Oligonucleotides detection by SERS analysis for biodiagnostic applications

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Surface-Enhanced Raman Scattering (SERS) spectroscopy plays an important role in materials science, biophysics, medical diagnostics, and molecular biology for high-sensitive label-free detection. In particular, SERS have been widely applied to biological fields for the detection of different biomolecules such as peptides [1] whole proteins, DNA, and even cells [2].

Detection of micro-RNAs (miRNAs), small, non-coding RNAs, by 24-nucleotide single-stranded sequences, is of great relevance in gene regulation affecting essential processes such as cell proliferation, cell death, tumor genesis, and mammalian cell development.

Herein, we present a protocol aimed to the immobilization of thiolated cDNA oligonucleotides (~22 bp) on plasmatic metal-decorated nanomaterials consisting of silver nanoparticles (AgNPs) sticked on porous silicon substrates (pSi). These sensing platforms were successfully tested as efficient SERS substrates for the detection of other biomolecules so that they could be considered promising tools for miRNA sequences hybridization with the respective immobilized complementary cDNA probes.

**Experimental: synthesis of Ag NPs sticked to the pSi surface**

**Biological Assay designed and optimized for solid SERS substrates**

The aim of our protocol is the immobilization of thiolated cDNA oligonucleotides (~22bp) (5′-Cy5GATTTCGATCGATGATGTACG-3′) by chemical binding between the thiol group (-SH) and the Ag nanoparticles, resulting in a self-assembled monolayer (SAM).

**Selected protocol for SERS analysis**

1. AgNPs substrates pre-wet in TE-leaver, pH 7.5 (5 min).
2. cDNA probes reduction in DTT and pre-treatment at 95 °C for 2 min to promote oligo uncoupling, followed by rapid cooling (30 sec in ice bath) [9].
3. Simultaneous DN incubation (co-immobilization) at room temperature of cDNA-SH probes 15 μl in TE-leaver with biotin as [8].
4. Wash (3x5 min) in TE-leaver 0.5 X, pH 7.5 to remove unspecific binding.
5. AgNPs substrates pre-wet in the SSC buffer, pH 7.5 (15 min).
6. miRNA pre-treatment at 95 °C for 2 min to promote oligo uncoupling, followed by rapid cooling (30 sec in ice bath) [9].
7. Incubation of miRNA sequences (15 μM in SSC 1X) at 65 °C for 5 min;
8. Cool down at room temperature (30 min);
9. Wash (3x5 min) in SSC 1X pH 7.5 to remove unspecific binding;
10. Rinse in ddH2O (5 min) before SERS analysis.

**SEM characterization: compatibility of our substrates with the experimental parameters of the biological protocol**

**SERS analysis**

Preliminary detection of cDNA-mRNA hybridization on AgNPs/pSi

Reproducibility test on different AgNPs/pSi treated with the same protocol

**Conclusions**

- SERS-active substrates were synthesized on macroporous silicon by immersion plating to obtain Ag NPs/pSi sensing surfaces suitable for cDNA-immobilization;
- A biological protocol to study the interaction between cDNA probes and complementary mRNA sequences was developed on AgNPs/pSi substrates;
- By means of binding agent/spacers and cDNA co-immobilization promising results have been obtained during preliminary analysis, although further investigations are required.