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Label-free SERS in biological and biomedical applications: Recent progress, current challenges and opportunities

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ABSTRACT

To achieve an insightful look within biomolecular processes on the cellular level, the development of diseases as well as the reliable detection of metabolites and pathogens, a modern analytical tool is needed that is highly sensitive, molecular-specific and exhibits fast detection. Surface-enhanced Raman spectroscopy (SERS) is known to meet these requirements and, within this review article, the recent progress of label-free SERS in biological and biomedical applications is summarized and discussed. This includes the detection of biomolecules such as metabolites, nucleic acids and proteins. Further, the characterization and identification of microorganisms has been achieved by label-free SERS-based approaches. Eukaryotic cells can be characterized by SERS in order to gain information about the outer cell wall or to detect intracellular molecules and metabolites. The potential of SERS for medically relevant detection schemes is emphasized by the label-free detection of tissue, the investigation of body fluids as well as applications for therapeutic and illicit drug monitoring. The review article is concluded with an evaluation of the recent progress and current challenges in order to highlight the direction of label-free SERS in the future.

1. Introduction

Access to information on the molecular level is crucial for the development of new strategies and ideas to prevent, diagnose and treat diseases. For example, the early detection of amyloid beta aggregates [1], one of the biomarkers of Alzheimer's disease, can increase the treatment success rate. The correct identification of pathogens causing an infection assures the proper selection of antibiotic treatment [2]. Insight into cellular apoptosis can offer information on cancer biology, immune response and pathogenesis [3]. The aim of the scientific community has always been directed toward developing reliable, fast and accessible analytical methods that can address different aspects of biology and medicine. Biological assays, polymerase chain reaction, chromatography, microscopy and immunoassays are just some of the methods implemented in clinical laboratories. A newly emerging technique that offers high specificity, and sensitivity and improved time to result is surface-enhanced Raman spectroscopy (SERS) [4–7]. The large number of reports describing its potential for drug monitoring [8–13], cancer diagnosis [14–17], pathogen identification [18–22] or analysis of cellular mechanisms [23–25] clearly illustrates the capabilities of the method.

SERS combines molecular information with the plasmonic properties of metallic nanostructures [26–29]. The Raman effect, based on inelastic light scattering, is inherently weak. Only one out of one million incident photons is in-elastically scattered and when successfully detected, it can contribute to the Raman spectrum. Generally, at the early stage of a disease, biomarker amounts with prognosis power are at low concentrations. Screening tests have to be very sensitive in order to give a reliable result. When these biomarkers are brought into the proximity of metallic nanostructures and a laser with a given wavelength is used as an electromagnetic source, enhanced Raman scattering can be observed [26,30]. Coinage metals shaped into spheres, rods, triangles, cubes, bow-ties or structured surfaces have been widely used as SERS active substrates [31–33]. The key parameter defining the magnitude of the Raman signal enhancement is the dielectric function of the metal and of the surrounding medium. In the case of spherical nanoparticles, a so-called plasmon resonance condition is fulfilled when the relation $\varepsilon(\lambda) = -2\varepsilon_m$ is met (here $\varepsilon(\lambda)$ is the wavelength-dependent dielectric function of the nanoparticle, while ε_m is the dielectric constant of the surrounding medium). The induced dipolar localized surface plasmon polaritons (LSPPs) give rise to high local field

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enhancements, and the sensitivity of Raman spectroscopy is improved by several orders of magnitude (10^6–10^8).

SERS-based strategies can be classified as label-free and label-mediated SERS approaches. For the latter case, the detection of a biomarker is performed indirectly. The SERS active substrate is functionalized with a label or a tag, also referred to as a reporter molecule, and the SERS signal of this molecule is detected. In this way, the specificity for a given substance is considerably increased. SERS-based immunoassays [34–36] are very successful in detecting a large variety of biomolecules in simplex and multiplex platforms. However, when the focus is on the direct detection of biomarkers, SERS active substrates free of any molecular species that could give rise to their own Raman signal are required.

Label-free SERS approaches can provide, among other features, information on protein structure [1,37,38], nutrient amounts present in food [39,40], and the identity of pathogens present in clinical samples [18–22] or biological processes taking place at the cellular level [23–25]. In the present review, the focus lies on giving an overview of the advances in the field of label-free SERS approaches reported during the last five years. Promising studies regarding the molecular detection in simple systems, such as low-molecular weight substances, nucleic acids and proteins, or in complex environments, cells and tissue section, are discussed, as well as the investigation of biological fluids and the potential of label-free SERS for the detection of therapeutic and illicit drugs.

2. Biomolecules

The detection and analysis of biomolecules and pathogens by means of molecular specific and highly sensitive SERS approaches is widely represented in the literature and well addressed by recently published review articles [41–45]. The term 'biomolecule' is defined as a molecule that is produced by a living organism, including the low-molecular weight substances referred as building blocks of life such as nucleotides, amino acids, lipids, monosaccharides, vitamins, metabolites and semiochemicals. Their atomic components are carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur. Moreover, macromolecules formed out of those monomers are biopolymers with high biological relevance, e.g., nucleic acids, proteins and carbohydrates. The detection of these biomolecules will be discussed within the following sections based on various label-free SERS approaches.

2.1. Low-molecular Weight Biomolecules

The molecule riboflavin (vitamin B2) is a vitamin found in food that is essential for human body functions. The detection of riboflavin can be based on a combination of SERS with an electrochemical detection scheme [39]. Here, a SERS-active electrode is embedded in a microfluidic environment, allowing for the characterization of the analyte molecule in the flow by using an injection volume of 100 nL. The profiles of the detected SERS spectra for various concentrations are changing, which is attributed to a reorientation of the molecule toward the metallic surface due to the applied potential of the SERS electrode. The detection of riboflavin and cobalamin (vitamin B12) was further pursued in the presence of a complex food matrix containing different monosaccharides and starch employing silver-coated nanostructured quartz surfaces as SERS substrates [40]. Here, the molecules could be differentiated based on the Raman marker bands, and qualitative detection is achieved for both molecules simultaneously in a cereal product following a food extraction protocol. The potential of the same SERS substrates for the detection of biomolecules was further demonstrated by the detection of lycopene and β-carotene [46]. In this study, it was shown, that both molecules possess a similar SERS response and their differentiation in a real food matrix, i.e., extracts from tomato samples, was successfully performed via a chemometric analysis. The results for the determination of lycopene and β-carotene were in good agreement with HPLC analysis.

The nucleoside adenosine was characterized by SERS [47]. Therefore, silver nanostructures were prepared within a microfluidic channel system to ensure controllable measurement conditions. Concentration-dependent SERS measurements down to a μM level were achieved. To identify 20 proteinogenic L-amino acids by employing SERS, simultaneous detection is complicated due to the similar molecular structures causing comparable Raman fingerprint information [48]. The Raman spectra of the investigated amino acids are dominated by the contributions of amine, carboxyl and side chain moieties. Thus, a separation technique combined with SERS is required. The authors developed a SERS measurement procedure based on the application of capillary zone electrophoresis (CZE) as a separation approach. It was shown that the combination of CZE with SERS allows for the separation and identification of all 20 L-amino acids in a mixture. The reproducibility of this CZE/SERS approach is related to the excellent behavior of the applied SERS substrate. Finally, the same approach was applied for the online detection and characterization of eight biologically active peptides [49]. The differentiation and identification was enabled by the characteristic SERS spectral profiles of the investigated peptides due to aromatic and sulfur-containing moieties as well as the vibrational modes of the side chains. This result was confirmed by a hierarchical cluster analysis, achieving 100% accuracy.

To achieve a deeper understanding of processes within the brain, the determination of the level of dopamine, the key neurotransmitter within the dopaminergic neural system, is of high medical importance. A SERS-based method to detect dopamine in artificial cerebrospinal fluid as well as in mouse striatum was introduced [50]. The surface of a magnetic Fe_3O_4/Ag nanocomposite was equipped with the dopamine selective recognition molecule iron nitriloacetic acid, allowing for the specific enrichment of dopamine and the separation from a complex matrix. For data processing, the marker mode at 1046 cm^{-1}, assigned to the C—N vibration of dopamine, was used, and the quantification of the analyte molecule was achieved by means of standard addition. As a gold standard, HPLC-MS was applied. Although a higher standard deviation was found when employing the HPLC-MS-based analysis requires time-consuming preparation protocols, which are not needed for the SERS-based detection scheme. Thus, SERS is a perfect analytical tool in bioanalytical detection schemes where the analysis time matters and only time-consuming HPLC-based approaches are available as the gold standard.

Furthermore, the metabolite glycerophosphoinositol has been studied; this molecule, a component of the cell cytosol, is associated with the oncogenic transformation of the Ras genes in epithelial cells [52]. The gold standard is radio labeling combined with HPLC-MS; thus, a less laborious method with molecular specificity and excellent sensitivity was investigated. By means of SERS, spectra of glycerophosphoinositol were recorded, and the Raman modes were assigned, taking the two molecular subunits *myo*-inositol and glycerol bridged via a phosphate group into account. Finally, mixed samples containing glycerophosphoinositol, *myo*-inositol and glycerol with varying concentrations were prepared, and the concentrations were predicted employing the partial least-squares (PLS) method. Pyocyanin, a metabolite specific for *Pseudomonas aeruginosa*, was investigated by means of SERS, and detection in the range of 10 μM was achieved using saliva as a complex matrix [53]. Further, the SERS-based detection of two metabolites, i.e., pyocyanin and violacein, specifically produced by *Pseudomonas aeruginosa* and *Chromobacterium violaceum*, was demonstrated by the application of SERS-active nanostructures embedded in agar [51]. Here, as a result of interspecies chemical interactions, both metabolites are identified based on their specific Raman fingerprint signals. The authors showed the spatial distribution for both metabolite molecules (see Fig. 1) in a co- and monoculture and confirmed the expression of violacein as well as the reduced expression of pyocyanin under coculture conditions. Thus, the proposed SERS-based detection scheme allows the in situ

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