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Surface Enhanced Raman Spectrosocpy (SERS) – an Alternative Tool for the Identification of Pathogenic Bacteria

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Rapid detection and differentiation of pathogenic bacteria has become an increasingly important task in the pharmaceutical industry, medical and veterinary diagnosis, water and food safety, and food-processing. In order to achieve this objective, alternative diagnostic methods like Raman spectroscopy are becoming attractive for analysis of chemical and biological components (Tu & Chang, 2012; Cheng *et al*., 2013). Conventional pathogen identification methods based on biochemical tests are generally laborious and time-consuming (Lazcka *et al*., 2007). These tests require a pure, isolated bacterial culture and involves a combination of various methods in order to accurately identify the bacteria, and the results occasionally take several days to be available (Walker *et al*., 1990; Oliveira *et al*., 2012). These limitations can influence medical diagnosis especially in emergency cases, when immediate action should be taken. Meanwhile microbial identification is still in process, and broad spectrum antibiotics are usually administered empirically in the patient. The consequences of this practice are the undesirable antimicrobial resistance, which often delay and disturb the resolution of the infection (Andersson & Hughes, 2010; Oliveira *et al.*, 2012).

Advanced methods employing molecular biology techniques and immunological detection generate results quicker than culture-based methods (Swaminathan & Feng, 1994; Lazcka *et al*.,2007; Wu *et al.,* 2013). On the other hand, they are expensive, often limited in accuracy, specificity and sensitivity (Siegel *et al*., 2012), and there are significant circumstances restricting their uses. The polymerase chain reaction (PCR) is a molecular biology technique that uses the process of nucleic acid amplification; however, this process it is unable to determine the difference from viable and nonviable microbes (Wu *et al.,* 2013). In addition, PCR requires considerable laboratory equipment, highly qualified personnel (Wu *et al.,* 2013), prior knowledge of bacteria genomes (Beier *et al*., 2012), species-specific probes (Cherkaoui, 2010; Münchberg *et al.,* 2014) and it still depends on a culturing step for samples with pathogen concentrations below 10^4 colony forming units per milliliter (CFU ml^{-1}) (Gilmartin and O'Kennedy, 2012; Drake *et al*., 2013). Immunological detection such as enzyme-linked immunosorbent assay (ELISA), is specific to microbial type and strain. Nevertheless, it also requires multiple steps, diverse chemical reagents, and incubation time

which makes this method unsuitable to satisfy today's real time detection requirements. (Ivnitski *et al*.,1999; Wu *et al.*, 2013).

The extensive demand for a method that can deliver fast pathogenic bacteria detection without the necessity of enrichment or culturing steps, and a detection limit in the 1–100 CFU ml^{-1} range (Gilmartin and O'Kennedy, 2012; Drake *et al.*, 2013) leads to alternative approaches to quickly analyze pathogens. One of these approaches are the vibrational spectroscopic techniques like Raman scattering spectroscopy. Raman spectroscopy has the potential to detect a broad range of biological and chemical substances (Efrima and Zeiri, 2009), and reveal the molecular composition of a sample at the micrometer scale in a nondestructive and noninvasive way (Vandenabeele, 2010).

In 1928, the Indian scientist Sir Chandrasekhara Venkata Raman demonstrated experimentally the Raman effect for the first time after it had already been predicted by Adolf Gustav Stephan Smekal. At the time, Raman worked with filtered daylight, and a few hours of irradiation were required to enable to record the spectrum of a huge volume of a pure liquid. Spectra were recorded on photographic plates. Thanks to instrumental improvements, now it is possible to record the spectrum of a micrometer-sized sample in a couple of seconds (Vandenabeele, 2010).

"Raman scattering is a basic property of the interaction of light with molecules. When light hits a molecule it can cause the atoms of the molecule to vibrate. This vibration will then change the energy of additional light hitting the molecule. This additional scattered light has characteristics that are measurable and are unique to the structure of the molecule that was caused to vibrate" (Siegel *et al.,* 2012). A molecular vibrational spectrum of biological samples comes from a superposition of the vibrational fingerprint of their molecular components such as DNA, proteins, lipids, and many other components of the cell (Münchberg, 2014), and those unique "fingerprint" peaks can be used to discriminate particular molecules being investigated. Nevertheless, Raman spectroscopy relies on the inelastic scattering interaction of excitation light and vibrational modes of molecular bonds (Wu *et al.,* 2013), and the spectrum of biological samples themselves are usually very weak (Harz *et al*., 2009), and it lacks in specificity and sensitivity for the detection of biological organisms and components (Siegel *et al.,* 2012).

Roughened metallic nano particles have been utilized to improve the Raman signal in Surface Enhanced Raman Spectroscopy (SERS) via surface plasmon resonance (SPR) (Campion & Kambhampati, 1998). SERS is a highly sensitive Raman detection technique based on metallic nano structured substrates (Wu, 2013), which increases the intensity of the Raman signal on the order of 10^4-10^6, and can be as high as 10^8 and 10^{14} for some systems (Moskovits, 1985; Kneipp, 1999), and has been utilized to identify trace levels of molecules or even a single molecule (Zhou et al., 2006). Fleischmann *et al.* first

discovered SERS in 1974. Intense Raman signals from pyridine absorbed on roughened silver electrodes in aqueous solutions were recorded by the authors. After that, several studies determined that conventional Raman spectroscopy could not assess these intense signals and indicated that the enhancement method using a metal surface would improve the scattering intensity from each adsorbed pyridine molecule (Albrecht & Creighton, 1977; Drake *et al.,* 2013).

Several forms of nano structures have been developed and used to enhance SERS bacterial spectra including silver metal deposits, silver colloid, and gold colloid solutions (Wu, 2013). The considerable improvement observed in the SERS technique has been correlated to two factors: electromagnetic (physical) enhancement, and chemical enhancement (Drake, 2013). The electromagnetic effect is dominant (Kambhampati, 1998), and it is a result from the localized surface plasmon resonance (SPR) combined with the metallic nano particles, which considerably enhances the electromagnetic field at the surface of the nano particles (Weaver *et al.,* 1978). On the other hand, the chemical effect arise from the changes to the adsorbate electronic states that leads to the component's chemisorption (Weaver, 2000), and its effect contributes only on one or two orders of magnitude for the enhancement (Kambhampati, 1998). The structural and molecular identification power of SERS can be used for numerous interfacial systems, including electrochemical, model and actual biological systems, catalytic, *in situ* and ambient analyses and other adsorbate-surface interactions (Weaver, 2000). Due to the sensitivity of SERS, detection of trace molecules can be accomplished as well (Kneipp, 1999).

In summary, rapid detection of bacteria is essential for many areas, such as food and pharmaceutical industry, and biomedical applications. Current methods to identify a microorganism in a sample based on either microbiological methods such as polymerase chain reaction (PCR) or biochemical techniques are time consuming, expensive and requires species-specific probes, and lower sensitivity. An alternative method of microorganism identification is Surface Enhanced Raman Spectroscopy (SERS), a sensitive, rapid, simple and straightforward method. SERS is also less expensive than the other regular methods because it does not require invasive procedures or other reagents. Furthermore, it can detect multiple microorganisms at once and has potential to identify a single cell.

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