Biosensing Based on Surface-Enhanced Raman Spectroscopy by Using Metal Nanoparticles

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Abstract

Surface-enhanced Raman spectroscopy (SERS) is a promising tool in the analytical science because it provides good selectivity and sensitivity without the labeling process required by fluorescence detection. This technique consists of locating the target analyte on nanometer range of roughed Au-nanoparticles. The presence of the metal nanoparticles provides a tremendous enhancement to the resulting Raman signal through an electromagnetic enhancement of both the laser excitation light and stokes-shifted light by 5–6 orders of magnitudes. SERS makes it possible to create a spectroscopic device that can act as a highly sensitive molecular detector using Raman signal as a fingerprint of the analyte. In this review, we present a general overview of the recent advancements in SERS as an analytical tool for identification of molecular species with concentrations below biological level in aqueous solution, with a particular attention to its potential applications in biomedicine.

Keywords

Analytes; Nanoparticles; Biosensor; Raman spectroscopy; Surface plasmon resonance

Introduction

Detecting certain molecules in a solution with high sensitivity and molecular specificity is of great scientific and practical interest in many fields such as chemistry, biology, medicine, pharmacology, and environmental science [1]. Raman spectroscopy (RS) has been employed in sensor technology for many years, as it provides many advantages over other spectroscopic techniques such as Fourier transform infrared (IR) spectroscopy, near-infrared (NIR) absorption, UV-vis absorption, fluorescence, nuclear magnetic resonance (NMR), x-ray diffraction, x-ray photoelectron spectroscopy or mass spectroscopy [2,3].

Raman studies of living cells were not complex due to the small probe volumes and low concentration of inherent cellular molecules [4]. The most important disadvantage of normal RS for diagnostic applications, *i.e.*, the intrinsically weak cross section, is solved in another technique. RS is a non-destructive, non-invasive technique, because the threshold intensity of Raman lasing is ultralow. Owning to the local electromagnetic field enhancements near the noble metal nanostructures, Raman signal could be considerably improved and novel optical labels based on surface-enhanced Raman spectroscopy (SERS) instead of using fluorescence labels were proposed [5-8]. SERS has been used as a signal transduction mechanism in biological and chemical sensing of trace analytes such as pesticides, anthrax spores [9], prostate-specific antigen [10],

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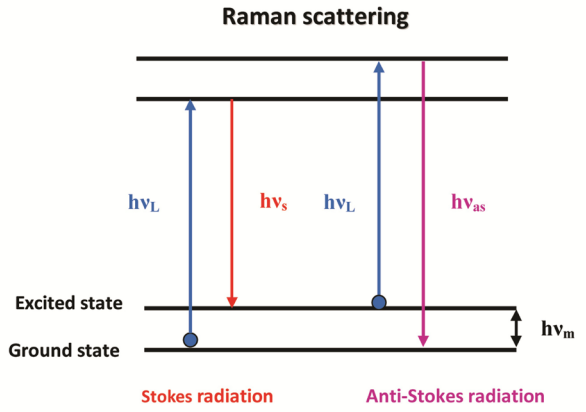

Figure 1: Schematic diagram showing Raman scattering.

glucose [11-12], nuclear waste [13], bacteria [14] genetic materials [15], and immune complex [15-18]. A miniature inexpensive SERS device can be used in clinics and field [19].

SERS can be used when the target analyte is brought to the surface of a noble metal nanostructure. When the molecules attach to the metallic nanostructure, the local electromagnetic field around the nanostructures improves RS by a factor of 10^6–10^8 for an ensemble of molecules [20,21], and by a magnitude of 10^{12}–10^{14} for a single molecule [22-24]. Under these conditions, the Raman cross section and, in turn, the signal intensity are extremely enhanced so that the level of detection down to a single molecule can be reached, while preserving all the structural information provided by RS [25]. Therefore, advancement in SERS detection is linked to the progress in the synthesis and optimization of the optical characterization of nanostructures. SERS

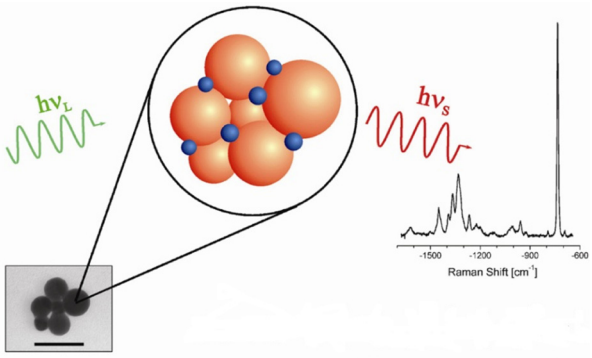

Figure 2: Schematic diagram of SERS [4].

can be achieved and maximized by controlling both the electrical and chemical effects, mainly through careful design of the optical substrates and improving the absorption of the analytes of interest. Therefore, preparing optical substrate with optimized properties is of paramount importance. In this review, we present the most recent advancements in the optimization of metal nanoparticles for SERS that are gaining popularity in bio-related fields such as chemical and molecular biology, diagnosis of diseases, biodetection or bioimaging. We also describe Raman scattering and SERS, and indicate that morphology of nanoparticles would affect SERS enhancement.

Surfaced-Enhanced Raman Spectroscopy (SERS)

RS is a popular technique; it identifies and indicates the unique binding energies of molecules and is highly selective. Raman scattering is an inelastic scattering between a photon and a molecule. During this process, the incoming photon with energy hv_L will have a shift in its energy by the characteristic energy of vibration of hv_M. These shifts depend on whether the molecule is in its vibrational ground state or in its excited state. In the first case, the photon loses energy by excitation of a vibrational mode (Stokes scattering); in the second case, energy gains by de-excitation of such a mode (anti-Stokes scattering) are possible [26]. Stokes and anti-Stokes radiation via Raman scattering are depicted in Figure 1. The typical Raman cross section is on the order of 10^{-30} cm^2 per molecule, which is about 14 orders of magnitude lower than the typical fluorescence cross section. Thus, conventionally RS has not been a useful technique for sensing molecules in extremely low concentrations. SERS has been considered a technique that provides the selectivity of RS while generating an extremely large enhancement to the Raman signal; it enables highly sensitive measurements [27-32]. SERS enhancements on the order of 10^{14} have been demonstrated for

molecules in the vicinity of metal nanostructures [33-35]. SERS utilizes a mutual electromagnetic enhancement due to surface plasmon resonance (SPR) and a chemical enhancement, both of which provided by gold or silver nanostructure. When biomolecules adsorb on to a metal nanostructure or move to a few nanometers of its surface, the electromagnetic field near the nanoparticle enhances locally, so the scattered Raman signal can be strong. Figure 2 illustrates a schematic diagram of SERS, where biomolecules (blue spots) are put near spherical gold nanoparticles (orange sphere). SERS spectrum shows a strong Raman signal near the gold nanoparticles. The peak enhancements are the result of target molecules located at the so-called "hot spots" which generally exist in nano-sized features of clustered nanoparticles.

The effect of nanoparticle morphology on SERS enhancement

The most important SERS substrates are made of noble metals such as silver and gold. although, silver is much more efficient than gold, collection of both metals yield the same electromagnetic enhancement factor (up to 10^{14}) at near-infrared (IR) frequency [36,4]. This feature makes the gold nanoparticles (1–100 nm) a very good candidate nanosensors for studying the internal organelles of the prokaryotic cells [23, 37]. Other key factors affecting SERS intensity are the size and shape of the nanostructures. Several authors have studied the dependence of the SERS signal on the size of silver [38] and gold [39] nanoparticles. Smaller nanoparticles are advantageous in protein labeling and cellular imaging, because their small surface area reduces non-specific interactions and enables more targeted binding. In addition, smaller particles generate more confined electromagnetic fields so that they are more sensitive to single biomolecules, which take up a greater portion of the sensing area. However, the nanoparticle absorbance and scattering depend on nanoparticle size, scaling with nanoparticle volume for absorbance and with volume squared for scattering. Absorbance and scattering cross sections become comparable when the nanospheres are about 60 nm in diameter for silver nanospheres and 80 nm in diameter for gold nanospheres [40, 41]. The third main factor affecting SERS intensity, which is regarded as the most important one, is the nanoparticle shape [42]. Controlling the morphology provides a method to tune the optical and spectroscopic response of nanomaterials [43], which is an essential requirement for a wide range of applications such as genetic diagnostics, immunoassay labeling, and detection of trace amounts of drugs, biomolecules and pesticides. The most popular nanoparticle shapes are spheroids, triangular prisms, rods, and cubes (Fig. 3).

Due to the inhomogeneity of the distribution of SPRs throughout the whole particle surface, electromagnetic field concentrates in certain regions of the particle. Such an electromagnetic field concentration has been observed at the corner of triangular particles [44], the end of nanorods [45], and the edges and corners of nanobars and nanocubes [46]. Complementary

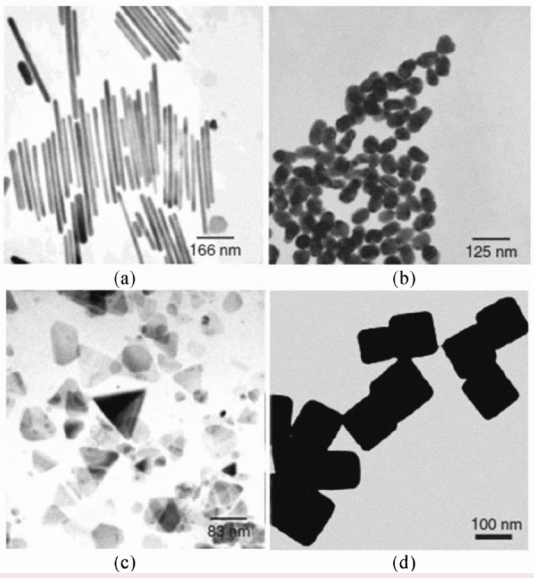

Figure 3: Nanoparticle geometries: a) Gold nanorods, b) Gold colloids, (c) Silver triangular prisms, and d) Silver nanocubes [47].

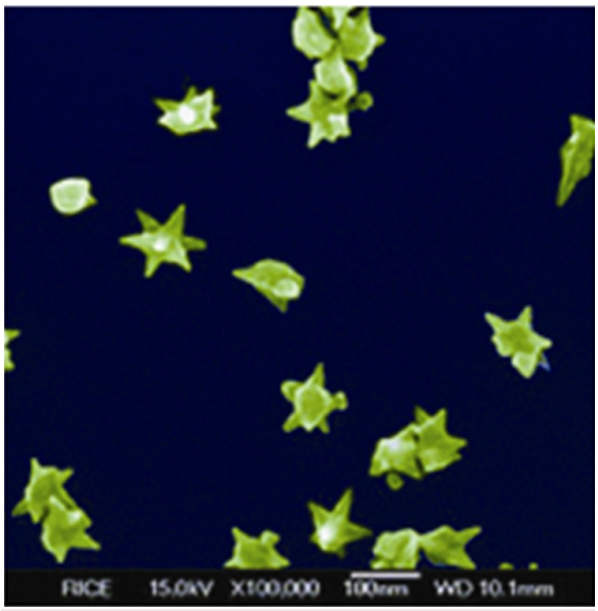

Figure 4: Gold nanostars

to these latest approaches for electromagnetic field concentration, remarkable progress has been directed to the controlled fabrication of the so-called "hot spots." Hot spots are defined as specific gaps between particles where the electromagnetic field intensity is extremely high due to coupling between their plasmon resonances [48]. Carefully designed hot spots can become much more active and even enable the possibility of single molecule spectroscopy [49]. It has been recently indicated that the electromagnetic field can be strongly localized at sharp apices of nanoparticles such as gold nanostars. Theoretical and experimental results [50] demonstrate such a field concentration (Fig. 4) that results in considerably higher SERS enhancement for stars than other geometries [51].

Principle of SERS nanosensors

The design of efficient and flexible nanosensors based on SERS, is one of the main challenges to be achieved before the technique can be widely applied. Due to the unique optical properties of noble metals, gold nanoparticles have been used in biodetection and bioimaging as SERS nanosensors [52].

The most important challenges encountered in the design of any nanosensor are the composition of the nanostructure and the selectivity of the supplying electromagnetic field [23, 52]. The small metal nanostructures can cross cell membranes and allow probing of cells with no deleterious effects [4,53]. By measuring the enhanced Raman signal near the gold nanoparticles, information about the morphology of molecular structure can be obtained.

Probing with SERS nanosensors

RS can provide intermolecular information about biological molecules by chemical and physical probing. In the vicinity of gold nanoparticles, Raman signal will be very strong, thus, obtaining the chemical information of cellular molecules is possible; therefore, it acts as an effective nanosesor [25, 51]. Gold or silver nanostructures attached to the reporter molecules can be considered as an intracellular pH meter, which illustrates a calibrated pH-dependence SERS spectrum [4,54-56]. A wide range of physiological and metabolic processes can be interpreted by SERS spectrum that reflects intracellular pH value. pH measurement by SERS spectrum of silver and gold electrodes attached to 4-mercaptobenzoic acid (pMBA) as a reporter has been reported [4,57]. SERS nanosensors studies on silver nanoparticle clusters functionalized with pMBA showed that the spectrum is sensitive to pH changes in the range of 6–8 [4].

In another SERS experiment, a single live NIH/3T3 cell was placed near a pH nanosensor for 4.5 hrs until the RS was performed. If the nanosensor could enter the internal organnels, it shows that pH is more acidic in the endosomes. The maximum value of pH (6.8) measured by sensors was recorded in early endosomes, while the minimun pH of 5.4 was measured after 4.5 hrs when delayed endosomes or lysosomes were developed [4]. Using pairs of thin spectrally line of Raman signal in the spectrum, SERS nanosensors can collect useful information. In general, estimating the local pH value by SERS nanosensors

led to probing sensitive target of molecular composition in a cellular partition.

In conclusion, in this review we demonstrated that SERS has been set up as a reliable diagnostic technique for the detection of an extremely low concentration of a wide variety of biomolecules. By utilizing the improved cross sections of SERS, the first study of a single molecule is achieved. This research has established novel optical nanosensors based on SERS for probing and detecting live cells. Theses nanosensors are composed of gold nanoparticles and reporter molecules, which can be detected based on the Raman spectrum of the reporter. By measuring SERS spectrum of the reporter, intracellular information can be attained. Therefore, the electromagnetic field of SERS, in particular for designing sensitive, label free, selective and real time nanosensors has great potential.

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