Surface enhanced raman scattering active chips for mycotoxin detection in food matrices

SURFACE ENHANCED RAMAN SCATTERING ACTIVE CHIPS FOR MYCOTOXIN DETECTION IN FOOD MATRICES

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ABSTRACT

Surface-enhanced Raman Scattering (SERS) is an excellent analytical tool that can be used to develop assays and biosensors for the sensitive detection of small molecules in complex samples, taking advantage of plasmonic nanostructures. In this work, an aptamer was exploited to develop a competitive assay for Aflatoxin B1 (AFB1) by means of SERS analysis. After the careful optimization of the SERS-based assay, its potentiality for food safety assessment was demonstrated by detecting AFB1 in different extracts of AFB1-spiked slurry of hazelnut below the threshold defined by the regulatory limits.

KEYWORDS: SERS, mycotoxin, food matrix, aptamer, plasmonic nanostructures, microfluidics

INTRODUCTION

The fabrication of increasingly homogenous and efficient plasmonic nanostructures for SERS allowed the spreading of this technique throughout several sectors demanding for reliable and rapid sensing devices (e.g. food safety). In particular, the detection of food toxins at low concentration in complex matrixes can benefit from the intrinsic sensitivity of the SERS technique, which can be further enhanced by its combination with aptamers, one of the most versatile biorecognition system. These artificial receptors consist in oligonucleotide sequences folded into a 3D structure, hosting a specific binding site for the target molecule, providing higher affinity and stability compared to conventional probes [1].

EXPERIMENTAL

The very high SERS efficiency and the good uniformity of silver nanoparticles (NPs) *in situ* grown on porous silicon membranes are exploited to develop a simple competitive assay for AFB1, taking advantage of its easy integration in a PDMS-based microfluidic chip (Fig. 1a) [2]. In detail, an aptamer [3] is immobilized on the surface of the Ag NPs and hybridized to a FAM-labelled "switch" sequence, complementary to the AFB1 binding site. In the presence of AFB1 the switch sequence is replaced by the target and a decrease of the SERS signal of FAM is observed, allowing the sensitive detection of the toxin (Fig. 1b).

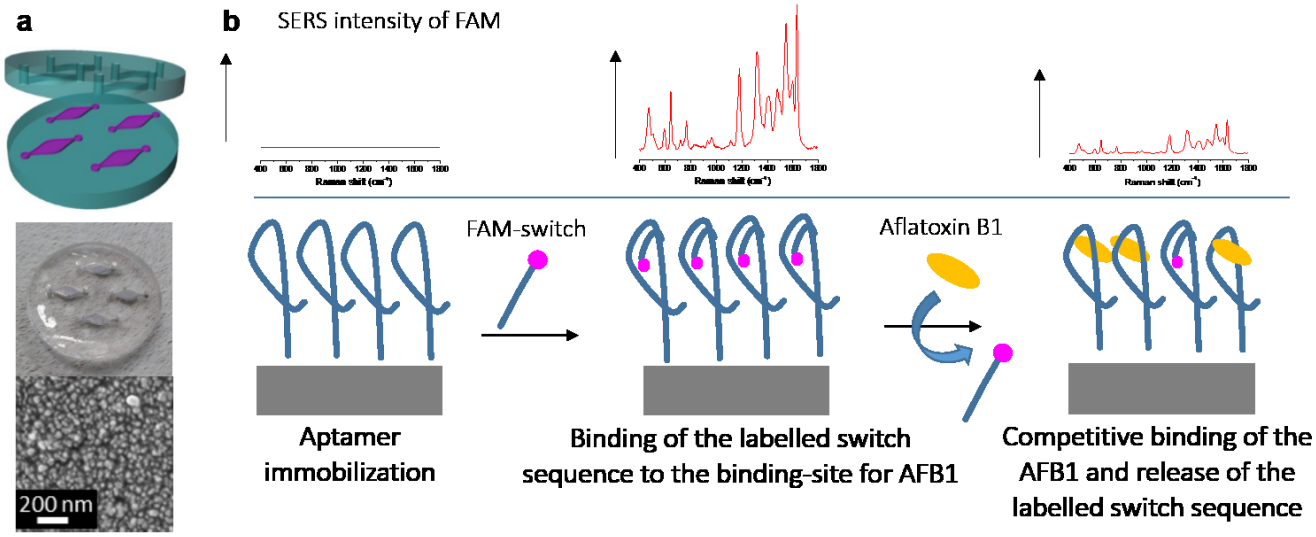

Figure 1: a) Microfluidic chip integrating porous silicon membranes decorated with silver nanoparticles; b) Scheme of the sensing principle exploited for the SERS detection of AFB1.

RESULTS AND DISCUSSION

All the steps of the functionalization protocol were carefully analyzed by SERS and optimized, starting from the immobilization of the aptamer on the plasmonic surface (Fig. 2a). Different orientations of aptamers, modified with an anchoring thiol group at the 3'/5' end, were studied using both labelled and unlabeled aptamers. A modified version of the 3' thiolated aptamer was also employed, shortening its sequence in order to boost the Raman signal of the labelled switch, which is strongly dependent on its distance from the Ag nanoparticles. Five FAM-switch sequences, differing in length (from 11 to 9 nt) and hybridization position, were then compared in order to find the best compromise between the stability of the pairing interaction and the ability to be displaced by the target.

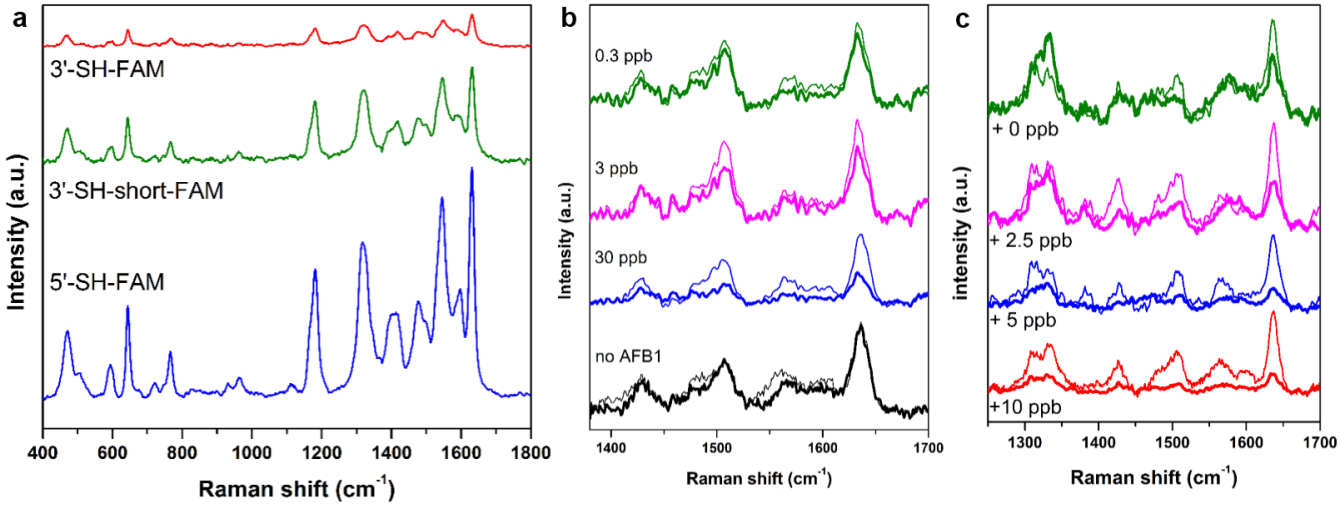

Figure 2: a) SERS analysis of immobilized FAM-labelled aptamers in the microfluidic chip; b) Average SERS spectra obtained by mapping the Ag nanoparticles at the switch hybridization step (thin line) and at end of the bioassay operated with different concentration of AFB1 in buffer (thick line); c) SERS analysis of extracts of AFB1-spiked hazelnut slurry.

The combination of SERS and ELISA analyses allowed to identify the 5' thiolated aptamer and 10 nt switch as the most performing system, which was employed to screen different concentrations of AFB1 in buffer (Fig. 2b). Finally, the possibility to detect AFB1 below the threshold defined by the regulatory limits (2 ppb for dried fruits, 8 ppb for hazelnut [4]) was proved by analyzing different extracts of AFB1-spiked slurry of hazelnut (Fig. 2c), highlighting the potentiality of SERS analysis for food safety.

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