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SERS-active metal-dielectric nanostructures integrated in microfluidic devices for ultra-sensitive label-free miRNA detection

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Abstract

In this work, silver decorated porous silicon membranes integrated in a polydimethylsiloxane multi-chamber microfluidic chip were functionalized with DNA-probes and used for the detection of miRNA by Surface-enhanced Raman Scattering analysis. An innovative biological protocol has been designed: the probe was divided in two short pieces that interact before and after the miRNA incubation. The optofluidic biosensor was applied for the label-free detection of miRNA sequences at *in vivo* concentrations.

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1. Introduction

Surface-enhanced Raman Scattering (SERS) plays an important role in the high-sensitive label-free detection of biomolecules. Nanostructured materials based on semiconductors and metal-oxides decorated by noble metal nanoparticles have been recently developed as SERS-active substrates [1,2].

In this work SERS is applied for the detection of miRNA, which consist of short regulatory sequences usually over- or under- expressed in connection with a particular disease (e.g. oncogenesis) [3]. In detail, new ultra-sensitive metal-dielectric substrates made of silver decorated porous silicon (pSi) membranes were functionalized and used to detect different miRNA sequences in a polydimethylsiloxane (PDMS) multichamber microfluidic chip.

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2. Experimental

The synthesis of the PDMS-supported Ag-pSi membranes (PSD) and of the PDMS multichamber microfluidic chip is reported elsewhere [2]. A protocol for miRNA detection performed by enzyme-linked immunosorbent assay (ELISA) was set-up on commercial plates by the overnight immobilization of a 5'-alkylthiol-capped DNA probe (25 μ M, in TE, 1 M NaCl, pH 7.5) corresponding to the antisense sequence of the miRNA222. The hybridization (22 $^{\circ}$ C, 60 min) was performed in SSC4x, 0.1% SDS, pH 7.5. Streptavidin-HRP was incubated (22 $^{\circ}$ C, 60 min) to detect biotin and a TMB substrate solution was used for the colorimetric reaction. Subsequently, the biological assay was transferred to the substrates used for SERS analysis with excitation at 514.5 nm.

3. Results and Discussion

The protocol developed in commercial ELISA plates was adapted to the PSD substrates and to the optofluidic biosensor (Fig.1a) in two operative approaches. In the first, the hybridization was assessed by incubation of the corresponding miRNA sequence. (Fig.1b). Due to the poor sensitivity of this method, the probe was divided in two pieces (half1 and half2) that interact before and after the miRNA incubation, to allow the label-free miRNA profiling in two hybridization steps (Fig.1c). The half2 was modified with biotin or with a Raman label (cy3). Afterwards, the ELISA and the SERS analysis (Fig.1d-e) were used to assess the hybridization between the grafted probe and the miRNA, allowing its label-free detection in a physiological range of concentrations (0.1-50 nM).

