CHARACTERIZATION OF THE SURFACE ENHANCED RAMAN SCATTERING OF BACTERIA ON A NOVEL SERS SUBSTRATE

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Abstract: The surface enhanced Raman scattering (SERS) of several Gram-positive and Gram-negative species and strains of bacteria on a novel gold particle (~80 nm) cluster covered SiO_2 substrate excited at 785 nm is reported. Strongly enhanced, reproducible SERS spectra of bacteria at the single cell level are obtained on these substrates. Advantages of the SERS methodology for the detection and identification of high priority bacterial pathogens is demonstrated by these results.

The development of SERS for the detection and identification of bacterial pathogens has attracted current research interest [1-5] motivated by both applications in clinical diagnostic microbiology and the recently heightened concerns about potential bio-terrorist attacks. The ability to provide unique vibrational signatures of a biologically relevant species lacking any visible chromophores in aqueous solutions at low concentrations illustrates the potential of SERS to be a valuable analytical and structural spectroscopic tool. Non-SERS or bulk Raman spectra of bacteria have been reported previously [6-8]. Vibrational fingerprints have been shown to serve as a basis for the classification of intact bacterial cells when reliable and reproducible protocols can be established. However, SERS should offer several advantages over bulk Raman observation for bacterial detection and identification purposes.

The SERS spectra of six species of bacteria in the 400 – 1700 cm^{-1} range acquired on a novel gold aggregate covered SiO_2 substrate are shown in Fig. 1. These spectra were obtained with ~ 2 mw of incident power at 785 nm in 10 seconds of data accumulation (Renishaw Raman microscope model RM-2000). As seen in this figure, each bacterial species is characterized by a unique SERS vibrational fingerprint. Systematic trends with phylogenic proximity are not found in the data. Furthermore, distinct spectral differences between Gram positive and Gram negative bacteria are not immediately striking, despite the difference in outer cell wall structure for these classes of microorganisms.

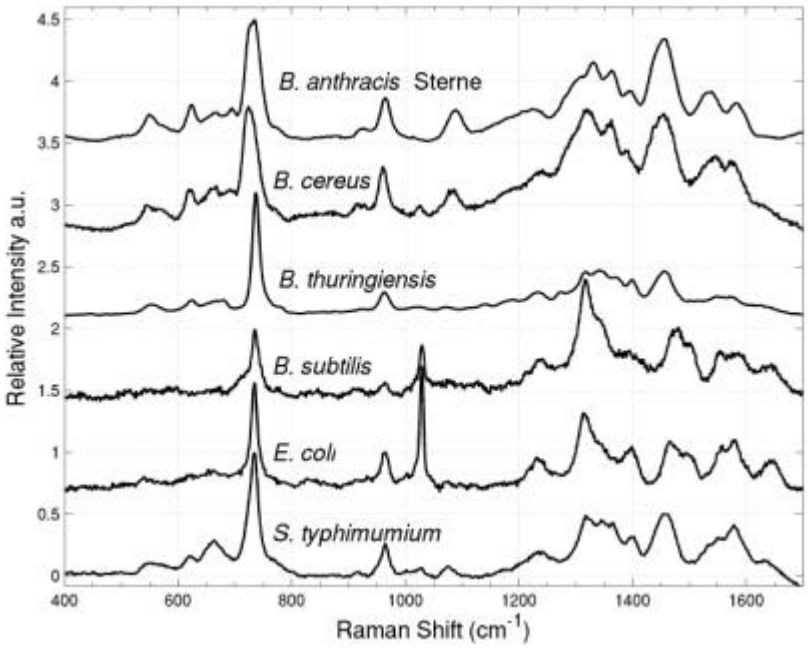

Figure 1. SERS spectra of six bacterial species obtained on gold aggregate coated SiO_2 substrates.

A metal doped, sol-gel procedure has been developed to produce the nanostructured SERS active substrates used to obtain these spectra. SiO_2 chips covered by aggregates of mono-dispersed

~80 nm gold particles results from this *in-situ* growth sol-gel recipe. Raman cross section enhancements in the range of $2 - 5 \times 10^4$ per bacteria are found for both G(+) and G(–) species on these SERS active surfaces for 785 nm excitation.

Due to the distance and orientation dependence of the electromagnetic and charge-transfer enhancement mechanisms, the number of vibrational bands and the relative intensities of the vibrational features are quite different in the SERS and non-SERS spectra of bacteria. The number of Raman transitions evident in the SERS spectra are significantly fewer than in the corresponding bulk Raman spectra. Furthermore, the SERS spectra of different species are more distinct than their corresponding bulk Raman spectra and thus imply that bacteria are more differentiated at their outer cellular layers than in the cytoplasm. These features alone make the SERS technique potentially a more powerful spectroscopic tool than normal Raman scattering for bacterial identification, aside from advantages due to improved Raman scattering efficiency.

Weak broad fluorescence overlaps the Raman region in the 785 nm excited emission spectra of *Bacillus* bacteria and severely limits the ability of bulk Raman observations to make detailed bacterial identifications in this excitation region. This broad emission feature, however, is not evident in the corresponding *Bacillus* SERS spectra despite the field enhancement mechanism which amplifies both the Raman and fluorescence components. In agreement with a density matrix formulism of resonant secondary radiation, [9] the emission is dominated by the Raman component for molecules in the proximity of nano-structure metal surfaces due to a rapid energy transfer process. Thus SERS is essential for the observation of vibrational signatures excited by red to visible radiation for some potential priority pathogens such as *B. anthracis*.

In addition to the species differentiation, the SERS fingerprints exhibit strain specificity and spectral differences between specific gene deletion bacterial mutants and their congenic parents on these gold cluster covered substrates. Raman spectra of bacteria at the single cell level have also been obtained on these SERS substrates. Aside from illustrating the sensitivity of this technique, the ability to observe vibrational signatures of single cells allows SERS to be used to identify members of a mixture of cell types, thus enabling rapid detection and identification of mixed infection components and the study of heterogeneities of microbial populations in general.

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