Utilizing Silver and Gold Nanoparticles for Investigation of Bacterial Cell Wall Biochemical Structure

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ABSTRACT

Due to its "fingerprinting" property, surface-enhanced Raman scattering (SERS) can give significant amount of molecular information about molecular structures in the close vicinity of gold or silver surfaces in a short time. The requirement that the noble metal nanoparticles must be close to the molecular structure for the signal enhancement in SERS can serve as a sensing tool. This study investigates the use of the gold and silver nanoparticles in a controlled manner to obtain molecular level information from microorganisms. The results demonstrate that pH of the sample, the type, surface charge, concentration and arrangement of nanoparticles have a great impact on SERS spectra of bacteria. The finding of these experiments can be used to monitor the changes on the bacterial cell wall and development of novel diagnostic tools for fast microbial identification and discrimination.

Keywords: *SERS, nanoparticles, bacteria, spectroscopy*

1 INTRODUCTION

Raman spectroscopy is utilized to investigate live cells and microorganisms due to its "fingerprinting" properties, immunity to water, and easy sample preparation [1-14]. While bulk Raman spectroscopy has the disadvantage of weak scattering which results long collection times and the use of high laser powers, surface-enhanced Raman scattering (SERS) mostly overcomes these problems. In SERS, the molecule of interest is brought to the close vicinity of a noble metal surface such as gold or silver [15-17]. Although a surface or a colloidal solution can be suitable for molecules, silver or gold colloidal solutions are preferred for bacterial SERS. This is mostly due to the simplicity of the sample preparation and suitability for the experimental configuration. When the silver or gold nanoparticles are mixed together, nanoparticles interact with bacterial cell wall and a fraction of the particles, depending on the colloidal solution concentration, get close enough to biochemical structures on the bacterial cell wall. Because of the distance dependence of SERS, the factors influencing the nature of the interactions can be manipulated to gather more information about the cell wall bio-structure and dynamics. The collected information can be used for identification, discrimination, classification, development of novel diagnostic tools, and fundamental studies for the bacterial cell wall process.

In this study, we investigated the use of the gold and silver nanoparticles in a controlled manner to obtain molecular level information from bacterial cell wall.

2 MATERIAL AND METHODS

2.1 Chemicals

$AgNO_3$ (99.5%), and nutrient agar are purchased from Fluka (Seelze, Germany). Sodium citrate (99%), and is purchased from Merck (Darmstadt, Germany). Sodium borohydride and $HAuCl_4.3H_2O$ are purchased form Alfa Aesar (Karlsruhe, Germany). HCl (37.00%) is purchased form Sigma-Aldrich (Hamburg (Proligo), Germany). All chemical are used as received without further purification.

2.2 Preparation of Bacteria Samples

All bacteria used in this study were obtained from our microorganism collection (Yeditepe University, Genetics and Bioengineering Department) and it was verified by Microbial Identification System (MIDI) before their use and were grown axenically and aerobically for 16-20 h at 37ºC on 20 mL nutrient agar. The bacteria were collected with sterile plastic inoculating loops solid culture plate. The collected samples were added into 1 mL d.i. water, vortexed and centrifuged for 5 minutes at 7500 rpm. The supernantent was discarded. This procedure was repeated three times. The 5 μL of the each washed bacterium was added into a 100 μL silver colloid solution. Then, it was mixed with a vortex to create a homegenous mixture. A 5 μL of this mixture was located onto a CaF_2 slide and, dried at room temperature before analysis.

2.3 Preparation of Silver and Gold Colloids

Ag colloids were prepared by the method reported by Lee [18] and the maximum of its absorption was recorded at 420 nm for the synthesized colloidal solution. Gold colloids were prepared by the method of citrate reduction [19]. Sodium borohydride reduced silver nanoparticles were prepared using the method by Creighton [20] and its maximum absorption was recorded at 395 nm.

2.4 Raman instrumentation

All measurements were performed using a completely automated Renishaw InVia Reflex Raman Microscopy

System (Renishaw Plc., New Mills, Wotton-under-Edge Gloucestershire, U.K.) equipped with an 830 nm diode and 514 nm Argon-ion lasers. The laser power was in the range of 0.2-6 mW and the exposure time was 10 sec for 830 nm diode laser. Our previous studies showed that with the use of an 830 nm diode laser resulted with better quality and more producible SERS spectra compared to a 514 nm Ar^+ laser with our sampling method. This can be explained with the shift of surface plasmon resonance wavelength of aggregated silver nanoparticles to longer wavelength and greater penetration depth of the light into sample at longer wavelengths. A 50x objective was used. The wavelength of the instrument was automatically calibrated using an internal silicon wafer and the spectrum was centered at 520 cm^{-1}.

2. 5 Experiments

Influencing the surface charges of silver nanoparticles and bacterial cell wall can affect the type and the strength of the interaction, thus, the proximity of the nanoparticles to the bacterial cell wall and SERS spectrum. In order to probe into these interactions, two types of experiments were conducted.

pH Experiments: A change in the sample pH influences both nanoparticle surface properties and ionization status of the functional groups such as –COOH and $–NH_2$ on bacterial cell wall. These experiments were performed to investigate the affect of pH on SERS spectra. Thus, pH of the colloidal solutions were adjusted with an HCl acid solution and mixed with bacteria sample. There is no significant pH change observed after mixing the bacterial sample with the silver colloid solution in the final solution. This was possibly due to the much greater volume of the colloidal solution (100 μL) compared to bacteria sample volume (5 μL). The pH of the sample without any pH adjustment was remained around 9, and any pH adjustment was not made.

Method of Nanoparticle Synthesis: With the different preparation methods, the surface charge properties of silver nanoparticles vary. Due to this variation, by utilizing colloidal solutions with different preparation methods, it is possible to probe into the type of interaction between bacteria cell and silver nanoparticles. In these experiments, sodium citrate and sodium borohydride reduced silver nanoparticles were used to obtain SERS spectra from both bacteria.

Colloidal Solution Concentration Experiments: The colloidal silver solution concentration was increased to obtain higher density of silver nanoparticles in the sample to increase the chance to acquire reproducible spectra and to probe the surface chemistry of bacteria by getting more nanoparticles closer to the bacterial cell wall. The silver colloidal solution was concentrated by centrifugation and the concentrated solutions were mixed with bacteria sample.

2.6 Scanning Electron Microscope

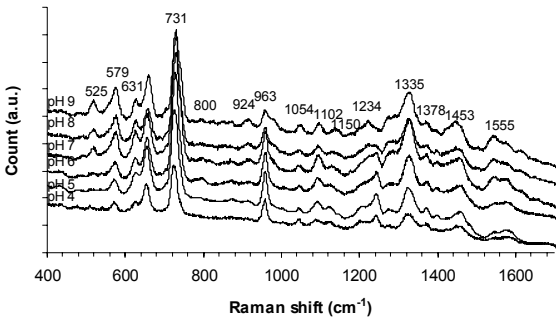
The prepared bacterial samples were spotted and dried on SEM specimen stub. A Karl Zeiss EVO 40 model SEM instrument was used. The accelerating voltage was in the range of 5-10 kV**.**

3 RESULTS AND DISCUSSION

3.1 Effect of pH on SERS Spectra

Increasing the pH may influence both nanoparticle surface properties and ionization status of functional groups on bacterial cell wall. The silver nanoparticles used in this experiment were produced with citrate reduction method and the surface of the nanoparticles is quite negatively charged in nature as synthesized. A pH increase will influence the double charge properties (zeta potential, ζ) [21] on the particle surface, thus the affinity of nanoparticles towards the bio-structures on the cell wall and their aggregation properties. In addition, cell metabolites and culture media carried over may influence the surface properties of the silver nanoparticles. Figure 1 shows the pH effect on SERS spectra of E. coli. The collapse of colloidal solution and loss of SERS activity is a known phenomenon at very low pHs [20]. However, after pH is 4 or higher, the changes in peak intensities and pattern can be attributed to the strength of the interaction of nanoparticles with bacterial cell surface. The peaks at 525 cm^{-1} is attributed to COC glycosidic ring deformations [22] of carbohydrate structure [23], 631 cm^{-1} COO^- bending, and 924 and 963 cm^{-1} C-N stretch [23], which are the components of the cell wall structure.

Figure 1: pH effect on SERS spectra of *E. coli*.

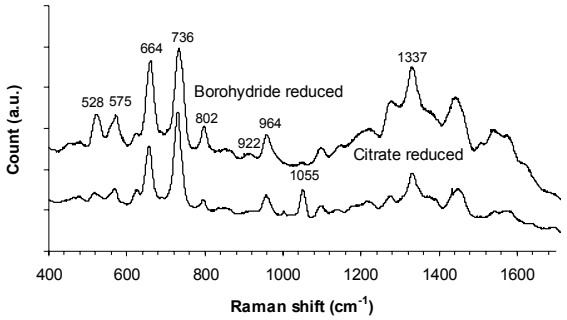


3.2 Influence of Particle Charge on SERS Spectra of Bacteria

The silver nanoparticles synthesized with two different synthesis procedure posses different surface charge properties. The citrate-reduced silver nanoparticles are more negatively charged compared to borohydride-reduced ones [24]. Thus, their interaction strength is expected to be different with biochemical moieties on the cell wall. This difference must be reflected on the SERS spectra. Figure 2

shows the SERS spectra of *Pseudomonas putida,* a Gram-negative bacterium, with citrate-reduced and brohydride-reduced nanoparticles. As seen, intensity of peaks at 528, 575, and 802 cm^{-1} is greater on SERS spectra obtained with borohydride-reduced nanoparticles, and the peak at 1055 cm^{-1} is absent.

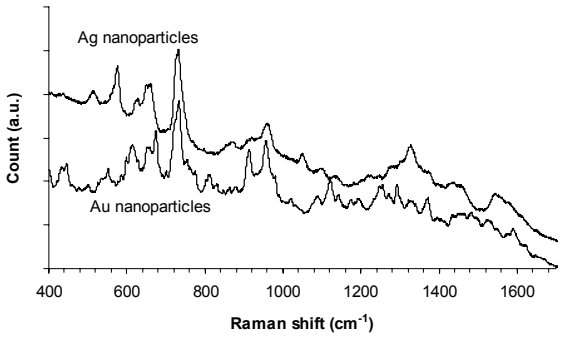
Figure 2. Chnages on SERS spectra with application of nanoparticles carrying different surface charges.



3.3 The Influence of Nanoparticle Type on Bacterial SERS

Surface plasmons, surface charge, and aggregation properties of nanoparticles have a definite effect on the SERS spectra of molecules. Thus, an observable effect should be detected with the use of either gold or silver nanoparticles on bacterial cell. Figure 3 show the SERS spectra of *E. coli* obtained with silver and gold nanoparticles. There are significant differences between two spectra and these differences must be the result of the several factors such as aggregation properties of nanoparticles, characteristics of surface plasmons, and surface charge differences present in such a complex environment.

Figure 3. Comparison of the influence of silver and gold nanoparticle on SERS spectrum of *E. coli*.



3.4 Influence of Nanoparticle Concentration and Arrangement Around the Bacterial Cell on SERS Spectra

We have observed that the use of an increased concentration of silver colloidal solution increases the number of nanoparticles in contact with the bacterial cell wall (submitted data). A simple mixing of bacteria with increased colloidal concentration generates a non-uniform mixture. Although the SERS spectra obtained from this non-uniform sample shows increased reproducibility and improved quality. Because the biomolecule composition of bacterial cell surface varies greatly, the acquisition of detailed SERS spectra might depend on the surface coverage of bacterial cell. We have developed a sample preparation method based on previously reported convective assembly [25, 26], which was used to prepare uniform coatings from modified and unmodified silica, and 3D SERS substrates. Figure 4 A and B show the SEM images of two samples prepared by simple mixing of bacteria and nanoparticles. Figure 5 shows the SERS spectra obtained from these samples. Convective assembly generates a uniform sample and not only limits the variations from sample-to-sample and spot-to-spot but also results with almost complete coverage of bacterial cell with silver nanoparticles.

Figure 4. SEM image of a sample generated with simple mixing of *E. coli* (A) and convective assembly (B).

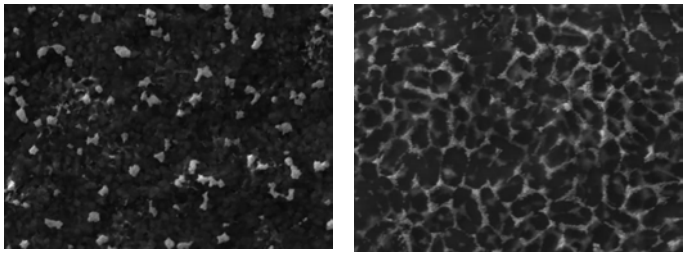
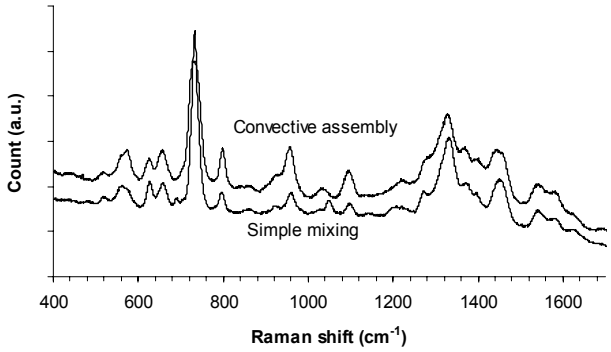


Figure 5. Comparison of SERS of E. coli obtained from a sample prepared with simple mixing and convective assembly.



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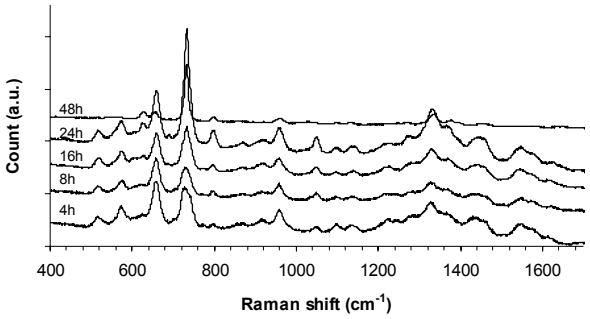
3.5 Monitoring Cell Wall During Bacterial Growth

Because the obtained SERS spectra mostly represents the information from bacteria cell wall. It is possible to monitor the changes on the cell wall during the growth of a bacterium. Figure 3 demonstrates the changes on the cell wall of *Bacillus megaterium* during its growth. From the SERS spectra, it is possible to deduce molecular changes during growth. For example, the SERS spectrum obtained during the 48th hour shows the death of bacterial cells. The death of bacterial cell during this period was also confirmed with the conventional bacterial growth studies. It is again there are several changes on the SERS spectra, which can be attributed to the changes in biochemical composition during the growth.

Figure 6. SERS spectra of *B. megaterium* during its growth.



4 CONCLUSIONS

This study demonstrates the influence of several experimental parameters to gather information about the biochemical structures and composition from bacterial cell wall. By changing the pH of the mixture, surface charges, type of nanoparticles and concentration, the molecular level information can be reflected to SERS spectra. This information can be used for fast identification and classification of microorganisms. A complete surface coverage of bacterial cells with silver nanoparticles improves the quality and reproducibility. Finally, the growth of bacteria was monitored with SERS.

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