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SURFACE-ENHANCED RAMAN SCATTERING: PRINCIPLES, NANOSTRUCTURES, FABRICATIONS, AND BIOMEDICAL APPLICATIONS

CLEMENT YUEN, WEI ZHENG and ZHIWEI HUANG*

Bioimaging Laboratory, Department of Bioengineering
Faculty of Engineering, National University of Singapore
Singapore 117576
** biehzw@nus.edu.sg*

This article gives an overview of the development and applications of the surface-enhanced Raman scattering (SERS) techniques in biomedicine. We first introduce the fundamental principles of the SERS mechanisms. We also present the different fabrication techniques of SERS nanostructures and substrates. Finally, the importance and potential roles of the SERS nanostructures and substrates in biomedical applications are summarized.

Keywords: Surface-enhanced Raman scattering; surface plasmon; nanotechnology.

1. Introduction

Raman spectroscopy is a vibrational spectroscopic technique that measures inelastic light-scattering processes. It is able to provide specific spectroscopic fingerprints of molecular structures and compositions of materials.[1] The inherent small Raman scattering cross-section or intensity of Raman signal from molecules can be significantly improved by surface-enhanced Raman scattering (SERS) effect. The SERS phenomenon originating from the electromagnetic and chemical enhancements between metal structures and molecules in close proximity[2,3] was first observed by Fleischmann *et al.* in 1974.[4] The average augmentation of the SERS effect in Raman intensity over an excitation area can achieve up to 8 orders of magnitude,[5] while an increase of 14–15 orders of magnitude has also been demonstrated in a localized area for a single molecule.[6,7]

The discovery of surface-enhanced phenomenon has triggered many highly sensitive detection technologies development in analytical chemistry,[8] biomedical engineering, and life sciences,[9] such as surface-enhanced second-harmonic generation, surface-enhanced fluorescence, surface-enhanced infrared spectroscopy, surface-enhanced sum frequency generation, and single-molecule detection.[10–13] Numerous

*Corresponding author.

research articles, reviews, and books on this subject have been published.[2–16] In this paper, we discuss the electromagnetic and chemical enhancement mechanisms responsible for the enhancement of Raman signal in SERS. We also review the advanced metallic structures and nanoparticles in SERS. Furthermore, fabrication techniques of different SERS substrates and metal nanoparticles are also presented. Finally, exploitation of the SERS substrates and nanoparticles in biomedical applications will be given, illustrated by several important examples in these arenas.

2. Fundamental Principles of SERS

The amplification of Raman scattering in SERS effect is generally generated by two mechanisms: (i) electromagnetic-field enhancement through the localization of optical fields in metallic nanostructures, and (ii) chemical or electronic enhancement due to the increase of Raman cross-section when the molecule or lattice is in contact with metal nanostructures.

2.1. *Electromagnetic enhancement*

Since Raman intensity of a molecule or a crystal is proportional to the incident electromagnetic field, the enhancement factor (EF) of each molecule or crystal is described by Eq. (1),[17]

$$EF \approx t|E(\omega)|^2|E(\omega')|^2 \tag{1}$$

where $E(\omega)$ represents the electric-field enhancement factor at excitation source frequency of ω, and $E(\omega')$ gives the EF of the Raman scattering fields at Stokes-shifted frequency of ω'. The magnitude of enhancement is approximately $|E(\omega')|^4$, when $\omega = \omega'$. Enhancement is possible even for the molecules that are not in direct contact with the metal surface. The enhancement deteriorates by a fraction of $[r/(r+d)]^{12}$, for a single molecule, is at a distance d from the surface of a metal sphere of radius r.[18]

Figure 1 shows the schematic of plasmon oscillation for small metallic spheres. The coherent oscillation results in conduction electrons of a small spherical metallic nanoparticle due to light irradiation. The oscillation of the electron cloud is caused by the Coulomb attraction between the electrons and nuclei.[19] The estimated enhancement in local electric field can be calculated from the sum of an incident field and a contribution from all other dipoles in the particles.[19]

Figure 2 gives the calculated contours of the local electromagnetic field for nanoparticles of different shapes and arrangements.[20] The peak value of $|E(\omega)|^2$ for dimmers of: (a) nanospheres is 1.1×10^4, and (b) prisms is 5.7×10^4, which are 2–3 orders of magnitude larger than the peak value of $|E(\omega)|^2$ for isolated single nanoparticles.[20] The value of $|E(\omega)|^2$ can be further modified by having more nanoparticles.[20]

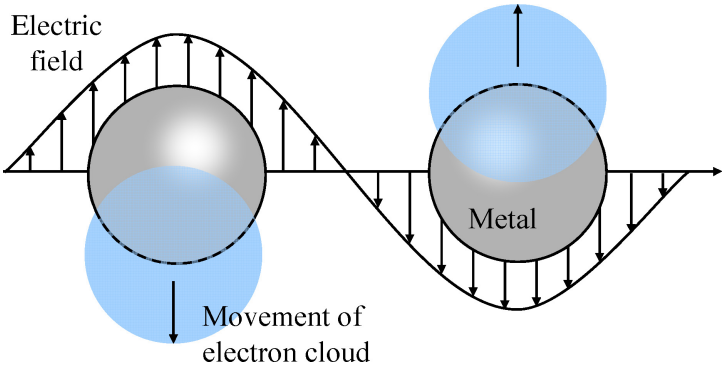

Fig. 1. Schematic of plasmon oscillation for small metallic spheres.[19]

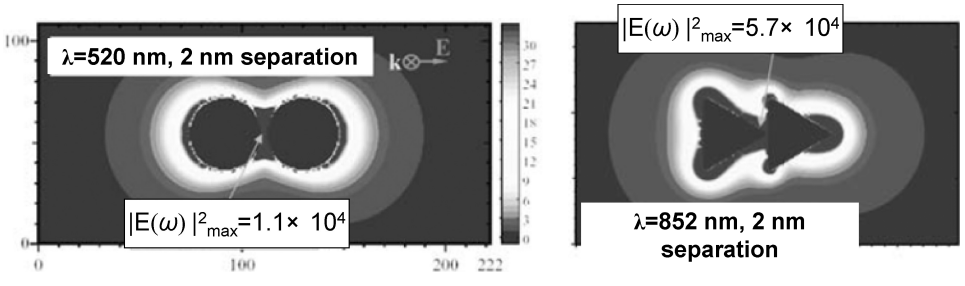

Fig. 2. Electromagnetic-field enhancement contours for dimmers of: (a) nanospheres (radius of 36 nm), and (b) triangular prisms (edge dimension of 60 nm with 2 nm snip and thickness of 12 nm) at specified wavelengths λ with peak value of $|E(\omega)|^2$.[20]

When these plasmonic-electromagnetic resonance waves are coupled to electromagnetic field of light at the surface of metallic structures mentioned above or other nanostructures as described in Sec. 3, an augmentation of the scattered Raman field will occur. The amplification can be realized via interactions with the metal surface, known as surface-enhanced Raman effect.[21]

2.2. *Chemical enhancement*

Chemical enhancement is another mechanism that is responsible for the SERS effect, in which the Raman polarizability of a molecule adsorbed on a metal surface is enhanced.[22] For instance, despite CO and N_2 having similar Raman cross-sections, the Raman intensity for the two different molecules can differ by a factor of 200.[23] Figure 3 shows the charge transfer (CT) model to explain the above observation, illustrating the electron transfer for metal with atomic scale roughness (ASR) adsorbed with analytes. When a photon impinges onto a metal, electron in the metal is excited and moves into electron affinity levels of the molecule adsorbed

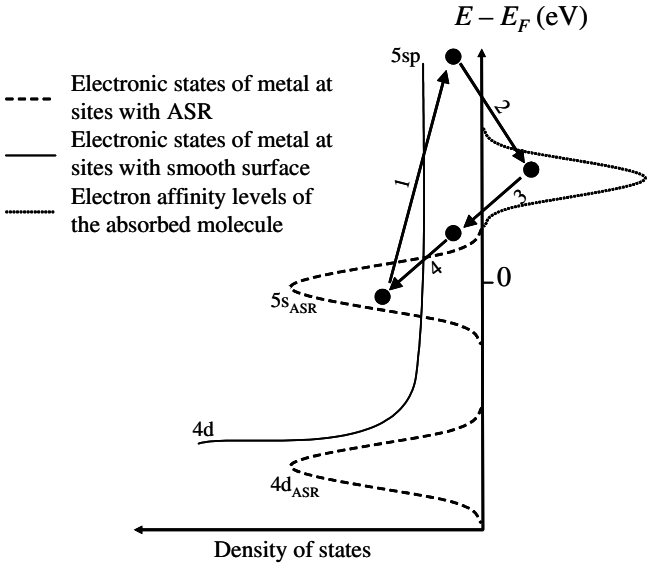

Fig. 3. Charge-transfer model of the electron transfer for the metals with atomic scale roughness (ASR) adsorbed with analytes.[24]

on the metal. Hence, relaxation of the molecule follows a different new equilibrium, unlike the normal route of the same molecule without any metallic contact.[24] The molecule may stay in the new excited vibrational state, even after the excited electron moves back to the metal and combines with a metal hole, rendering a Raman-scattered photon.[24] Therefore, the difference in the SERS effects between CO and N_2 can be explained by the dissimilar adsorption levels of CO and N_2 on the silver substrate.[23] Since CO can be relatively better adsorbed onto the silver substrate than N_2, charge transfer can take place between the metal and absorbate and thus, chemical enhancement is realized and lead to SERS enhancement.[24]

In addition to the CT models, selection rules,[23] microensemble,[25] inelastic Mie scattering,[26] modulation of metal-surface reflectivity,[27] coherent parametric excitation,[28] cooperative effects,[29] surface-polarizability contribution,[30] and forbidden bands[31] are other examples of models in chemical enhancement that have been employed to account for Raman signal amplification.

3. Advanced SERS Structures

Different types of nanoparticles give rise to different Raman enhancement characteristics,[32] as the amplification of Raman signals depends on the interactions of plasmonic waves with electromagnetic waves on the surface of metal nanostructures. Hence, optimizing and tuning of the SERS characteristics require an appropriate control of SERS structures in different aspects, such as size, shape, spacing between nanoparticles, interaction between nanoparticles and substrate,

solvent, dielectric overlayers, and molecular absorbates, employed in the fabrication procedures.[32] Theoretical work hypothesizes that SERS intensity should increase with increasing nanoparticles aspect ratios.[23,33] Moreover, the resonant position of surface plasmon modes and enhanced electric fields are different in nanoparticle clusters of dissimilar shapes.[34] As a result, a variety of nano-scale structures with a high ratio of surface area to volume cross-section, including nanoparticles,[35] nanocavities,[36] nanorods/nanowires,[32,37] and nanoshells,[38,39] have been proposed for SERS applications.

3.1. *Nanoparticles*

Surface electric field is enhanced on the surface of metal nanoparticles, and extinction spectra of nanoparticles can be modified by changing the size and dielectric environment.[35] In addition, nanoparticles with different numbers, inter-particle distances and arrangements can affect the strength of inter-particle electromagnetic coupling and thus, the degree of SERS enhancement.[40]

3.2. *Nanocavities*

In contrast to utilizing nanoparticles in SERS, plasmonic structures containing nanocavities have also been demonstrated for surface plasmons enhancement.[41] Cavities[42] have been found to cause an effective excitation of surface plasmons, and the periodic corrugations surrounding the void can facilitate the coupling of propagating light to surface plasmons.[43] With more than one aperture being arranged in an array, the period, the shape, and dimensions of nanocavities can be tuned to control the optical characteristics[44,45] well.

3.3. *Nanorods/Nanowires*

In nanorods, two surface plasmon bands exist due to the electron oscillations in the long and short axes of nanoparticle. By changing the dimensions of nanorod, the plasmon resonance frequency can be modified and a red shift in the resonance band can be observed due to the increased aspect ratio of nanorod.[46] For aligned nanowires or nanorods, maximum SERS intensity is observed for the polarization direction perpendicular to the long axis of the nanostructures.[46]

3.4. *Nanoshells*

Nanoshell is another type of configuration that can tune the resonance wavelength by modifying the geometries of the dielectric core and the shell,[38] thereby providing a wide spectrum of plasmon-resonance wavelengths ranging from 2.3 eV to 0.45 eV and below[39] for a gold-coated silica-core nanoshell. Since the extinction spectra can be tuned effectively, nanoshells can be used as biomarkers and SER nanotags for *in vivo* tumor targeting and detection in the near-infrared (NIR) range.[47]

Furthermore, a lot of other nanoparticle geometries, such as nanospheres, nanoprisms, bipyramids, nanocrescents, nanotriangles, nanorices, and polyhedrals[38,48–51] have been demonstrated to efficiently improve the SERS effect. Therefore, fabrication techniques of effective SERS probes are highly desirable for optimizing and controlling the interspacings and configurations of nanostructures to significantly enhance the SERS performance.

4. Fabrication Techniques of SERS Nanoparticles and Substrates

Different sizes, shapes, and other configurations of the nanostructures as described in Sec. 3 affect the peak plasmon-resonance wavelength and consequently, the SERS performance. Hence, developing fabrication method yielding reproducible, reliable, and stable SERS nanostructures/substrates is critical for predictable and repeatable SERS performance. Generally, the commonly practised techniques for fabricating SERS nanoparticles and substrates can be categorized as follows: (i) oxidation-reduction cycling on a metal electrode,[4] (ii) vapor deposition,[52] (iii) reduction of Au or Ag with various chemicals,[53] and (iv) sol-gel process.[9]

4.1. *Oxidation-reduction cycling on a metal electrode*

Surface-enhanced Raman scattering was first observed on a roughened Ag electrode prepared from oxidation-reduction cycles (ORC), which pyridine solution on the roughened electrode experienced an intense Raman scattering.[4,54] In this method, the surface morphologies of the electrode can be controlled by the different potential sweep and potential step applied.[55] However, the surface roughness is also sensitive to the variation in temperature, concentration, and electrochemical property of the electrolyte.[56] In addition, the ORC method is difficult to have consistent surface roughness as compared to the substrates fabricated by vapor-deposition method.[56]

4.2. *Vapor deposition*

Vapor deposition is another method to produce SERS nanostructures/substrates.[57] With different deposition rates as well as substrate temperature and deposition geometries, the morphology and the optical properties of the deposited films can be varied.[57,58] In addition, vapor-deposition method has demonstrated to fabricate nanorods, nanobelts, and nanosheets that exhibit an enhanced resonance.[58]

4.3. *Reduction of Au or Ag nanoparticles with various chemicals*

In chemical synthesis, apparatus required can be more compact and economical than vapor deposition, but the method is more chemically intensive. For the chemical reduction method, the typically employed reducing agents are citrate,[59] borohydride,[60] hydroxylamine,[61] and oxalate.[62] The advantages of the chemical technique are many folds: (i) facile preparation and characterization processes,

(ii) control of particle shape and size,[63] and (iii) acceptable reproducibility and stability.[64] However, extra ions may be generated from the reaction process.[64]

4.4. *Sol-gel process*

Sol-gel process is another technique that can fabricate SERS nanostructures/substrates.[9] Different precursors are mixed and reacted to form the sol that can be coated onto a substrate.[65] After drying or after a thermal treatment, the gel is formed and the metal nanoparticles can be realized by immersing the sol-gel film into a reducing agent.[65] Advantages of employing the sol-gel method for preparing for the SERS substrates are: (i) clustering is prevented by matrix isolation, (ii) porosity of the coating facilitates the analyte to be in close contact with the metal particles, and (iii) bifunctional ligands can be integrated into the sol-gel matrices for an effective analyte adsorption on substrates.[65]

Furthermore, a number of other advanced fabrication techniques, such as micelles,[66] self-similar,[67] electron-beam lithography,[68] soft lithography,[69] nanosphere lithography,[70] metal film over nanosphere[56,71] and focused-ion beam,[41] have been developed for SERS substrates fabrication. Each of these techniques has its own unique advantages and disadvantages in giving rise to different resonance wavelengths, such that these different SERS substrates prepared can be utilized in a wide spectrum of biomedical applications.

5. SERS in Biomedical Applications

One of the ultimate goals in fabricating different advanced SERS nanostructures is to formulate nanostructures and devices that serve as signal enhancers to sensitively probe molecular structure and conformation of the samples with low concentrations (even down to single-molecule levels).[6,7] Based on the characteristics of SERS performance, different nanostructures have been applied for biomedical applications, including: (i) tip-enhanced Raman spectroscopy (TERS),[72] (ii) Fourier Transform (FT)–SERS microspectroscopy,[73] (iii) SERS,[74] (iv) SERS-scanning near-field optical microscopy,[75] (v) bioanalysis,[8,76] (vi) quantification,[77] (vii) single-molecule detection,[6,7] (viii) SERS mapping and imaging,[78] (ix) labeling in position tracking of molecules,[79] (x) SERS gene probe in diagnosis,[80] and (xi) DNA mapping and sequencing.[81]

5.1. *Tip-enhanced Raman spectroscopy (TERS)*

In the case of a nanotip, the nanostructure can be employed in TERS.[82] During measurements, the tip is illuminated and localized surface plasmons are excited at the apex and the enhanced field is confined at close vicinity of the tip for an effective SERS enhancement, achieving a 10^7-fold amplification in Raman signal with < 5 nm spatial resolution.[72] Tip-enhanced Raman spectroscopy (TERS) can be carried out on a metallized atomic force microscopy (AFM) tip[83] (Fig. 4), scanning near-field

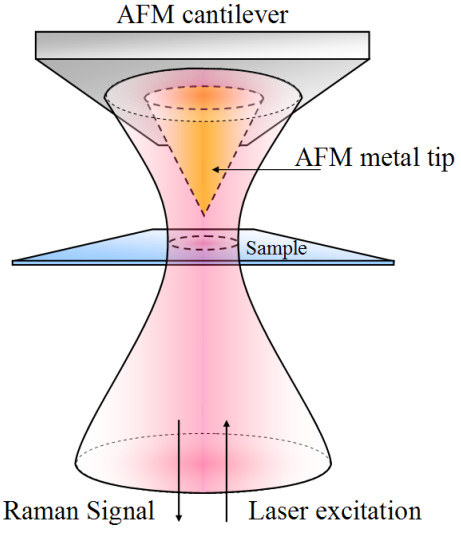

Fig. 4. Schematic diagram of a tip-enhanced Raman scattering setup of an AFM metal tip working in back-reflection mode.

optical microscopy with a metal-coated tip,[84] or a scanning-tunneling microscopy with an etched wire.[85]

5.2. *FT–SERS microspectroscopy*

Colloids and island films have been used as SERS-active substrates in Fourier transform-SERS (FT–SERS) microspectroscopy.[73] Beljebbar *et al.*[73] have developed FT–SERS microprobe with resolution on the micrometer scale and acquired the FT–SERS spectra of crocetin, mitoxantrone, and mitoxantrone/DNA complex, demonstrating a mass detection limit of $\sim 5 \times 10^2$ molecules. The technique is sensitive to the interaction within supramolecular complexes and provides an additional molecular structural information about the samples.

5.3. *Surface-enhanced resonance Raman spectroscopy (SERRS)*

Surface-enhanced resonance Raman spectroscopy (SERRS) is a technique that combines resonance Raman and SERS for highly sensitive detections of bioanalytes[86,87] with a limit of detection of $\sim 10^{-16}$ M and an enhancement factor of $\sim 5 \times 10^{11}$. Maeda *et al.*,[74] have prepared self-assembled negatively charged monolayers (SAMs) of mercaptoalkanoic acids adsorbed on Ag colloid to characterize cytochrome *c* by SERRS.[74] The SERRS technique associated with the use of SAM-modified Ag has shown potential to investigate the structure of proteins (cytochrome *c*) based on biomimetic membrane (SAM) without the disruption of cell activity.[74]

5.4. *SERS-scanning near-field optical microscopy (SERS–SNOM)*

Surface-enhanced Raman scattering–Scanning near-field optical microscopy (SERS–SNOM) is another type of hybrid system that exploits the SERS effect. Zeisel *et al.*[75] have developed a SERS–SNOM system to realize an enhancement factor of 10^{13} by modifying an existing SNOM.[75] In this system, a laser beam excites rhodamine 6G (R6G) probe molecules adsorbed on silver-island films deposited on glass, via a metallized fiber tip scanning on the sample surface.[75] The scattered light is then collected by a microscope objective and projected onto a silica fiber by a dichroic mirror, in order to be detected by a charge-coupled device (CCD) camera. Exposure time of 60 seconds and a signal to noise ratio of 25 are achieved.[88]

5.5. *Bioanalysis*

Exploiting on the advantage that SERS is highly sensitive,[7] components of drugs can be selectively detected and analyzed by SERS. Levi *et al.*,[76] have utilized silver colloids to study the SERS spectra of anti-tumor drugs as well as their complexes with DNA. Additionally, SERS technique facilitates the investigation of interactions between different molecules.[89] For example, Miskovsky *et al.*[89] have investigated the interaction of hypericin with human serum albumin, based on SERS for cancer therapy.[89] The binding place for hypericin has been identified and an albumin–hypericin complex model has also been illustrated, which is crucial for a better understanding of the pharmacokinetics and therapeutic (antibacterial, antiviral, and antitumoral) properties.[90]

5.6. *Quantification*

Surface-enhanced Raman Scattering is capable of detecting chemicals in trace amount and is able to accurately quantify the amount present.[91] For instance, Shafer-Peltier *et al.*[77] employed silver film over nanosphere surface absorbed with an alkanethiol monolayer as SERS substrate for glucose quantification. The concentration of glucose can be predicted quantitatively over two different ranges: (i) concentration of 0–250 mM with a root-mean-squared error of prediction (RMSEP) of 3.3 mM, and (ii) concentration of 0–25 mM with RMSEP of 1.8 mM. The above SERS detection sensitivity is comparable to that (1 mM) required in medical applications.[77]

5.7. *Single-molecule detection*

Surface-enhanced Raman scattering (SERS) can detect chemicals in small amount and even down to the single-molecule level.[6,7,92] By coupling single molecules of analyte to metal nanoparticles,[7] the SERS signal can be amplified tremendously and the size-dependent properties of the nanostructures can be studied. In order to achieve detection at low level, the commonly used detection system is integrated

with sensitive optical imaging and spectroscopy for molecular information.[6] An AFM can also be used to investigate the dimension of particles or molecules.[7] The Raman enhancement factors of single analyte molecules adsorbed on selected particles can be in the order of 10^{14} to 10^{15} M,[7] with a detection limit of 10^{-14} M for the probe molecules.

5.8. *SERS mapping and imaging*

Apart from investigating the SERS spectral characteristics of a localized point in single-molecule detection, SERS spectra over an area of a sample can also be achieved, where locations at which the spectra are collected form a grid pattern, known as the SERS mapping.[93] Since the inherently weak Raman signal from biological samples limited the spectral-acquisition speed, enhancing the Raman signal can shorten the mapping time for a single cell by achieving 1 second per mapping point with 1 μm lateral resolution in scanning range of 400–1800 cm^{-1}.[93] Alternatively, the biosample surfaces of interest can be globally illuminated and imaged on to a CCD detector in SERS imaging technique,[78] to study the different spatial distributions and components of chemicals in tissue.

5.9. *Labeling in position tracking of molecules*

From SERS mapping or imaging of cells or biosamples, examination of intra-cellular and inter-cellular processes have been made possible via the tracking of the position of molecules inside the cells, tissues or bio-samples.[94] In order to track the molecules effectively, SERS-label strategy has been employed as suggested by Vo-Dinh *et al.*[79] Their group has prepared SERS active labels (e.g., cresyl fast-violet dye) to facilitate gene probing.[79] The probes do not suffer radioactive decay and gene under investigation can be detected by more than one probe with different labels, because SERS labels do not have broad spectral bandwidth, and the labels are not susceptible to the photobleaching or quenching effect.

5.10. *SERS gene probe in diagnosis*

Surface-enhanced Raman scattering (SERS) spectroscopy has also shown potential for biomedical diagnosis, for example, in cancer detection based on probing the minute variations and the changes of biochemical structures and constituents associated with pathologic changes.[80] Moreover, the discriminations and characterizations of different cancer stages are not just limited to the cellular level; the analysis and diagnosis can also be carried out even at the single-molecule level or gene level.[79,81,95] The SERS-active substrate as a gene probe was fabricated by depositing a thin film of Ag on a glass via physical vapor-deposition technique.[96] This type of SERS gene probe has been used to identify the breast cancer-susceptible gene with high sensitivity.

5.11. *DNA mapping and sequencing*

Surface-enhanced Raman scattering (SERS) effect is not only limited to the fabrication of gene probe. Surface-enhanced Raman scattering has also shown potential to be invaluable in the research field of genomics, such as determining the sequence of DNA.[81,97] Single-DNA base molecule can be detected by the SERS technique without any labelings,[81] and DNA sequences can be identified with the help of SERS gene probe.[79] In addition, SERS is useful for DNA mapping through the simultaneous detection of multiple bacterial artificial chromosomes clone-labeled probes that can eventually be developed into maps of human chromosomes.[98]

Apart from the above-mentioned biomedical applications, SERS has also shown potential in the following fields: studies of living cells,[90] characterizations of drugs,[99] SERS immunoassays,[100] proteomics,[101] analysis of hemoglobin,[102] enzymes,[103] nucleic acid[104] and saliva,[71] geometry prediction of molecules,[105] discrimination of bacteria[106] and other biomedical applications. Surface-enhanced Raman scattering is important in the field of biomedical applications mainly due to the fact that SERS technique is highly sensitive for bioanalysis where minute chemical variations can be discriminated.

6. Conclusions

Surface-enhanced Raman scattering (SERS) technique has found a wide range of applications in biochemical and biomolecular sensing. For instance, SERS can be used to monitor surface-adsorption dynamics at electrodes, charge-transfer kinetics upon adsorption of organic molecules on colloidal metal particles. Surface-enhanced Raman scattering has also shown great promise for basic research in genomics and proteomics. For example, the SERS-based DNA label systems enhance the utility of gene-probe technology for gene diagnostics. The multiple SERS probes can also be employed for the detection of a single gene, DNA, proteins, and lipids that are unlikely to be realized if the conventional fluorescent or chemiluminescent labelings are used. Very recently, SERS technique incorporated with near-field optical techniques (e.g., AFM, SNOM), and non-linear optical microscopy (e.g., coherent anti-Stokes Raman scattering (CARS))[107–111] is on the rise to study near-field SERS activities with enhanced factors of up to 10^{12}–10^{15} and a limit of detection down to 10^{-10}–10^{-14} M, particularly useful for highly sensitive single-molecule detection. Therefore, with further advancements of the fabrication of highly sensitive SERS-active probes, SERS technique may open up a new direction for high-sensitive, high-throughput signal detection in biomedical engineering, such as chemical sensors and biological sensors, nanomedicine, drug delivery, and bioimaging.

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