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Rapid identification of antimicrobial drug resistance strains of *E-coli* using SERS nanowire chip

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ABSTRACT

We have investigated the enhanced Raman spectra of AMR bacteria strains of *E. coli* using silver coated silicon nanowires SERS assay. Three different *E. coli* strains, *E. coli CCUG17620*, *NCTC 13441*, and *A239*, were detected using two different excitation laser wavelengths. We found stable and enhanced SERS spectrum using 785 nm laser as opposed to 532 nm. Future development of SERS-chip could offer a reliable platform for direct identification of the pathogen in bio-fluid samples at strains level.

Keywords: Raman spectroscopy, SERS, Nanowire, pathogen detection, AMR

1. INTRODUCTION

Antimicrobe-resistant (AMR) bacteria cause 1.27 million deaths annually [1]. Most infectious pathogens like *Escherichia. coli, Staphylococcus aureus*, *Klebsiella pneumoniae, Psudomonas aeruginosa, Enterobactor spp*, etc., have different antibiotic sensitivity profiles [2]. *E. coli* in particular causes various gastrointestinal infections, and some of the resistant genes are easily transferrable to other strains via horizontal gene transfer [2]. The strain of *E. coli* bacterium can be extremely dangerous to children and the elderly, leading hemolytic uremic syndrome, which causes kidney damage [2]. Currently, the identification of strains of bacteria and their sensitivity profile relies on time-consuming culture-based methods (3-5 days), which can lead to a more severe infection in the patient [3]. Thus, the bacterial strain must be identified immediately to treat an infected patient with the right antibiotic. Raman spectroscopy is becoming a popular technique, where the real-time, nondestructive optical method is utilized to detect the spectra of bacteria without the requirement of extended sample preparation [4,5]. The Raman spectroscopy is an elastic scattering of light containing vibrational and rotational energy levels in terms of molecular fingerprint. Raman spectroscopy has found usage in diversified applications and have been integrated with different functions such as optical trapping [6] and microfluidics. But the inherently low Raman scattering signal is inefficient to detect molecular spectrum for sample having lower concentration [7].

The surface-enhanced Raman spectroscopy (SERS) technique offers enhanced Raman spectra utilizing the nanostructure of metallic nanoparticles [8-10]. In SERS, metallic nanoparticles provide a hotspot with an enormously high local electric field due to localized surface plasmon resonance (LSPR) condition. Here we utilized an Ag coated Si NW SERS array for the detection of Raman spectra of three strains of *E. coli* (*CCUG17620, NCTC13441, and A2-39*) using 532 nm and 785 nm wavelengths, respectively. Various works have reported of SERS technique to detect virus, fungi, and bacteria [11]. Wang et al. introduced a flexible nanosticker to detect pathogenic bacteria [12]. Detection of *E. coli* bacteria up to 100 colony forming unit (CFU) /ml using Ag capped Al nanorods and a portable spectrometer has been

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also demonstrated [13]. Previous studies have also demonstrated the SERS detection of bacteria for various classes of different pathogenic bacteria including tuberculosis pathogens, urinary tract infection pathogens. More recently, another study demonstrated the superior performance of Ag coated Si NW SERS assay and machine learning enabled detection of pathogenic bacteria at class and strain level [14]. Here, we investigate the dependency of laser wavelength for bacterial detection using the fabricated Si NW SERS chips. The different excitation lasers excite slightly different vibrational bands of bacteria since the Raman cross section is dependent on the excitation laser [15]. Thus, the SERS spectra of multi-excitation adds extra molecular label information. The utilized SERS substrate exhibits distinct and reproducible results at 785 nm, benchmarking the strain level information using complementary whole genome sequencing (WGS). We further investigated the stability of enhanced Raman spectra as a function of laser powers. The enhanced Raman spectra of different strains of *E. coli* species is elaborated in the subsequent sections.

2. METHODOLOGY

2.1 Bioinformatics and Whole genome sequencing (WGS) data of E. coli strains

The AMR genes and plasmid sequences of *E. coli* were thoroughly discussed in previous publication [16]. We used the WGS data of *E. coli* strains, named, *E. coli CCUG 17620*, *E. coli NCTC 13441* from NCBI assembly database (SAMN02929659, SAMEA2432036, SAMN02604091) and *E. coli A2-39* from European nucleotide archive (PRJEB49072 and PRJEB45084), respectively. *E. coli CCUG17620* is a wild type of *E. coli* strain. *E. coli A2-39* is resistive to cefotaxime, ceftazidime, and *E. coli NCTC 13441* is resistive to cefotaxime, ceftazidime and fluoroquinolone, respectively.

2.3 SERS substrate fabrication

The silicon nanowire (Si NW) assay was fabricated using an optimized two steps metal assisted chemical etching (MACE) technique. MACE is entirely wet-chemical process and is convenient for the fabrication of large area Si NW arrays at room pressure and temperature. The two steps MACE consists of 1) Ag nanoparticles (NPs) deposition on the Si wafer in the first step, and 2) etching of Si wafer in an etchant solution in the presence of Ag NPs in the following step [17]. After these steps, a removal and redeposition of the Ag nanoparticles ensure for an optimal coverage of the standing nanowires for spectra enhancement. Prior to the deposition, a P-type Si wafer having <100> crystal orientation was ultrasonically cleaned using acetone. propanol, and DI water, respectively. The cleaned wafer was kept inside piranha (H_2SO_4 and H_2O_2 in 3:1 ratio) for 30 min for removal of the native oxide. The wafer was washed with DI water and kept inside an etchant solution of $AgNO_3$ (12.5 mM) and HF (22.6 M) for Ag NPs coating on a wafer surface. The Si wafers were then washed immediately and kept inside an etchant solution of 30 % H_2O_2, 40 % HF, and DI water in a volumetric ratio of 1:2:1 for 90 min. The fabricated Si NW assays were after dipped inside 60% HNO_3 solution to remove extra Ag^+ ions. The cleaned Si NW assay were finally dipped inside a fresh $AgNO_3$ and HF solution to coat Ag nanoparticles on Si NWs for an optimized immersion time of 100sec. A more in-depth explanation and optimization study is provided elsewhere [14].

2.4 SERS spectra acquisition

A bacteria solution of 2 µl in volume was drop-casted on a SERS substrate and dried at room temperature before taking measurements. The Raman spectra were recorded using a tabletop micro-Raman spectrometer (Renishaw inVia, used objective: 50X, 0.75 NA). Before drying, bacteria count present under the microscope were calculated to be 5541 for *E. coli CCUG17620,* 4872 for *E. coli NCTC 13441*, and 3120 for *E. coli A2-39*, respectively. The utilized excitation wavelengths were 532 nm and 785 nm (Spectrum range: 600-1800 cm^{-1}). The laser power was varied from 0.5 mW up to 10 mW. The integration time was kept at 10 s.

2.5 SERS spectra analysis

Figure 1 shows the SERS spectra of *E. coli* for the two different excitation laser wavelengths of 532 nm and 785 nm, respectively. *E. coli CCUG17620* shows distinct and enhanced peaks at 762 cm^{-1} indicating the porphyrin breathing mode. The mode at 1280 cm^{-1} is attributed to deformation of Amide III protein. The mode at 1361, 1473, and 1634 cm^{-1} attributed to Tryptophan guanine for DNA, protein deformation, respectively. The *E. coli NCTC* 13441 gives peaks at

1106 cm^{-1} indicating vibration of porphyrin, 1175 cm^{-1} for stretching of (C=C) of lipids, and (C-C) stretching of carotenoid. Raman shifts at 1301, 1324,1348, and 1380 cm^{-1} are assigned for Amide III protein, deformation of fatty acids and proteins, and stretching of guanine in DNA, respectively. The modes at 1623 and 1634 cm^{-1} represent vibration of Amide I protein. *E. coli A2-39* shows peaks at 1284, 1352, 1618 cm^{-1} indicating CH deformation of protein, guanine DNA and Amide I protein respectively. The broad peak at 1000 cm^{-1} is coming due to the Si wafer background. The 785 nm laser gives a Raman peak of *E. coli CCUG17620* at 738 cm^{-1} for the stretching of peptidoglycan. The peak at 738 cm^{-1} is shifted to 730 and 735 cm^{-1} *for E. coli NCTC* and *A239* strains. Table 1 provides an overview of the Raman shift and assigned vibrational modes of bacteria. Figure 2 shows the SERS spectra stability of bacteria using varying laser power for both the excitation laser wavelengths. The SERS peaks position changes for all strains of *E. coli* for a change in excitation power having 532 nm laser, as shown in Figure 2 (a-c). No change in peak position occurred when excitation power was changed using 785 nm laser, as shown in Figure 2 (d-f).

Table 1: Assigned Raman bands of bacteria [14, 18-19]

Raman shift (cm^{-1})	Assigned vibrational modes
623	Twisting of CC- tryptophan
659	Tyrosine
674	(C-S) Stretching, Guanine
724-728	Peptidoglycan
734-738	Stretching of Adenine, peptidoglycan
1183	Stretching of C-C tyrosine, phenylamine, tryptophan protein
1175, 1180-1183	Stretching of C-C and C-H bonds of lipid
1280-1282	CH deformation of protein
1333	Deformation of Fatty acids and protein
1447-1449	Lipids and proteins deformation
1523	Exopolysaccharides, tryptophan
1583, 1591-1592	Ring stretching of C-C, phenylamine, tryptophan, and tyrosine
1603, 1618-1623	Amide I Protein

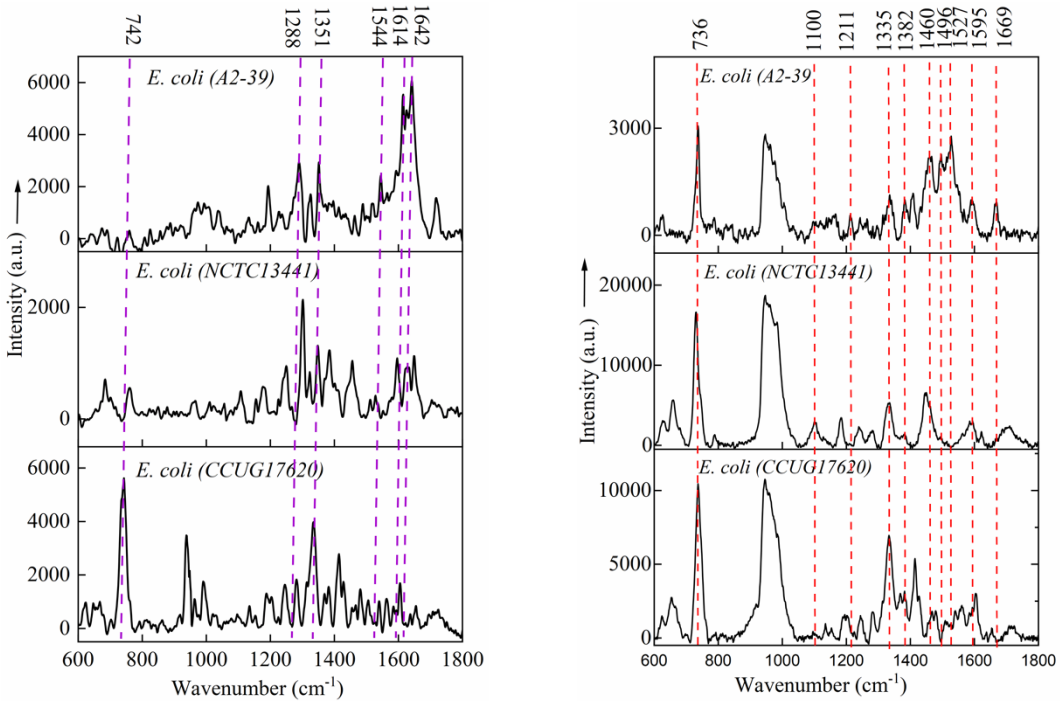

Figure 1. Enhanced SERS spectra of three different strains *of E. coli*, for (a) 532 nm and (b) 785 nm excitation laser wavelengths. The two lasers excite slightly different vibrational bands for each strain of *E. coli*. The two wavelengths excitation SERS adds extra information of vibrational bands coming from bacteria cell wall.

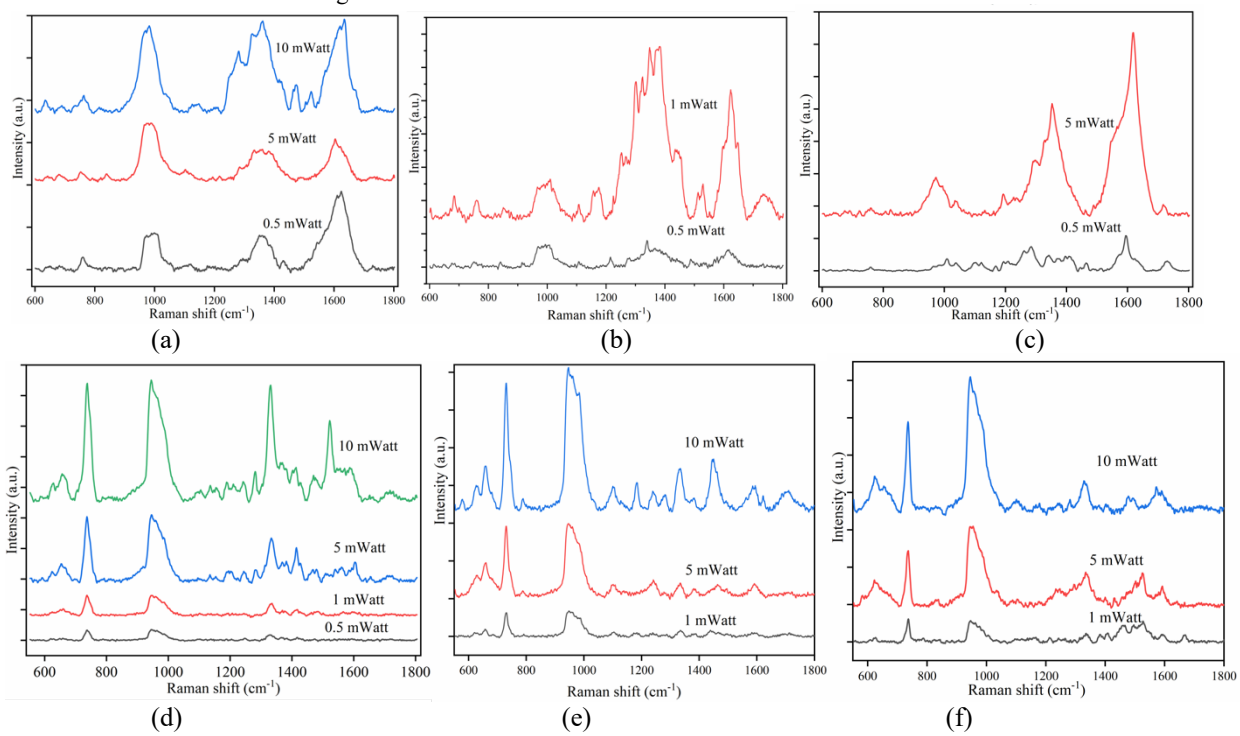

Figure 2 (a-f). Investigation of SERS spectrum stability for (a, d). *E. coli (CCUG17620)*, (b, e) *E. coli (NCTC13441)*, and (c, f) *E. coli* (*A2-39*) using varying laser power for 532 nm (a-c), and 785 nm (d-f) excitation laser wavelengths. The SERS spectra are stable for 785 nm laser compared to 532 nm laser since 532 nm laser causes enhanced autofluorescence background, causing inconsistency in the Raman spectra.

3. CONCLUSION

Here, we developed MACE fabrication process to fabricate SERS chip that does not require expensive vacuum equipment. The SERS chips were further used for the detection of Raman spectra of three different stains of *E. coli*, covering both the wild types and the antimicrobial resistant pathogens. The detailed SERS spectrum analysis reveals that the excitation laser wavelength of 532 nm exhibits an enhanced autofluorescence background while 785 nm laser wavelength does not. The slightly different wave shifts give strain specific information and each bacterium. At 532 nm, the SERS spectra were instable, and the peak positions slightly change with an increase in the incident laser power, possibly due to the autofluorescence background. However, the SERS spectra acquired using 785 nm excitation is stable with varying incident power. The proposed workflow is rapid and could be useful for the identification of AMR bacteria. Future studies will be carried out on larger data set, in real samples such as urine and possibly also for mixed infection conditions.

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