitive Raman detection

The PATP SAMs coated on Ag or Au NPs can be easily removed by using O₂ plasma cleaning [148], leading to exposure of the Ag or Au NPs. After 1 min O₂ plasma treatment, Raman signals of PATP cannot be observed (curve b in Figure 4-18) from the high-density Au nanogaps fabricated by four rounds of Au deposition (curve 4 in Figure 4-14C), indicating the successful removal of PATP molecules after O₂ plasma treatment. If the aforementioned O₂ plasma-treated substrate was coated with PATP again, the Raman signal of PATP can be observed again. But the signal intensity (curve c in Figure 4-18) was decreased by 40% compared to that from the original high-density Au nanogaps without O₂ plasma treatment (curve a in Figure 4-18), which maybe arise from the decreased

Figure 4-18. Raman spectra of PATP on high-density Au nanogaps (a) before and (b) after O₂ plasma treatment, (c) Raman spectrum after re-dipping the (b) in PATP ethanolic solution.

number of PATP molecules since it is difficult for PATP molecules to form SAMs in the very closed nanogaps again.

These O₂ plasma-treated substrates can be employed as efficient SERS substrates for detection of other molecules due to the dramatic Raman enhancement from the high-density nanogaps. As a proof of concept, methylene blue, rhodamine 6G, 3,5-dibromopyrene and pyrene were detected (Figure 4-19) by simply dropping the respective solutions (1 μ M) onto the fabricated SERS substrates.

Figure 4-19. Raman spectra of (A) methylene blue, (B) rhodamine 6G, (C) 3,5-dibromopyrene and (D) pyrene on the fabricated SERS substrates prepared by dropping 50 μ L of respective ethanolic solutions (1 μ M) on the substrates.

4.2.3. *Short summary*

In summary, we developed a simple, convenient but efficient method to fabricate high-density Ag or Au nanogaps on Si for SERS applications. The high-density Ag or Au nanogaps on Si were obtained by repeating the electroless deposition of NPs and the coating of PATP SAMs. The as-fabricated SERS substrate with high-density Ag or Au nanogaps dramatically enhanced the Raman signal of the coated PATP (Raman probe). After removal of PATP molecules with O₂ plasma, the fabricated substrate can serve as an efficient SERS substrate for detection of other molecules.

4.3. SERS of Ag NP-Decorated Reduced Graphene Oxide for Detection of Aromatic Molecules

4.3.1. Background

In the past decades, various SERS substrates have been fabricated by using e-beam lithography, colloidal lithography, nanoimprint lithography and templated assembly of NPs [16,18,23]. However, due to the less efficient adsorption of some molecules, *e.g.* non-thiolated aromatic organic molecules, onto the plasmonic metals [149], it is still a challenge to fabricate a SERS substrate for detection of these non-thiolated molecules [18].

Graphene, an atomic single-layer of graphite, has been intensively studied in the past years because of its unique electronic, optical, thermal and mechanical properties [150-156] and potential applications in the fields of sensing, electric devices and biology [67,124,153,157-167]. Among these applications, it has been reported that the mechanically cleaved graphene can serve as a SERS substrate for detection of aromatic molecules with EF of 2-17 [69]. It was demonstrated that graphene shows efficient adsorption towards many molecules, especially aromatic molecules. A chemical enhancement mechanism was proposed since the surface plasma of graphene is in terahertz range [69] rather than in the visible range. Although the mechanically cleaved graphene can enhance the Raman signals of adsorbed molecules, the EF is too small and the yield to produce graphene is very low, limiting such method for practical applications. Compared to the mechanical cleaved graphene, reduced graphene oxide (rGO), obtained by

reduction of graphene oxide (GO) [120,124-125], can be produced with high yield, giving more chance in practical applications. Although graphene with much fewer defects can be obtained using chemical vapor deposition (CVD) [168-170], it is normally formed on metal substrates and has to be transferred onto other substrates with complicated processes. Recently, the Raman property of NP-coated graphene or rGO was investigated [171-175]. However, those reports mainly focused on synthesis of the composite of NP-graphene or rGO, and enhanced Raman signal of graphene or rGO. Till now, little work has been carried out to study the Raman signal of adsorbed molecules on NP-decorated rGO.

Figure 4-20. Schematic illustration of the Au or Ag NP-decorated rGO/Si for enhancement of Raman signal of adsorbed molecules.

Herein, we develop a simple and convenient method to fabricate Ag or Au NP-decorated single-layer rGO film on Si surface. The as-fabricated SERS substrates show dramatic Raman enhancement and efficient adsorption of rhodamine 6G (R6G), methyl violet (MV), rhodamine B (RB) and methylene blue (MB) owing to Ag or Au NPs and rGO, respectively. Subsequently, the SERS substrates are

used to detect R6G, MV, RB and MB. It is found that the detection limit could be as low as nM.

4.3.2. Results and Discussion

4.3.2.1. Fabrication of Ag NP-decorated rGO/Si

GO sheets were firstly assembled onto a freshly cleaned Si wafer using Langmuir-Blodgett (LB) method [122-123] to form single-layer GO film. Note that a few double layers might be also obtained, which arose from the irreversible stacking of the size mismatched GO sheets due to face-to-face interactions during the LB process [116,122]. After GO was reduced by hydrazine [120,124-125], the formed reduced rGO-covered Si wafer was decorated with a layer of Ag NPs by simply immersing the wafer into a mixed solution of HF and AgNO₃ [114,116,137]. At the reaction time of 10 s, Ag NPs can be easily deposited onto both rGO sheets and exposed Si region (Figure 4-21A). The size and density of Ag NPs formed on rGO sheets are smaller and lower than those on the exposed Si region, respectively (Figure 4-21B, C). It indicates that Ag⁺ was reduced and Ag NPs formed on Si surface, which is consistent with the previous report [114], while rGO hindered the reduction of Ag⁺, resulting in the smaller size and lower density of the formed Ag NPs. As the reaction time increases, the Ag NPs on both single-layer rGO and exposed Si regions continue growing and the size difference of the Ag NPs becomes smaller (Figure 4-21D-F). These results demonstrate that the size and density of the deposited Ag NPs can be controlled by the rGO sheets and reaction time. In the following experiments, the fabricated substrate in Figure 4-21E was used if there is no special mention.

Figure 4-21. (A) SEM image of Ag NPs formed on rGO films on Si surface at reaction time of 10 s. Magnified SEM images of (B) the exposed Si and (C) single-layer rGO in (A), scale bars = 200 nm. (D-F) SEM images of Ag NPs formed on rGO films on Si surface at reaction time of 30, 60 and 120 s, respectively. (G) SEM image of Au NPs formed on rGO films on Si surface at reaction time of 30 s. Insets in (D-G) show the magnified images of the NPs formed on exposed Si (top) and rGO (bottom), scale bars = 200 nm.

4.3.2.2. Adsorption of aromatic molecules on rGO sheets

The adsorption abilities of different kinds of aromatic molecules on rGO were investigated. The rGO-covered Si wafers can be coated with rhodamine 6G (R6G), methyl violet (MV), rhodamine B (RB) or methylene blue (MB) by immersing the wafers into the respective aqueous solutions (400 μM) for 1 h. The AFM images show that the height of rGO increased from 1.1 to 1.8 nm (Figure 4-22), confirming that R6G was successively adsorbed onto rGO sheets, due to the π - π interaction between R6G and rGO [69]. Similarly, MV, RB and MB can also be efficiently adsorbed on rGO sheets.

Figure 4-22. AFM images of rGO sheets on Si substrate before (A) and after (B) adsorption of R6G.

4.3.2.3. Raman enhancement on Ag NP-decorated rGO sheets

The as-fabricated Ag NP-decorated rGO film on Si wafer, referred to as Ag NPs-rGO/Si, was used as SERS substrate for the aforementioned aromatic molecules. The Raman intensities of R6G, MV and RB on bare rGO adsorbed on

Si surface, referred to as rGO/Si, are hardly observed, in contrast to the strong Raman signals on the Ag NPs-rGO/Si surface (Figure 4-23A-C), indicating the significant role of Ag NPs on the dramatic Raman enhancement. In addition, the strong fluorescence of R6G, MV and RB on Si was observed (Figure 4-23A-C). Therefore, the exact enhancement is difficult to be determined. However, the Raman signal of MB on rGO/Si was identified (Figure 4-23D), allowing us to determine the enhancement of the fabricated SERS substrate.

Figure 4-23. Raman spectra of (A) R6G, (B) MV, (C) RB and (D) MB on Si, rGO/Si and Ag NPs-rGO/Si.

The MB molecules were adsorbed on the exposed rGO region and Ag NPs on Ag NPs-rGO/Si, due to the π - π interaction and covalent bonding [147], respectively. Based on the Raman band of MB at 1625 cm^{-1} (C-C ring stretching mode), the Raman intensity increases 350 times on the Ag NPs-rGO/Si substrate compared to that on rGO/Si (Figure 4-23D), confirming the significant role of Ag NPs on the Raman enhancement of the fabricated SERS substrate. Similarly, the Raman signals of other three molecules (R6G, MV and RB), which do not adsorb efficiently on Ag [149], are also dramatically enhanced on the Ag NPs-rGO/Si substrates (Figure 4-23A-C). These results proved the ability of Ag NPs-rGO/Si to enhance the Raman signal of aromatic molecules, which in general do not adsorb efficiently on the plasmonic metals.

4.3.2.4. Effect of Ag NP sizes on Raman enhancement

Furthermore, Raman intensity of the adsorbed molecules varies with the Ag NP size, which was easily controlled by the reaction time when rGO/Si was immersed into a mixed solution of HF and AgNO_3 [114,116,137] (Figure 4-24). As the size of Ag NPs increases with the reaction time, the gaps between Ag NPs become smaller, leading to fewer adsorbed molecules and stronger coupling between NPs [176]. At the beginning, Raman intensity of R6G increases with the size increase of Ag NPs, due to the stronger coupling between the NPs. The Raman intensity of R6G on Ag NPs-rGO/Si after 30 s reaction is 6.3 times larger than that on Ag NPs-rGO/Si after 10 s reaction. At 30 s-reaction time, although the area of exposed rGO decreases, the stronger coupling between Ag NPs [176] outdoes this drawback and hence, the substrate gives larger Raman enhancement. As the reaction time is prolonged, the Raman intensity becomes stable due to the

combination effects, *i.e.* the stronger coupling between Ag NPs and the smaller area of exposed rGO for adsorption of R6G. These results demonstrate the significant role of rGO sheets and Ag NPs, used for the adsorption of aromatic molecules and Raman enhancement, respectively. Besides Ag NPs, Au NPs can also be coated onto single-layer rGO sheets (Figure 4-21G) in a similar way, simply by replacement of AgNO₃ with HAuCl₄ in the electroless deposition procedure. These Au NP-decorated rGO sheets can also be used for highly sensitive Raman detection.

Figure 4-24. Raman spectra of R6G on rGO/Si and Ag NPs-rGO/Si with Ag NPs formed at reaction time of 10, 30, 60 and 120 s.

4.3.2.5. Quantitative detection using Ag NP-decorated rGO/Si

Importantly, quantitative detection of R6G, MV, RB and MB can be carried out by using the as-fabricated SERS substrates. The SERS substrates were immersed into aqueous solutions of aforementioned molecules with different

concentration for 1 h, and subsequently washed with Milli-Q water and dried by N_2 . Figure 4-25 shows that R6G, MV, RB and MB can be detected on Ag NPs-rGO/Si SERS substrates even at a low concentration. For example, the Raman signal of R6G could be detected at concentration of 10 nM, but no signal of R6G was observed on rGO/Si even at concentration of 1 mM. The Raman signals for MV, RB and MB can be detected at concentration of 10, 1 and 1 nM, respectively, indicating that the as-fabricated SERS substrate can be used to detect molecules with high sensitivity.

Figure 4-25. The plots of Raman intensity vs. concentration of (A) R6G, (B) MV, (C) RB and (D) MB, which were adsorbed on Ag NPs-rGO/Si in Figure 4-21E. Inset images show Raman spectra of the respective molecules at concentration of 10 nM, 10 nM, 1 nM and 1 nM, respectively.

4.3.2.6. Ag NP-decorated rGO on Si nanowells

The Raman enhancement can be further increased by replacing the flat Si wafer with Si nanostructure, *e.g.* Si nanowells. The Si nanowells were fabricated based on modification of the previous method [115-116]. GO sheets were then assembled onto Si nanowells using LB method [122-123], and subsequently reduced to rGO with hydrazine vapour [120,124]. The rGO sheets covered on top of Si nanowells (Figure 4-26A, B). Based on the band at 1625 cm^{-1} , Raman intensity of adsorbed MB on rGO sheets on Si nanowells increases about 6 times compared to that on rGO/Si (Figure 4-26C), demonstrating that Si nanowells can increase the Raman signal of adsorbed molecules, due to the enhanced light absorption, which is consistent with the previous report [141].

Figure 4-26. SEM images of (A) top and (B) side view of rGO sheets assembled on Si nanowell substrates. Inset in (A): A magnified top-view image. (C) Raman spectra of adsorbed MB on rGO sheets coated on Si and Si nanowells.

After the rGO-coated Si nanowells were decorated with Ag NPs using the aforementioned method, it further enhanced the Raman signal. The Raman

intensities of R6G, MV and RB on Ag NPs-rGO on Si nanowells increased for about 5 times compared to that on Ag NPs-rGO/Si (Figure 4-27A-C). As for MB, the Raman enhancement is even higher, *ca.* 15.9 times (Figure 4-27D), since more MB was adsorbed onto Ag NPs *via* the covalent bonding [147]. These results demonstrate that the Ag NP-coated Si nanowells can significantly enhance the Raman signal of adsorbed molecules, due to the enhanced light absorption, which is similar to that on bare Si nanowells, but much stronger Raman signal was obtained in the Ag NP-coated Si nanowells. Therefore, the Ag NPs-rGO on Si nanowells can serve as a more efficient SERS substrate for detection of

Figure 4-27. Raman spectra of (A) R6G, (B) MV, (C) RB and (D) MB on Ag NPs-rGO on Si nanowells (upper curves) and Ag NPs-rGO/Si (bottom curves). The reaction time for formation of Ag NPs is 60 s.

molecules, especially for aromatic molecules. Since previous reports demonstrated that DNA and protein can be efficiently adsorbed onto graphene surface [158,177-178], we believe our Ag NPs-rGO on Si or Si nanostructures will have great potential for detection of DNA or protein.

4.3.3. Short summary

A simple, convenient and efficient method is developed to fabricate Ag NP-decorated rGO sheets, by electroless deposition of Ag NPs on rGO sheets coated on Si substrates, which exhibit strong adsorption of aromatic molecules and dramatic Raman enhancement, owing to the nature of rGO and the deposited Ag NPs, respectively. The adsorbed aromatic molecules are easily detected with high sensitivity. Importantly, the enhancement can be further increased if Si nanostructures are used to replace flat Si substrates. Moreover, our developed SERS substrates might have great potential to detect biomolecules, such as DNA and protein.

4.4. NP-Coated PDMS Elastomers for Enhancement of Raman Scattering

4.4.1. Background

Although lots of metallic nanostructures have been proven to be SERS-active [15,45,48,51,56], the applications of SERS are still limited due to the following reasons. First, the metallic nature of the currently used SERS substrates makes them difficult to strongly adsorb many molecules, such as aromatic molecules, DNA, protein, *etc.* [18,21,59]. Second, the detected molecules are normally coated on the SERS-active substrates [32], so it is difficult to get chemical images by using the conventional SERS method.

Recently, tip-enhanced Raman spectroscopy (TERS) has been developed to overcome the aforementioned drawbacks [29,32-33,36-37,113]. Thanks to the local electromagnetic near-field enhancement provided by an illuminated STM or AFM tip, TERS, as a widely applicable spectroscopy and microscopy tool, has been used to detect various molecules on various substrates [32-33,113]. Besides these, TERS can also be used for surface chemical imaging at the nanometer scale [29,36-37,113]. However, a special setup for TERS is required, and fabrication of the tip is quite complex. Furthermore, the total Raman scattering signal from the tip area (about 20-50 nm in diameter) is rather weak [32,36-37]. Therefore, the applications of TERS are limited.

In this section, after Au or Ag NPs are adsorbed onto the 3-

aminopropyltriethoxysilane (APTES)-modified polydimethylsiloxane (PDMS) elastomer, it is used as a novel tool for the enhancement of Raman scattering. This method can be used for the sensitive Raman detection of molecules adsorbed on solid substrates by simply covering the NP-coated PDMS elastomer onto the detected surface. Importantly, this method is also used for chemical imaging to distinguish the micropatterns of PATP and MB with high Raman sensitivity. Compared with TERS, the total Raman scattering volume in our method is much larger, leading to much stronger Raman signal collected.

4.4.2. Results and Discussion

4.4.2.1. Fabrication of NP-Coated PDMS elastomers

The Au or Ag NP-coated PDMS elastomer was fabricated and used for highly sensitive Raman detection and chemical imaging of molecules. Briefly, the thin PDMS elastomer was prepared by curing the mixture of Sylgard® 184 elastomer and curing agent (w/w = 10:1) at 70 °C for 12 h [130], followed by O₂ plasma treatment and then APTES modification [118]. Afterwards, the Au or Ag NPs were adsorbed onto the APTES-modified PDMS elastomer (Figure 4-28A), thanks to the electrostatic interaction between the negative charged NPs and the positive charged APTES SAMs [118-119]. The as-prepared NP-coated PDMS elastomer serves as a flexible, convenient and efficient tool for highly sensitive Raman detection and chemical imaging of molecules adsorbed on solid substrates by simply covering this elastomer onto the detected surface (Figure 4-28B).

Figure 4-28. (A) Au or Ag NPs were adsorbed on APTES-modified PDMS elastomer. (B) Raman detection by covering the as-fabricated NP-coated PDMS elastomer (A) onto the detected substrate.

4.4.2.2. Au NP-coated PDMS elastomers for Raman enhancement

It has been reported that Au NPs can enhance the Raman signal of molecules, which adsorbed on or were close to the Au NPs [112,176]. On the surface of the as-fabricated Au NP-coated PDMS elastomer, lots of Au NPs are coated, which serve as the hot spots in Raman detection. When the Au NP-coated PDMS elastomer covers on the detected substrate, the Au NPs contact with or are close to the molecules on the solid substrate, resulting in Raman signal enhancement of the molecules. As shown in Figure 4-29A, the highly sensitive Raman intensity of MB monolayer on Ag surface was measured with the aid of the 38 ± 3 nm Au NP-coated PDMS elastomer. The EF can be calculated using this equation, $EF=(I_{SERS}/I_{bulk})\cdot(N_{bulk}/N_{SERS})$ [68]. Before covering the 38 ± 3 nm Au NP-coated

PDMS elastomer, the EF of MB on Ag surface is 3.2×10^4 (based on the band at 1625 cm^{-1}), while it increases to 3.8×10^6 after covering the $38 \pm 3 \text{ nm}$ Au NP-coated PDMS elastomer, resulting in 120 times increased (Figure 4-29A). We believe that the Raman enhancement arises from the formed Ag-MB-Au NP sandwich structures between Ag surface and Au NPs on the PDMS elastomer.

Figure 4-29. (A) Raman signals of MB on Ag surface before (a, $\times 50$) and after (b) it was covered with $38 \pm 3 \text{ nm}$ Au NP-coated PDMS elastomer. (B) Raman mapping of MB on Ag surface which was covered with $38 \pm 3 \text{ nm}$ Au NP-coated PDMS elastomer. Inset: The cross section analysis of the white line in (B).

In a typical TERS, the EF around the tip is about 10^4 [29]. However, due to the limited tip area (*ca.* 20-50 nm in diameter) [32,36-37] and the only one tip serving as the Raman hot spot, the total Raman scattering signal is quite weak. In our experiment, there are about 200 Au NPs (size of $38 \pm 3 \text{ nm}$) in $1 \mu\text{m}^2$ area (Figure 4-30C) serving as Raman hot spots, resulting in the dramatic enhancement of Raman signal in our experiment since the size of a typical laser beam in a confocal Raman spectrometer is *ca.* $1 \mu\text{m}^2$.

As shown in Figure 4-29B, the detected Raman intensity of MB on Ag surface after covering the 38 ± 3 nm Au NP-coated PDMS elastomer is quite uniform, indicating the uniform Raman enhancement of our method. Although the adsorption of 38 Au NPs on PDMS elastomer is not uniform in the nanometer scale (Figure 4-30C), it is relative uniform in the micrometer scale. Since the diameter of laser beam is in the micrometer range, the Raman mapping of MB shows quite uniform Raman intensity after covering Au NP-coated PDMS elastomer. All the aforementioned experimental results demonstrate that the Au NP-coated PDMS elastomer can serve as a simple, efficient and convenient tool to uniformly enhance Raman signal of molecules on solid substrates in the micrometer scale. In addition, the Au NP-coated PDMS elastomer can be reused, since the MB molecules are not adsorbed on the elastomer, and only exist on the underneath Ag substrate.

In our experiments, the PDMS elastomer was chosen to adsorb Au NPs and then used for enhancement of Raman signal of molecules adsorbed on solid substrates, because the PDMS elastomer is transparent, soft and easy to be attached onto the solid substrates, leading to the uniform contact with the underneath detected surface. As a comparison, glass, much more rigid than PDMS, is not suitably used in this method, since the Au NP-coated glass cannot contact very well with the underneath detected surface, leading to much larger distance between Au NPs and the molecules on the solid substrate. Therefore, the Raman intensity does not show the obvious enhancement.

4.4.2.3. Effect of Au NP size on Raman enhancement

As shown in Figure 4-30, the size of adsorbed Au NPs plays an important role on the enhancement of Raman signal. The 13 ± 1 nm Au NP-coated PDMS elastomer (Figure 4-30A) gives poor Raman enhancement, which is only 13 times increase for MB (band at 1625 cm^{-1}) after covering it on the MB-coated Ag surface (curve a in Figure 4-30D). However, the Raman enhancement is much larger for larger Au NPs. Figure 4-30D shows the results for the 16 ± 8 and 38 ± 3 nm Au NP-coated PDMS thin elastomers, and the Raman intensity of MB increased 32 and 120 times (band at 1625 cm^{-1}), respectively. The better performance of larger Au NPs is due to the stronger coupling between the larger Au NPs [176]. For PATP, the Raman signal can hardly be observed before covering the Au NP-coated PDMS thin elastomer. However, after covered with the Au NP-coated PDMS thin elastomer, the Raman intensity of PATP increased dramatically and were easily observed (Figure 4-30E). Similarly, the 38 ± 3 nm Au NP-coated PDMS elastomer shows largest enhancement of PATP coated on Ag substrate, resulting in EF of 4.6×10^6 and 7.9×10^7 for bands at 1072 and 1141 cm^{-1} , respectively.

Figure 4-30. SEM images of Au NPs with size of (A) 13 ± 1 , (B) 16 ± 8 and (C) 38 ± 3 nm adsorbed on PDMS elastomers which were coated 10 nm Pt film before imaging. Raman detection of MB (D) and PATP (E) on Ag substrates which were covered by the Au NP-coated PDMS elastomer. Curves (a-c) were obtained with different sized Au NP (A-C) coated PDMS elastomers.

4.4.2.4. Ag NP-coated PDMS elastomers for Raman enhancement

Besides Au NPs, Ag NPs can also be adsorbed onto the APTES-modified PDMS elastomers for highly sensitive Raman detection. Figure 4-31D-E show the Raman intensity of MB and PATP after covering the Ag NP-coated PDMS elastomers on MB or PATP coated Ag substrates. The 18 ± 6 nm Ag NP-coated PDMS elastomer (Figure 4-31A) gives poor Raman enhancement, which is only 7.5 times increased for MB (band at 1625 cm^{-1}) (curve a in Figure 4-31D). However, the 42 ± 12 and 68 ± 20 nm Ag NP-coated PDMS elastomers (Figure

4-31B-C) provide 47 and 71 times increase of Raman signal for MB (band at 1625 cm^{-1}), respectively (curves b and c in Figure 4-31D). Similarly, the Raman intensity of PATP pre-coated on Ag surface is also dramatically enhanced (Figure 4-31E) after covering the Ag NP-coated PDMS elastomers.

Figure 4-31. SEM images of Ag NPs with size of (A) 18 ± 6 , (B) 42 ± 12 and (C) 68 ± 20 nm adsorbed on PDMS elastomers which were coated 10 nm Pt film before imaging. Raman detection of (D) MB and (E) PATP on Ag substrates which were covered by the Ag NP-coated PDMS elastomer. Curves (a-c) were obtained with different sized Ag NPs (A-C) coated PDMS elastomers.

4.4.2.5. Au NP (or Ag NP)-coated PDMS elastomers on Au (or Ag) substrates

The Raman enhancements of molecules on Au or Ag substrates after covering Au or Ag NP-coated PDMS elastomers were compared in Figure 4-32. The Raman enhancements on both Ag and Au substrates achieved by covering the 42 nm Ag NP-coated PDMS elastomers (curves c and d in Figure 4-32) were larger than that achieved by covering 38 ± 3 nm Au NP-coated PDMS elastomers (curves a and b in Figure 4-32).

Figure 4-32. Raman spectra of MB on Au (curves a and c) and Ag (curves b and d) substrates after being covered by the 38 ± 3 nm Au NP (curves a and b) and 42 nm Ag NP (curves c and d)-coated PDMS elastomers.

As shown in Figure 4-30C and Figure 4-31, the density of 38 ± 3 nm Au NPs on PDMS elastomers was higher than that of 42 nm Ag NPs, indicating that the major reason for the larger Raman enhancement achieved by covering the Ag NP-coated PDMS elastomer is the stronger plasmon coupling between Ag NPs on

PDMS elastomer and underneath Ag or Au substrates. By covering the 38 ± 3 nm Au NP-coated PDMS elastomer, the Raman enhancements of MB on both Au and Ag substrates were comparable (curves a and b in Figure 4-32) with slightly stronger Raman signals on Ag surface.

4.4.2.6. Raman enhancement on dielectric surface

The Raman enhancement of reduced rGO on SiO_2 surface, which is dielectric, was also investigated but not shown in main text. Briefly, rGO was adsorbed on APTES-modified SiO_2 substrate. The Raman signals of D and G bands of rGO increased for about 12 times after covering our 38 ± 3 nm Au NP-coated PDMS elastomers (Figure 4-33A), which is smaller than that on Ag surface. After adsorption of MB on this rGO-coated SiO_2 substrate, the Raman signal of MB increased for about 12 times after covering the 38 Au NP-coated PDMS

Figure 4-33. (A) Raman spectra of rGO coated on SiO_2 substrates before (curve a) and after (curve b) being covered by the 38 ± 3 nm Au NP-coated PDMS elastomers. (B) Raman spectra of MB adsorbed on rGO/ SiO_2 surface before (curve a) and after (curve b) being covered by the 38 ± 3 nm Au NP-coated PDMS elastomers.

elastomers (Figure 4-33B). Abovementioned results proved that our NP-coated PDMS elastomers could be used to detect molecules on both metal and dielectric substrates. However, the enhancement on Ag or Au is much larger, due to the formed sandwich structures between NPs on PDMS and Ag or Au substrate.

Above results indicate that the as-fabricated SERS membrane can be employed as a flexible and convenient tool to investigate the adsorption behavior of rGO sheets, which is important for application of graphene, especially in biomedical application [158,177]. In future, this might be useful to investigate the adsorption of DNA or protein on rGO or graphene surface.

4.4.2.7. Chemical imaging on solid substrate

Our experimental results mentioned above indicate that the Au or Ag NP-coated PDMS elastomer can be used for highly sensitive Raman detection of molecules on Ag substrates. More importantly, by using this method, the highly sensitive chemical imaging can be easily achieved. Figure 4-34 demonstrates the detection of PATP and MB micropatterns on Ag surface. The patterns were fabricated *via* microcontact printing (μ CP) of PATP dots, followed by passivation with MB (Figure 4-34A). In normal condition, the as-fabricated PATP-MB micropatterns cannot be imaged with confocal Raman microscopy even with a long integration time of 0.5 s (Figure 4-34B). However, after covered with the 38 ± 3 nm Au NP-coated PDMS elastomer, the patterns can be easily imaged even with a short integration time of 0.01 s. Figure 4-34C-D show the Raman imaging of MB (bands: $1600\text{-}1645\text{ cm}^{-1}$) and PATP (bands: $1550\text{-}1580\text{ cm}^{-1}$), respectively. The Raman signals of PATP and MB micropatterns are easily detected (Figure

4-34E). It is noted that, in PATP dot patterns, the Raman signal of MB are also detected, and its intensity (band at 1625 cm^{-1}) is $\sim 1.6\%$ of that in the pure MB region. This indicates the defects existed in the formed PATP SAMs generated by μCP , which were then partially covered by MB, resulting in the weak Raman signal of MB observed in PATP patterns.

Figure 4-34. (A) Schematic illustration of micropatterns of PATP and MB formed on Ag substrate. (B-C) Raman mapping of MB (bands: $1600\text{--}1645\text{ cm}^{-1}$) before and after covering $38\pm 3\text{ nm}$ Au NP-coated PDMS elastomer. (D) Raman mapping of PATP (bands: $1550\text{--}1580\text{ cm}^{-1}$) after covering $38\pm 3\text{ nm}$ Au NP-coated PDMS elastomer. (E) Raman signals in MB and PATP patterned regions.

As discussed above, the Au NP-coated PDMS elastomer can be used for chemical imaging of molecules with high sensitivity, which is the major advantage of our method. Using the conventional SERS method, the detected molecules must be adsorbed onto the SERS-active substrates, so it is difficult to be used for chemical imaging (see Figure 4-34B). Unlike the conventional SERS, TERS can be used for chemical imaging, but the total Raman scattering signal from the tip area (about 20-50 nm in diameter) is rather weak [32,36-37], resulting in the quite low sensitivity. Compared with conventional SERS and TERS methods, our method can be used for highly sensitive chemical imaging by simply covering the Au NP-coated PDMS elastomer onto the detected substrates. Thanks to the high-density Au NPs coated on PDMS serving as Raman hot spots, the collected Raman signal can be dramatically enhanced, which is much higher than that obtained in typical TERS. Even though the lateral resolution is sacrificed compared with TERS, our method is simple and convenient in contrast to the special setup and complex operation of TERS. Therefore, our method can be used as an efficient and convenient tool for chemical imaging of molecules with high sensitivity.

4.4.3. Short summary

In conclusion, a simple and convenient method has been developed to successfully enhance the Raman signals of molecules on solid substrates by covering the Au or Ag NP-coated PDMS elastomer onto the detected surface. By using this method, the chemical imaging are also achieved, which is difficult from the conventional SERS and low sensitive TERS methods. As a proof of concept,

the micropatterns of PATP and MB are imaged. These promising results demonstrate that the NP-coated PDMS elastomer can be employed as a simple, efficient and flexible tool for highly sensitive Raman detection and chemical imaging of molecules on solid substrates.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

In this thesis, several new and simple methods were developed for fabrication of SERS substrates for highly sensitive Raman detection, and an efficient tool was developed for highly sensitive Raman detection and chemical imaging on solid substrate.

First, a simple, convenient and tuneable method was developed to fabricate SERS substrates with high average EF up to 10^8 by the electroless deposition of Ag NPs on catalytic etching-induced Si nanowells. An even higher average EF was obtained by producing Ag NP-PATP-Au NP sandwich structures *via* adsorption of Au NPs onto the Ag NP-coated Si nanowells. Moreover, the fabricated SERS substrate can be easily tuned for proposed applications by varying the pore size and depth of the Si nanowells as well as the size of the Ag NPs coated on Si nanowells, confirming our method to be a simple and convenient one for fabrication of efficient SERS substrates.

Second, a simple, convenient but efficient method was developed to fabricate high-density Ag or Au nanogaps on Si for SERS applications, by repeating the electroless deposition of NPs and the coating of PATP SAM. The fabricated

SERS substrate with high-density Ag or Au nanogaps dramatically enhanced the Raman signal of the coated PATP (Raman probe). After removal of PATP molecules with O₂ plasma, the fabricated substrate can serve as an efficient SERS substrate for detection of many molecules.

Third, a simple, convenient and efficient method is developed to fabricate Ag NP-decorated rGO sheets, by electroless deposition of Ag NPs on rGO sheets coated on Si substrates, which exhibit strong adsorption of aromatic molecules and dramatic Raman enhancement, owing to the nature of rGO and the deposited Ag NPs, respectively. The adsorbed aromatic molecules are easily detected with high sensitivity. Importantly, the enhancement can be further increased by replacing flat Si substrates with Si nanostructures. The developed SERS substrates might have great potential to detect biomolecules, such as DNA and protein.

Finally, a simple and convenient method has been developed to successfully enhance the Raman signals of molecules on solid substrates by covering the Au or Ag NP-coated PDMS elastomer onto the detected surface. By using this method, the chemical imaging are also achieved, which is difficult from the conventional SERS and low sensitive TERS methods. As a proof of concept, the micropatterns of PATP and MB are imaged. These promising results demonstrate that the NP-coated PDMS elastomer can be employed as a simple, efficient and flexible tool for highly sensitive Raman detection and chemical imaging of molecules on solid substrates.

5.2. Recommendations for Future Work

Simple methods have been developed in this thesis for fabrication of new and novel SERS substrates for molecular detection, and a convenient and flexible tool has been developed for highly sensitive Raman detection and chemical imaging on solid substrates. Hence, the following future works are recommended.

First, the average EF obtained is relatively large on our fabricated Si nanowell-based and high-density nanogap-based SERS substrates. Therefore, it will be interesting to detect biomolecules, carbon nanotubes and other nanomaterials by using these SERS substrates.

Second, it is of great importance and interest to detect biomolecules, such as DNA, RNA, protein, using the composite of rGO and Au (or Ag) NPs, due to the present of strong adsorption and dramatic Raman enhancement arising from the nature of rGO and deposited NPs, respectively.

Third, it is interesting to detect nanomaterials or chemical nanofeatures using the fabricated NP-coated PDMS elastomers, due to the simplicity, flexibility, reusability and convenience of the NP-coated PDMS elastomers. Moreover, it is possible to enhance the fluorescence of molecules on solid substrates using the NP-coated PDMS elastomers, which will be of great interest and importance in field of surface enhanced fluorescence (SEF).

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