SERS active Ag/silicon based nanostructures for biosensing applications

Abstract

Surface Enhanced Raman Scattering (SERS) has recently been proven to be a powerful tool for analytical and bioanalytical applications. Thanks to the huge signal enhancement provided by the resonant excitation of surface plasmons localized on the nanostructures it allows to investigate trace levels of analytes, such as important biomarkers. Especially concerning solid SERS platforms, the performances of the substrates can be finely tuned by varying the size, shape, and spatial arrangement of the nanoparticles. In this work, porous silicon (pSi) membranes supported on polydimethylsiloxane (PDMS) substrates decorated with silver nanoparticles are exploited. Such substrates show intense Raman enhancement and good uniformity of the SERS signal intensity, coupled with their cheapness and ease of fabrication. A first goal of this thesis was to determine the effect of the synthesis conditions onto the morphological and optical properties of the pSi-based substrates. To this aim three synthesis parameters such as temperature, silver precursor concentration, and flow rate were chosen and varied. In order to control the influence of these parameters on the substrates morphology, linked to their SERS efficiency, a microfluidic approach was used, by hosting the pSi-PDMS membrane in a microfluidic cell, in which a silver nitrate solution was injected. Each synthesis parameter was then varied in order to understand its effect onto the optical and morphological characteristics of the obtained sample. The evolution of the localized surface plasmon resonance (LSPR) was exploited to control the synthesis of the nanoparticles and it was monitored on-line thanks to UV-Vis transmittance spectroscopy. A careful morphological characterization was carried out taking advantage of a MATLAB routine for the image analysis of the FESEM micrographs of the synthesized samples. Finally, the SERS efficiency and uniformity of each substrates was tested using 4-MBA acid as molecular probe. Thanks to such experimental protocol it was possible to determine the effect of each of the three selected variables on the reaction outcome, yielding the potential relationship among them. Temperature was proven to be the most influencing parameter, since a strong increment of the reaction rate as well as the production of bigger nanoparticle was observed as the temperature was raised. The obtained samples also showed a broader distribution of the silver particle size. A similar, even though less marked result was obtained by raising the Ag precursor concentration, leading to the production of bigger particles. At last, the flow rate variation was proven to be the less influent variable, as its effect was often overwhelmed by the temperature and concentration raise. It is worth to notice that, compared to the other synthesis parameters, an opposite dependence was observed, as a flow rate increment led to the fabrication of smaller and more homogenous nanoparticles. These differences, concerning the samples morphology can influence their SERS efficiency. Indeed, lower enhancement and higher variability of the SERS signal were observed for the samples exhibiting less homogeneous nanoparticles distributions.

Once depicted the influence of each synthesis parameter on the fabrication of the pSi-based substrates, the focus was shifted towards their biological applications. To this aim the pSi/PDMS substrates (PSD) produced by immersion plating were used, in order to exploit their greater stability and reproducibility of the SERS signal. These substrates were employed in different biological fields of application: the selective detection of miRNAs, and in particular miR222, and the discrimination of different bacterial strains, namely *Escherichia coli* and *Staphylococcus epidermidis*.

A brief characterization of the developed PSD substrates coated with Ag nanoparticles synthetized by immersion plating was performed. FESEM images were first acquired to depict the morphological properties of the samples and afterwards their SERS efficiency and uniformity were evaluated using Rhodamine 6G (R6G) and 4-mercaptobenzoic acid (4-MBA).

Concerning the miRNAs detection, two assays were designed exploiting the hybridization of the target miRNA with a complementary DNA probe. The first protocol concerned the functionalization of the NPs surface with a thiol-capped probe enabling the selective recognition of the target miRNA labelled with a Raman reporter. On the other side, the second assay concerned the split of the probe in two halves, enabling a label-free detection of the target. Indeed, the first half was immobilized on the surface allowing to capture the target miRNA, whereas the second half, which was labelled with a Raman reporter, enabled the SERS detection. Each step of the bioassays was carefully optimized to maximize the amount of hybridized molecules, and different Raman labels, namely Cyanine 5 (Cy5), Cyanine 3 (Cy3) and R6G, were employed to investigate the effect of the potential electronic resonance and of the emitted fluorescence by specific dyes. In fact, using a laser line at 514 nm, Cy3 and R6G resonant Raman conditions can be exploited, while Cy5 exhibits a lower fluorescence background. Moreover, the position of the Raman reporter along the probe was studied, in order to substantially lower the detection limits. Finally, the two-step assay was exploited to determine the amount of miR222 into cellular extracts, in order to avoid complex and expensive chemical processes to label the microRNA.

Concerning the discrimination of different bacterial strains, a simple yet reliable protocol was designed to enable an *in-situ* label-free analysis, minimally affecting the bacterial environment and their metabolism. *E coli* and *S epidermidis* were selected as representative strains of Gram-negative and Gram-positive bacteria, respectively. The strains used for the experiments were not pathogenic, reducing any risk for the operator. The investigated bacteria species show several differences concerning both the cell wall architecture and the composition of the extracellular matrix during the evolution over time of the bacteria population. Such characteristics are clearly recognizable in the SERS spectra of the different bacteria. A complete characterization of the obtained spectra was carried out allowing to determine the contribution of each vibrational feature. Moreover, the growth of the bacteria population over time as well as the evolution of the extracellular matrix was taken into account, collecting samples after 12, 24, and 48 hours. Selected vibrational features related to the principal molecular components of living bacteria, such as proteins, polysaccharides, DNA and phospholipids, were monitored. An increase in the intensity of these bands was observed over the first 24 hours, while a net decrease was detected after 48 hours of culturing, in accordance with the standard bacterial growth curve. To further corroborate such results, fluorescence images of the bacterial cultures were collected and analyzed.

In this work the potentialities of the pSi-based SERS substrates were exploited in different bioanalytical fields of application, thanks to the great efficiency and reliability of these platforms. Such characteristics allowed the development of an assay for the label-free detection of miR222, whose application have been extended to real biological samples, as well as the *in-situ* discrimination of different bacterial strains, highlighting the flexibility of developed nanostructures