Detection and Differentiation of Foodborne Pathogenic Bacteria in Mung Bean Sprouts Using Field Deployable Label-Free SERS Devices

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Supplementary Material

S.1 Optimizing AgNR substrates for detection of bacteria

S.1.1 Optimizing AgNR length

Conventionally, AgNR substrates have been developed with a length maximized for chemical detection and sensing, e.g. BPE (Trans-1,2-bis(4-pyridyl)ethylene) and Rhodamine 6G (R6G), which allows small chemical molecules to diffuse in between the nanorods and reside in the small gap [1]. Compared to small chemical molecules, bacteria are much larger and the conventional AgNR substrates produce a relatively weak SERS signal of bacteria due to limited contact between bacteria and substrate surface which only takes place on the tips of the Ag nanorods. Therefore the length of the AgNR may need to be optimized in order to acquire the maximum SERS intensity and improve the LOD for bacteria. The length of the AgNR is proportional to the thickness the AgNR layer which is monitored by the QCM *in situ* during the deposition. To optimize the SERS substrates, AgNR of 200, 300, 400, 500, 600, 700, 800, and 900 nm QCM reading were deposited. Then a droplet of 10 μL 10^8 CFU/ml *E. coli* O157:H7 was applied to each of the substrates and their SERS spectra were collected under the same conditions and compared, as shown in Figure S1A. The spectra of *E. coli* has significant peaks at $\Delta\nu$ = 652 cm^{-1} (C-O-C from tyrosine), 728 cm^{-1}(ring breathing and N-H wagging mode from adenine), 893 cm^{-1} (C-O-C stretch from carbohydrates), and 955 cm^{-1} (C-C stretching), which are consistent with previous reports [2,3]. These peaks for *E. coli* O157:H7 samples are present for all AgNR substrates with thickness from 400 nm to 900 nm, but not for 200 nm and 300 nm substrates. For the 200 and 300 nm substrates, the shorter AgNR length makes the shape of the

substrate more like an islanded film rather than nanorods and the SERS signal enhancement is limited. In order to determine the optimal AgNR length for bacteria detection, we quantified the intensity of the SERS spectra by using the peak intensity at Δv = 728 cm^{-1} (I_{728}), which is the most significant peak from *E. coli* O157:H7 samples. The I_{728}, obtained by peak fitting, was plotted against the AgNR thickness in Figure S1B. As the length of the AgNR increased, the SERS intensity of *E. coli* O157:H7 increased, and it reached a maximum intensity at 600 nm AgNR thickness (QCM reading). The SEM images of the 600 nm AgNR are shown in Fig. S2. At 600 nm, the AgNR has an average diameter of 52 nm, an average length of 487 nm, and an average density of 18 rods/μm^2. The diameter of the bacteria is generally around 0.5 μm, so this AgNR length does not exceed the diameter of the bacteria. This morphology has enough "hot spots" to generate strong SERS signal, yet not too long to decrease the SERS signal. The morphology of the AgNR at other different thickness was previously reported [4]. Thus, the 600 nm-thick AgNR layer was found to be the optimal substrate for bacterial detection.

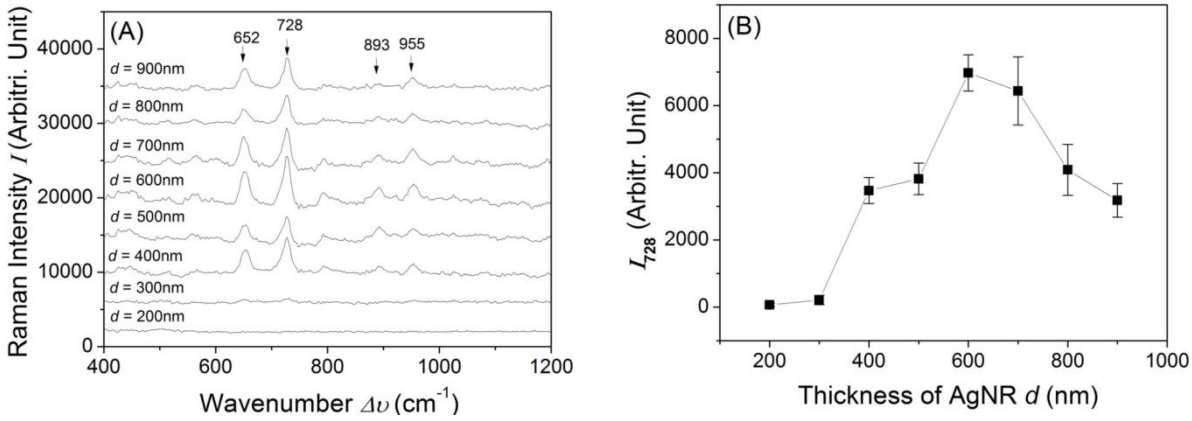

Fig. S1 A) SERS spectra and B) the SERS peak intensity I_{728} of 10^8 CFU/ml *E. coli* O157:H7 samples on AgNR with QCM thickness *d* of 200, 300, 400, 500, 600, 700, 800, and 900 nm, respectively. Spectra were measured by Enwave Raman system.

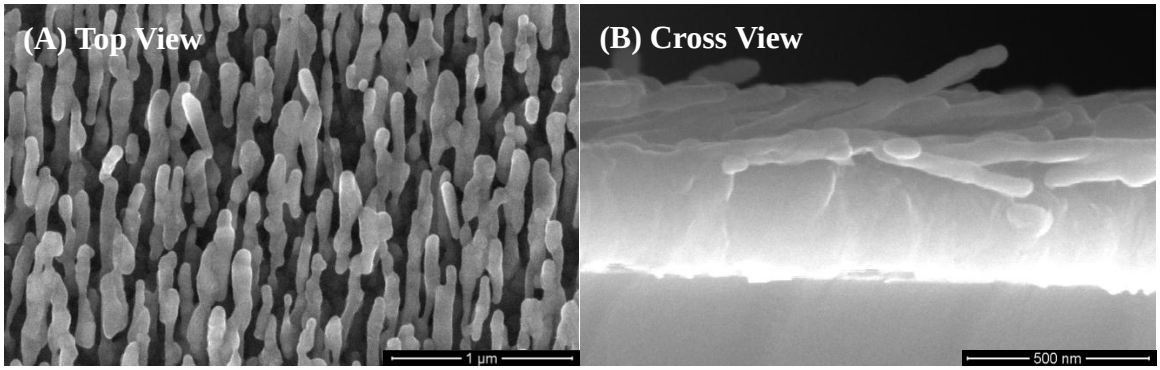

Fig. S2 SEM images of AgNR with 600 nm thickness (QCM reading). A) Top view. B) Cross view.

S.1.2 Optimizing AgNR functionalization using vancomycin

The mechanism of affinity capture of bacteria by vancomycin-modified nanoparticels is studied by Kell *et al* [5]. Liu *et al*. demonstrated that coating the Ag nanoparticles with vancomycin (VAN) provided a layer of capture agent for bacteria, which increased the detection sensitivity of bacteria [6]. Theoretically, coating the AgNR substrates with vancomycin would play the same role, and the optimal condition of such a coating on AgNR was explored in this study. The 600 nm AgNR substrates were coated with six different concentrations of vancomycin (0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 mM). After vancomycin coating, these functionalized substrates was immersed in 0.5 ml of 10^8 CFU/ml *E. coli* O157:H7 solution for 2 hours at 37 °C with shaking at 200 rpm. The inoculated substrates were then rinsed with DI water, followed by drying with Nitrogen. SERS measurements were carried out under the same conditions after drying. In order to determine which concentration of vancomycin would yield the greatest SERS intensity of bacteria, again we used the I_{728} peak from *E. coli* O157:H7 to quantify the SERS response of the bacteria on the VAN substrates, and Figure S3 shows I_{728} as a function of the vancomycin concentration C_V. Similar to results from Liu *et al*.,[6] the SERS intensity of the bacteria increased as the concentration of vancomycin increased until it reached a maximum at 1 mM. Ellipsometry was performed on a Ag thin film with 600 nm thickness (data not shown), and we found that at 1mM, the surface coverage of the vancomycin was close to 1. Therefore when the concentration of vancomycin is less than 1mM, the amount of bacteria captured on the AgNR

surface will be determined by the amount of vancomycin on the surface, i.e., the surface coverage of vancomycin, so the SERS intensity increases with vancomycin concentration initially as shown in Fig. S3.

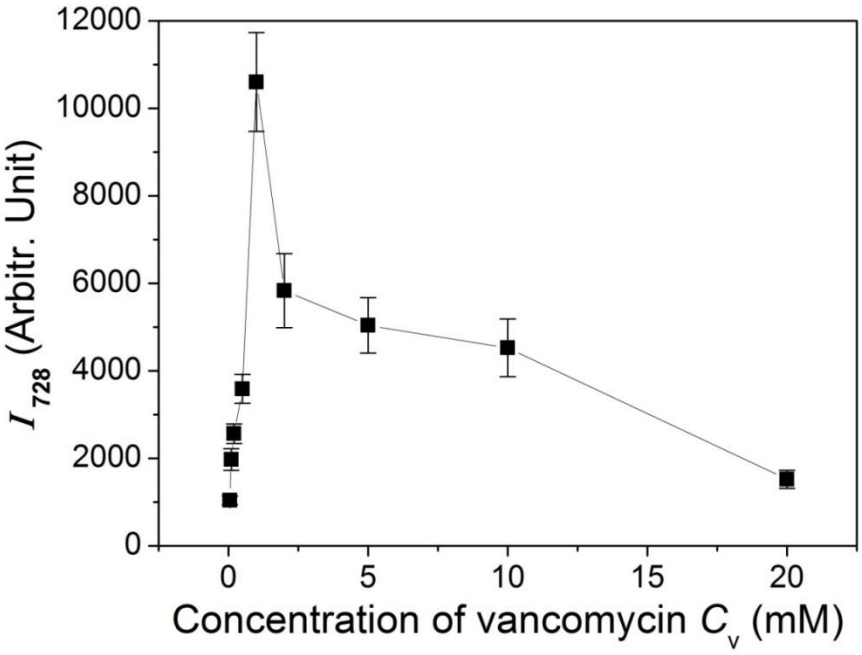

Fig. S3 The change of the SERS peak intensity I_{728} of 10^8 CFU/ml *E. Coli* O157:H7 as a function of the Vancomycin concentration C_V.

When the concentration of the vancomycin exceeds the 1mM, there will be multilayer vancomycin coating on AgNR surface. Although the amount of bacteria captured could stay the same, but some of them are captured by vancomycin molecules on the second layer. The SERS signal is very sensitive to the distance, and their relationship is approximately $I \propto (1/R)^{12}$, where I is the SERS intensity, R is the distance between the Ag surface and the absorbed molecule(s) [7]. Thus the bacteria captured by second layer would contribute less SERS intensity compared to those from the first layer. Thus, with the increase of the vancomycin concentration, more vancomycin molecules are on the second layer, less are on the first layer, then the effective SERS signal decreases. If third layer or forth layer of vancomycin is built, the SERS signal will become even weaker. Thus, the maximum I_{728} is found when 1 mM vancomycin was used, and is determined to be the optimal vancomycin treatment for the 600 nm AgNR.

S.1.3 Background of the optimized AgNR substrates

The background SERS spectrum of the optimized vancomycin coating (1mM) on 600 nm-thick AgNR substrates is shown in Figure S4. In Figure S4, the SERS peaks of the VAN-coated substrates are identified at Δv = 1243, 1321, 1369, 1476, 1595 cm^{-1}, respectively, and these peaks are consistent with previously reported Raman peaks of vancomycin [8]. Those peaks should be excluded from any bacteria SERS spectra generated on the VAN-coated substrates.

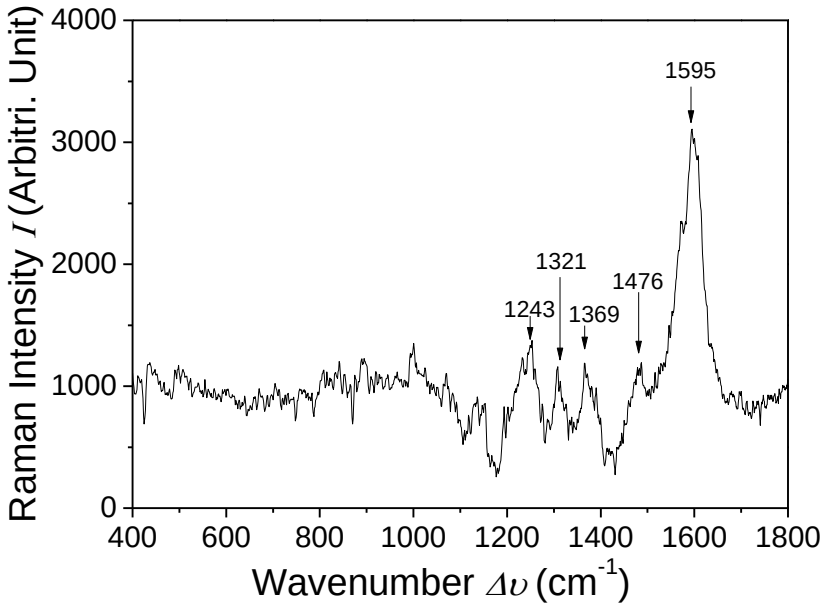

Fig. S4 The SERS spectrum of the 1mM Van-coated 600 nm AgNR substrates collected by using Enwave Raman spectrometer. The spectrum is baseline-corrected, and the significant peaks are identified.

S.2 Detection of *E. Coli* O157:H7 in mung bean sprouts using a handheld Raman spectrometer

In order to use our detection platform in field applications, the system should be light-weight and portable. The FirstDefender RM is a handheld Raman spectrometer that is easy to handle and suitable for field use because of its tough design and ease of mobility. To validate the use of this handheld Raman spectrometer we used it to acquire SERS spectra in conjunction

with the previously described procedures for inoculation and filtration. Figure S5 shows the SERS spectra of *E. coli* O157:H7 recovered from inoculated mung bean sprout samples (10^2, 10^3, 10^4, and 10^5 CFU/ ml) using the handheld Raman spectrometer. Similar to the results acquired using the Enwave spectrometer in Figure 3A in main text, significant peaks for *E. coli* O157:H7 SERS spectra collected by FirstDefender RM were found at Δv = 654, 730, 797, 895, and 957 cm^{-1}, with peak intensity increasing as the bacterial concentration increased. For the 10^2 CFU/ml sample, the spectrum has all these signature peaks of the *E. coli* O157:H7, thus the LOD for *E. coli* O157:H7 using the handheld Raman spectrometer should also be 10^2 CFU/ml.

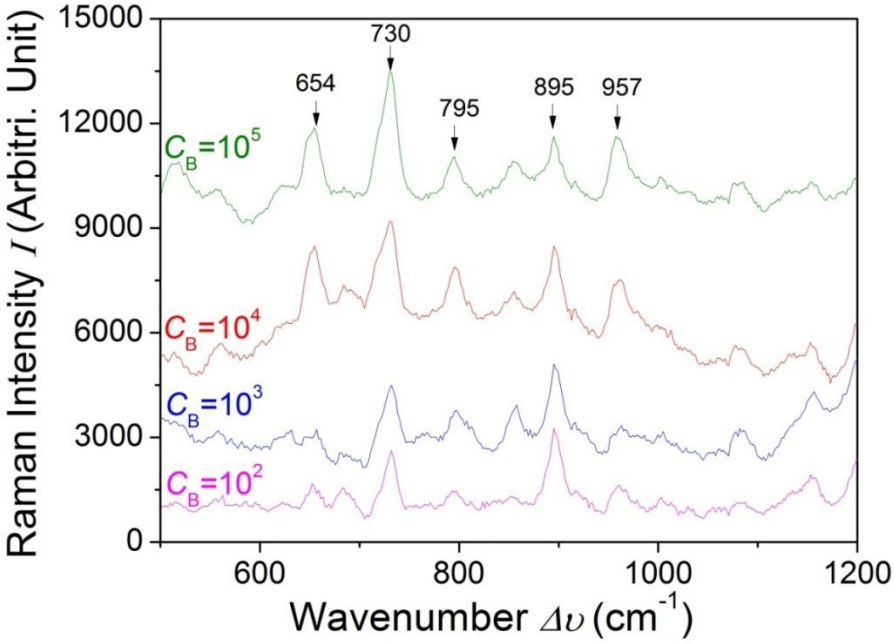

Fig. S5 SERS spectra of *E. Coli* O157:H7 recovered from mung bean sprout samples inoculated at different rates (C_B = 10^2, 10^3, 10^4, 10^5 CFU/ml) followed by a two-step filtration, collected using the FirstDefender RM handheld Raman spectrometer.

In order to see the spectral differences statistically, we also performed PCA and PLS-DA analysis. Figure S6A shows the plot of PC1 versus PC2. Samples inoculated with *E. coli* O157:H7 at rates as low as 10^2 CFU/ml formed a cluster separate from the control samples. As shown in Figure S6B, PLS-DA also confirmed the differences between the bacteria sample spectra and the control. Furthermore, the PLS-DA plot discriminates spectra between the control and bacterial samples inoculated over 10^2 CFU/ml with 100% sensitivity and specificity. PCA

and PLS-DA indicate that VAN-coated substrates can detect as low as 10^2 CFU/ml or 10^3 CFU/g *E. coli* O157:H7 in mung bean sprout samples using the handheld Raman spectrometer.

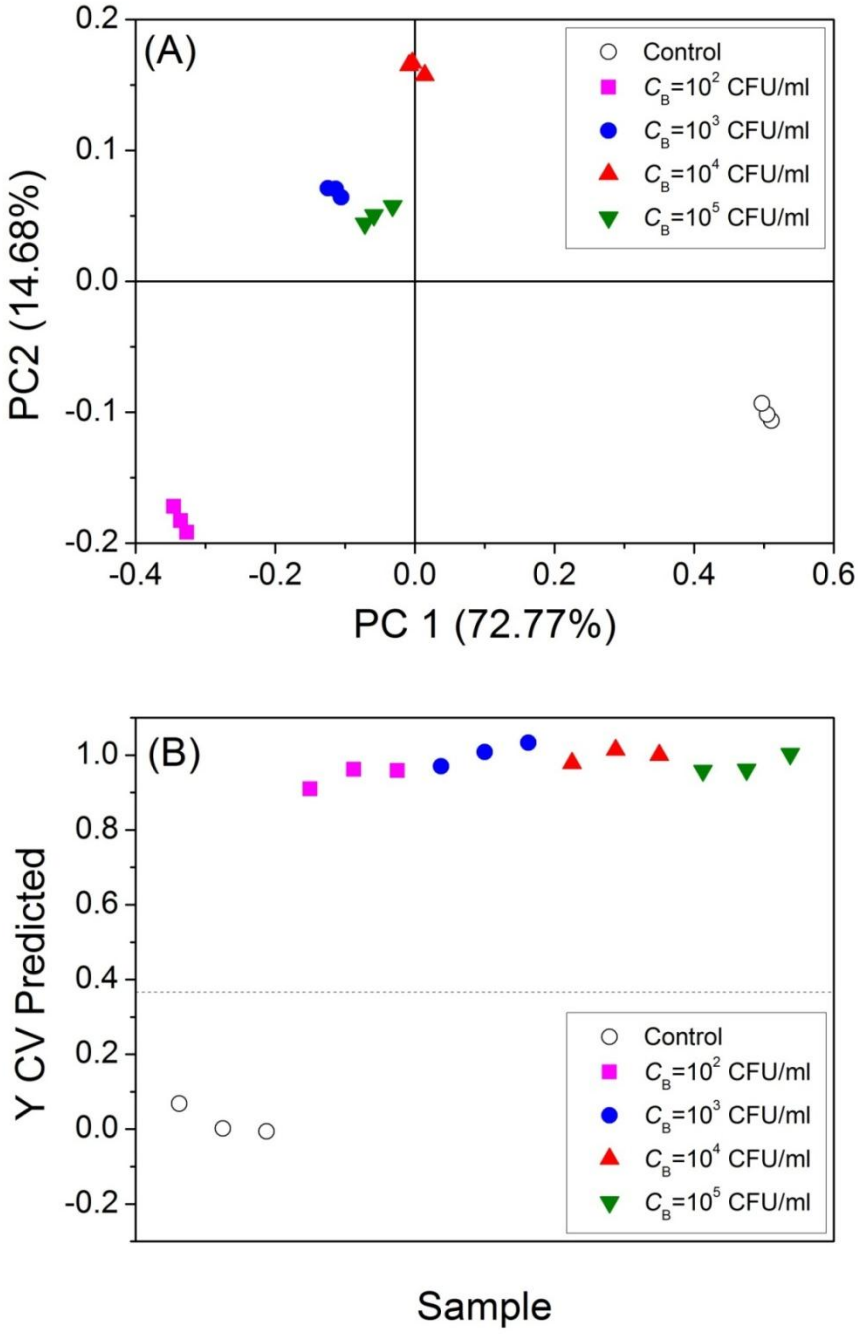

Fig. S6 A) PCA and B) PLS-DA plot of SERS spectra of *E. coli* O157:H7 recovered from mung bean sprout samples inoculated at different bacteria concentrations (C_B = 10^2, 10^3, 10^4, 10^5 CFU/ml) and sterile DI water inoculated in mung bean sprout samples (control) followed by a two-step filtration. Spectra were collected by the FirstDefender RM handheld Raman spectrometer.

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