Sers-Based Biosensor for Bacterial Pathogens Detection in Contaminated Foods

Giorgi Shtenberg[1], Divagar Muthukumar[1], Narsingh Raw Nirala[1], Ofer Nehushtai[1]
[1]Institute of Agricultural Engineering, ARO, Volcani Institute, Rishon LeZion, Israel
giorgi@agri.gov.il

Abstract
The current study presents a highly sensitive and selective surface-enhanced Raman scattering (SERS) method for detecting food- and water-borne pollutants in actual samples. An indirect assay evaluated the residual unreacted bio probes (antibodies or aptamers) using optimized silver-decorated porous silicon (Ag-pSi) platforms. Under optimized conditions, the SERS effect was augmented up to 7.7 orders of magnitude while offering means to detect Escherichia coli and Staphylococcus aureus as low as 3 and 1 CFU mL^{-1}, respectively. Considering the complex media analysis, the miniaturized SERS platform is highly reliable, rapid and accurate and could be applicable for routine on-site analysis of various emerging pathogens.

Introduction
Bacterial infections and contamination of food and water sources are common concerns for public health and the environment [1]. Specifically, the widespread of pathogenic microorganisms in water sources promotes the propagation of infectious diseases causing the loss of millions of lives annually. At present, all microbial contaminants are identified by conventional culture and molecular diagnostic techniques, which are highly efficient and precise using laboratory-based facilities, yet they are impractical for rapid and straightforward field diagnosis of the causative hazard [2]. Recent advancements in analytical methods miniaturization have revolutionized early-stage detection of target contaminants in complex matrices. Surface-enhanced Raman Scattering (SERS) is a reliable and attractive detection technique capable of differentiating bacterial load and quantifying minute content. This is mainly ascribed to the augmented enhancement factor (EF) that can reach values up to 10^{11} over the conventional inelastic scattering of the Raman spectrum. An essential prerequisite for efficient SERS detection is to produce a highly reliable, reproducible and homogeneous SERS substrate in terms of signal intensity and stability with high affinity toward the target analyte [3].
Herein, we present an efficient sensing approach for the sensitive and selective detection of two predominant bacterial pathogens (*Staphylococcus aureus* and *Escherichia coli*) associated with food and water-borne infections. The SERS active platform was constructed using noble metal (silver nanoparticles, AgNPs) coated porous silicon (pSi) thin films optimized for abundant signal enhancement, thus revealing the competence of the sensing approach to assess bacterial load with high sensitivity. The selectivity, shelf life and feasibility of SERS-based bioassay to assess foodstuff samples contaminated with the target pathogens were thoroughly evaluated.

Results and Discussion
The SERS substrate fabrication process was carried out with slight modifications based on our previous work [4]. Initially, bulk silicon substrates were subjected to electrochemical anodization using a constant current density of 530mA cm^{-2} for 9 sec. The freshly prepared pSi substrates were soaked in 1.5 mM silver nitrate solution (50% ethanol) to incorporate AgNPs within the porous scaffold. The Ag-PSi platforms were modified with 1 µM 4-aminothiophenol (4ATP, Raman tag) in ethanolic solution for 1 hr, followed by ethanol rinsing (trice) and dried at 100ºC for 10 min. Herein, the optical performance of the developed SERS platform was assessed through EF calculation [5, 6]. Figure 1 presents SERS and Raman spectra of 4ATP/Ag-pSi together with plain pSi and bulk Si (both omitting noble metal from the surfaces), respectively. The latter two Raman spectra show characteristic peaks of 4ATP (*i.e.,* 1087 and 1598 cm^{-1}); see Figure 1 inset. The computed EF of the developed 4ATP/Ag-pSi with respect to bulk Si and pSi are 5.6×10^{7} and 4.6×10^{7}, respectively. The resulting intensified signal enhancement is associated with the optimized surface environment that generates hot spots for the profound SERS effect. Immediately, secondary antibodies or complementary aptamers were applied on the Ag-pSi surfaces to functionalize their inherent voids. The sensitivity of the porous transducer was evaluated using the optimized experimental conditions. Different *Staphylococcus aureus* and *Escherichia coli* spiked samples (10^{1}, 10^{2}, 10^{3}, 10^{4}, and 10^{5} CFU mL^{-1}) were mixed with anti-bacterial target bio probes (antibodies or aptamers), passed through a 0.22 µm pore-size polyvinylidene fluoride filter (Millex) syringe filter and applied on the selective pSi scaffold. Note: conventional microbiological plate assay verified the different *Staphylococcus aureus* and *Escherichia coli* concentrations. Figure 2 depicts the relative intensity responses of the surfaces toward different filtrated solutions that vary in their unreacted bioprobes content. The optical responses gradually increase with the elevated bacterial load as fewer free antibodies/aptamers could interact with the pSi and thus inhibit the overall SERS intensity. The corresponding values were used to calibrate the optical responses of the platforms while depicting detection limits of 3 and 1 CFU mL^{-1} for *Escherichia coli* and *Staphylococcus aureus*, respectively.
We further confirmed the selectivity of the assay by testing it against other bacterial strains (*e.g., Staphylococcus epidermidis, Bacillus subtilis, Salmonella enterica Serovar Typhimurium* and *Listeria monocytogenes*), demonstrating its ability to specifically target the intended pathogens without significant cross-reactivity with other common interfering pathogens.

To ensure the practicality of the assay, we studied its shelf-life span, which was found to be stable for up to 8 weeks of storage time. Finally, we assessed the applicability of the developed assay using real food and water samples, including fish, tahini, groundwater, and milk samples. The milk samples were spiked with bacterial cells from various dairy livestock sources, such as bovine, goat, and sheep, as well as commercial pasteurized milk. The results were evaluated and compared to the standard plate counting method, showing a recovery range of 92% to 111%.

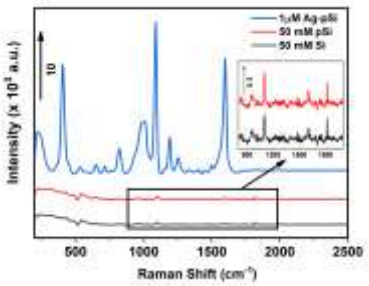

Figure 1. The SERS and Raman spectra of 4ATP molecules measured on the developed Ag-pSi SERS plat-form (acquired for 3 s) along with plain pSi and bulk Si (50 mM of 4ATP each, acquired for 30 s), respectively.

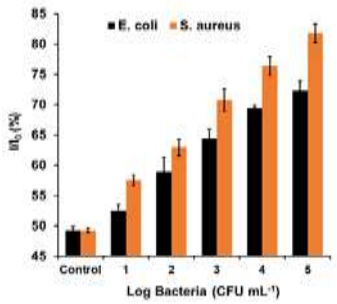

Figure 2. Optical responses toward different Escherichia coli and Staphylococcus aureus concentrations. I/I_0 was recorded at 1598 cm^{-1}. Data are reported as mean ± SD (n = 3).

Conclusions

In summary, a portable sensing approach was developed for rapid and reliable detection of *Escherichia coli* and *Staphylococcus aureus* contaminants in various food samples. The indirect assay was coupled to a highly efficient SERS-based substrate to evaluate minute bacterial content through optical signal inhibition. The presented sensing concept showed several profound advantages for field analysis, such as the separation process to exclude microorganisms from the complex media using the syringe filter and hence minimize the interference with the Ag-pSi surface, which is a rapid, straightforward and cost-effective method. Moreover, no enrichment process (to augment the bacterial score) was involved in the pre-treatment procedure, which simplified the execution and practicality of the presented approach. Finally, the presented proof-of-concept can be easily adapted for detecting other potent microorganisms in most complex media without hindering its spectral resolution.

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