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Rapid single-cell detection and identification of bacteria by using surface-enhanced Raman spectroscopy

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Abstract

Recently, the possibility of developing surface-enhanced Raman scattering (SERS)-based biosensors for rapid detection of bacteria is widely explored. With this purpose, we used SERS spectroscopy along with chemometric techniques to detect and identify by their spectral profiles relevant pathogens grown in different cultivation conditions by using *in situ* synthesized silver colloid (Bacteria@AgNPs) and incubation in silver colloid [1, 2]. Enhanced darkfield hyperspectral microscopy analysis was employed for characterizing the interaction between the bacteria and silver nanoparticles (Bacteria@AgNPs system). Moreover, a label-free SERS-based protocol was optimized and the influence of taxonomic affiliation and time-dependent effects of incubation in silver colloid were monitored.

By using SERS-based protocol with the optimized experimental parameters, the label-free detection and identification of the most common pathogens (*E. coli*, *Aeromonas*, *M. morganii*, *E. lactis, L. casei* and *L. monocytogenes*) was assessed. The reduced sample volume required, the rapid spectral acquisition (within 5 minutes), and the use of chemometric techniques for an unbiased analysis of the SERS single-cell spectra, provided the optimum platform for developing SERS-based biosensors for food safety, water research, or health care real-life applications.

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1. Main text

In this work, surface-enhanced Raman scattering (SERS) was employed for the identification and discrimination of pathogenic bacteria, based on their specific SERS fingerprint recorded at single-cell level. Several genera of both Gram-negative (*E. coli*, *Aeromonas* and *M. morganii*) and Gram-positive (*E. lactis L. casei* and *L. monocytogenes*) bacteria that are found in most of the isolated infections in bacteraemia were successfully identified in less than 5 minutes without using labels, antibodies or other specific receptors. The SERS direct detection platform is unique by considering the facile, ready-to-use SERS substrate, produced with low costs, with a high enhancement capability, which enables single-cell detection. The innovative approach of detection is based on the *in situ* synthesis of silver nanoparticles (AgNPs), generated in direct contact with the bacterial membrane. We have previously clarified that by inducing a positive charge on the glass slide surface, Gram-negative bacteria were successfully immobilized, without the need of a specific receptor [3]. However, this immobilization procedure based on electrostatic forces did not work when dealing with Gram-positive bacteria. Therefore, in this study, microscope adhesion slides with permanent positive charge were used, providing a more efficient bacterial cell adhesion, therefore enhancing the single-cell events for SERS investigation.

Nomenclature

SERS	surface-enhanced Raman scattering
NPs	nanoparticles

1.1. State of the art

Biosensors with integrated nanotechnology address the analytical needs crucial in practical pathogen diagnosis by superseding the conventional culture-based assays due to clear advantages like high throughput, single-cell detection, reduced sample volume required and the possibility of label-free, fast detection [4, 5] with ultra-sensitivity and multiplex capability [6]. There is still significant effort directed towards the development of SERS-based biosensors for pathogenic microorganisms' detection. The most relevant uropathogens were detected by means of label-free SERS-based detection and classification at strain level by using microarray immobilization of single bacterial cells coupled with *in situ* AgNPs synthesis and PCA data discrimination [1, 3, 7] for Gram-negative bacteria, and SERS mapping by using silver dendrites [8] both for Gram-negative and –positive bacteria. The label-free SERS-based detection by incubating the bacteria with AgNPs [2] was also recently tested on *E. coli*. For the Gram-positive bacteria, the protocol needed improvement as reported by Wang et al. [8] as well. They used silver dendrites for SERS detection and the results obtained were not promising for Gram-positive bacteria, probably due to their different membrane structure, which features less outside proteins.

Despite of these constant practical challenges, the use of *in situ* synthesized Ag colloids for bacteria detection was demonstrated in several assays only in our group [1, 3, 7, 9, 10]. Recently, a label-free NIR-SERS detection and discrimination of bacteria after pre-treatment of bacterial cell membrane with disrupting agents was presented, with a measuring time of less than 5 min [11]. Latest works have tested the applicability of the *in situ* synthesized Ag colloids in environmental research, for instance, for the detection of bacteria in plant roots [12] and for pesticide monitoring in edible leaves [13]. These results demonstrate the applicability of SERS-based non-invasive detection approaches for identification of pathogens and detection of their secreted metabolites, if required. The *in situ* synthesis of Ag colloid ensures the structural integrity of the coated bacterial cells and helps through a rapid generation of hotspots at the membrane surface to acquire unique whole-body spectral signatures with high sensitivity and specificity.