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SURFACE ENHANCED RAMAN SPECTRA OF *ESCHERICHIA COLI* CELLS USING ZnO NANOPARTICLES

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Recently Raman spectroscopy has attracted great interest and emerging as a potentially powerful whole-organism fingerprinting tool for the rapid identification of bacteria and fungi. Since, bacteria and fungi commonly show very week Raman signal, it needs to be greatly enhanced by attaching or placing some molecules/metal nanoparticles microscopically close to or by suitably roughened the bacteria/fungi surface. This is well known surface enhanced Raman scattering (SERS) technique. In this paper, we are reporting the enhancement in Raman signal of *Escherichia coli* bacteria by incorporation of ZnO nanoparticle. The surface enhancement effect allows the observation of Raman spectra of single bacterial cells excited at low incident powers and short data acquisition times. A Very intense surface-enhanced Raman signals are observed for ZnO incorporated *Escherichia coli* single cells. We believe this to be the first report demonstrating SERS due to ZnO nanoparticle.

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Keywords: ZnO nanoparticles, *Escherichia coli*, Surface-enhanced Raman spectroscopy.

1. Introduction

To analyze the cellular components of biological cells or characterizing molecular interactions in the biological cell is currently a challenging task. Recently Raman spectroscopy has attracted great interest and emerging as a potentially powerful whole-organism fingerprinting tool for the rapid identification of bacteria and fungi. The Raman microscopy enables us to determine the interaction of different microorganisms and other substances with nanoparticles and biological cells just by its Raman vibrational signature. Raman spectroscopy is a nondestructive analytical technique that provides fingerprint spectra with spatial resolution of an optical microscope with almost no sample preparation. In past, Raman spectroscopy, have proven effective for the rapid identification of bacteria and fungi[1-7]. Raman spectroscopy is based on the measurement of the vibrational energy levels of chemical bonds by measuring the inelastically scattered light following excitation. Biologically associated molecules such as nucleic acids, protein, lipids, and carbohydrates all generate strong signals in Raman spectra. Therefore, the Raman spectroscopic method can be used to generate "whole-organism fingerprints" for the differentiation of biological samples or in analyzing the effect of nanoparticle's interactions with biological cells or characterizing molecular interactions in the biological cell. Since, bacteria and fungi commonly show very week Raman signal, it needs to be greatly enhanced by attaching or placing some molecules/metal nanoparticles microscopically close to or by suitably roughened the bacteria/fungi surface. This is well known surface enhanced Raman scattering (SERS) technique. SERS can tolerate water molecules and can generate more sharp and distinguishable bands of specific molecules. The surface enhancement effect allows the observation of Raman spectra of single bacterial cells excited at low incident powers and short data acquisition times.

Several investigations into SERS of bacteria have been undertaken[8-11]. Most of the SERS studies rely on the use of gold and silver nanoparticles. SERS relies on either the adsorption or close proximity of an analyte to a metal substrate[12, 13]. The substrate can be in the form of a

roughened metal surface, a colloidal solution, or a roughened electrode. Previous workers have shown that the total enhancement of the SERS effect is explained by two processes; a charge-transfer mechanism, known as *chemical enhancement*, and an *electromagnetic enhancement* effect. Chemical enhancement is thought to take place at sites of atomic-scale roughness on the metal surface and involves electronic coupling (an exchange of electrons) between the metal substrate and the analyte. Electromagnetic enhancement, thought to contribute more to the overall magnitude of enhancement, takes place on the nanometer scale and can be attributed to surface Plasmon oscillations. These provide a higher "local" optical field, due to redistribution and concentration of electromagnetic energy. Coupled with the incoming electric field from the incident radiation, this generates a larger spectroscopic signal for an analyte "caught" with Biological cells are complex mixtures of a large number of bio-molecules enclosed in the cell membrane, including nucleic acids, proteins, polysaccharides, and lipids.

In this paper, we are reporting the enhancement in Raman signal of *Escherichia coli* bacteria by incorporation of ZnO nanoparticle. A Very intense surface-enhanced Raman signals are observed for ZnO incorporated *Escherichia coli* single cells.

2. Experimental Section

2.1. Materials and Methods

Bacterial culture *Escherichia coli* (DH 5α) were obtained from Centre for Cellular and Molecular Biology (CCMB), Hyderabad, India. Agar, Yeast extract, Peptone and NaCl were purchased from Alfa Aesar, Germany and were of bacteriological grade. We used the spherical ZnO nanoparticles having 25 nm mean size synthesized by our group[14] to study the effect of interaction of these ZnO nanoparticles on the *Escherichia coli.*

Bactericidal effect of ZnO nanoparticles was studied against the Gram-negative bacteria *E coli* (DH5α). These nanoparticles were dispersed in autoclaved deionized water by ultrasonication. Aqueous dispersions of ZnO nanoparticles of desired concentrations were made. An axenic culture of *E coli* (DH5α) was grown in liquid nutrient broth medium containing NaCl 5 g, peptone 5 g and yeast extract 2.5 g. For this experimental investigation, freshly grown bacterial inoculums of *E coli* were incubated in the presence of 5 μg/ml of ZnO nanoparticles and the bacterial cell growth was observed at 37˚C with constant shaking. One flask was taken as control, with no nanoparticle load. Bacterial cell growth enhances the turbidity of the liquid nutrient medium. Samples were collected and coated onto glass slides by using standard spin coating unit to form thin and uniform films for SERS analysis.

2.2. Characterizations Used

Surface enhanced Raman spectra was recorded on a Renishaw make micro - Raman (model RM-2000) integrated with Nanonics AFM model Multiview 2000, capable of ~ 2 λ spatial resolution having piezo-electric scanner's with < 0.005 nm (Z), < 0.015 nm (XY), < 0.002 nm (XY) low voltage mode resolutions . 514 nm Ar^+ laser was used as excitation source for SERS analysis. Typically, the incident laser power was attenuated to 2 mW and acquisition time was set to 10 S. The true confocal performance of the instrument allows the analysis of single cells 1μm or smaller size. This confocal Raman microscope was equipped with an integrated Lica microscope having three distinct microscope objectives as 5×, 20× and 100× respectively. In the current work all the confocal Raman data were acquired with 100× objective. The laser beam was targeted on the single bacterial cell visually in videoscope mode on computer screen. Raman signal was optimized by adjusting the laser focus; the spectrum was then acquired between the range 800 – 2000 cm^{-1}.

3. Results

3.1. Normal (Bulk) Raman spectra Vs Surface-Enhanced Raman Scattering:

The typical unprocessed normal (bulk) Raman spectra and SERS spectra of selected individual bacterial cell from without ZnO treated and 5 µg/ml ZnO treated sample is shown in figure 1.

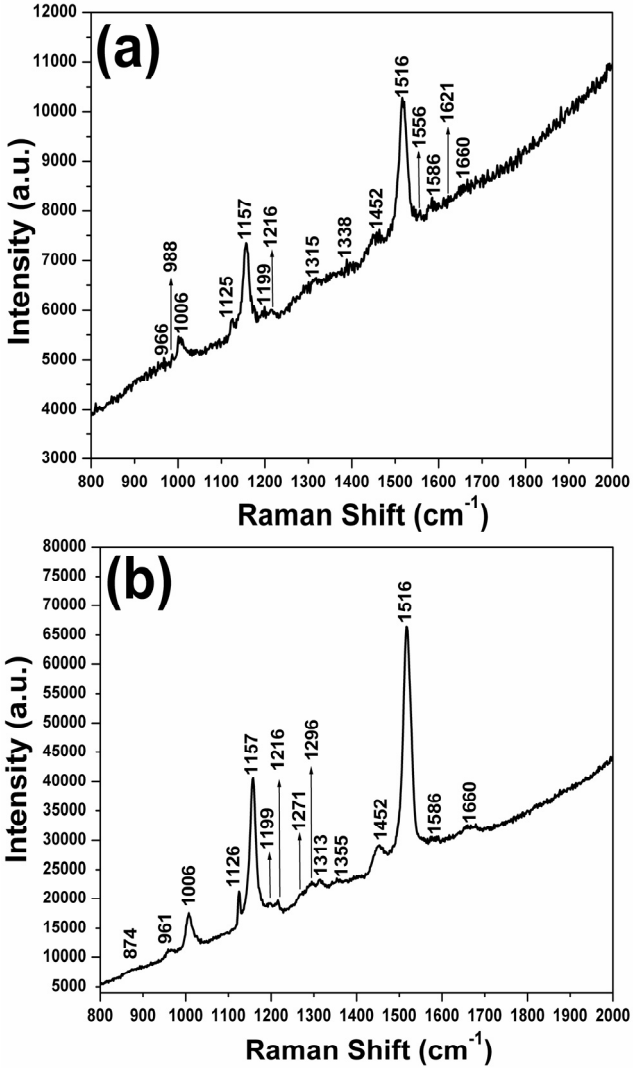

Fig. 1. Comparison of relative Raman spectra of the (a) normal (Bulk) Raman spectra and (b) SERS spectra of E coli single cells.

This figure depicts comparative Raman spectra of the **(a)** normal (Bulk) Raman spectra and **(b)** SERS spectra of *E coli* single cell treated with ZnO. The Raman spectra showed the main vibrational bands at 874, 961, 1006, 1126, 1157, 1199, 1216, 1271, 1296, 1313, 1355, 1452, 1516, 1586 and 1660 cm^{-1}.

3.2. Discussion

3.2.1. Normal (Bulk) Raman spectra Vs Surface-Enhanced Raman Scattering:

Some of the major bands observed by us can be associated with nucleic acids (1516[15], 1586 cm^{-1}) and peak at 1006[16]cm^{-1} was due to aromatic amino acid. The peak at 1516[17] is

also considered for aromatic amino acids according to some authors. We do see an intense band at 1157[16, 18] cm^{-1} associated to protein (C-N, C-C stretching). Whereas peak at 1126[19, 20] cm^{-1} can also be associated with C-N, C-C stretching. Raman band at 1452[19, 21] cm^{-1} is due to presence of lipids (CH_2 deformation). Our results also showed peak at 961 cm^{-1} and according to Spiro and Gaber[22] this peak is due to the C-C stretch (or C-C-N stretch) for various proteins that are present in the cell wall. Some different peaks are seen as side bands which are may be due to different methods of sample preparation, measurement and some possible experimental conditions. Both, the normal (bulk) Raman spectra and SERS spectra can be easily discriminated. SERS spectra [figure 1 (b)] is much clearer and sharper, with many fold enhanced Raman signals when compared to the normal (bulk) Raman spectra [figure 1 (a)]. Thus we can conclude that the difference in both the spectra is associated with the ZnO incorporation and ZnO significantly enhances the Raman signal obtained from the surface of the *E coli* single cell. We believe this to be the first report demonstrating SERS due to ZnO nanoparticle.

4. Conclusions

Thus we successfully demonstrated the SERS of *Escherichia coli* bacterial cells using ZnO nanoparticle. The surface enhancement effect allows the observation of Raman spectra of single bacterial cells excited at low incident powers and short data acquisition times. In future, this can be further utilized in enhancing the week Raman signals obtained from different bacterial cells and enables detection of both the spectral features of cells and interior organelles. The molecular composition, structure and interactions of cells with nanoparticles can be precisely identified using SERS by incorporating ZnO nanoparticle to that bacterial species.

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