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Extended Abstract

Detection of SARS-CoV-2 by plasmonic optical fibers and molecularly imprinted polymers †

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Abstract: A plasmonic sensor with a synthetic receptor for SARS-CoV-2 has been realized by combining a D-shaped plastic optical fiber (POF) with a specific molecularly imprinted polymer (MIP). The used MIP was properly designed for the molecular recognition of the Subunit 1 of the SARS-CoV-2 Spike protein. In order to characterize the developed optical chemosensor, preliminary tests were carried out using solutions spiked with the Subunit 1 of the SARS-CoV-2 Spike protein. Afterwards, real nasopharyngeal (NP) swabs collected in UTM (universal transport medium) and physiological solution (0.9% NaCl) were tested. The proposed POF-MIP sensor proved to effectively detect SARS-CoV-2 virions in biological samples according to the obtained results.

Keywords: Surface Plasmon Resonance; Plasmonic sensors; Plastic Optical Fiber; Molecularly Imprinted Polymers; SARS-CoV-2.

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1. Introduction

The pandemic of Coronavirus Disease 2019 (COVID-19), caused by the pathogen SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), has triggered a global public health crisis. [1]. Reference diagnostic techniques for virus determination are based on RT-PCR real time (Reverse Transcription Polymerase Chain Reaction) analysis that must be performed in an equipped laboratory using reactive and implying at least 3-4 hours, with possible further extended times in case of samples storage before analysis and/or lack of reagent. Thus, the availability of simple, low-cost, small-size, rapid and point-of-care diagnostics has become of great interest [2,3]. Surface plasmon resonance (SPR) sensors can be used to monitor specific interactions between an analyte in solution and a molecular recognition element (MRE) immobilized on the SPR sensor [4,5].

In this work, we report on an SPR-based optical fiber sensor with a specific molecularly imprinted polymer (MIP) receptor realized for the detection of SARS-CoV-2 virus in several aqueous solutions. In particular, a specific SARS-CoV-2 sensor has been developed from a plasmonic plastic optical fiber sensor [6] coupled with a synthetic MIP, able to molecularly recognize the Subunit 1 of the SARS-CoV-2 Spike protein [7].

A preliminary experimental phase has consisted in testing buffer solutions containing increasing concentrations of the Subunit 1 of the SARS-CoV-2 Spike protein, obtaining

a dose-response curve. Subsequently, tests on real samples were performed by testing nasopharyngeal (NP) swabs collected in two different media: UTM (universal transport medium) and physiological solution (0.9% NaCl) [7].

2. Materials and Methods

2.1. SPR-POF platform

The SPR platform is based on a D-shaped POF with 980 μm core of poly-methylmethacrylate (PMMA) and a 10 μm cladding of fluorinated polymer. A polishing process along half of the circumference of the POF, with 5 μm and 1 μm polishing papers, allowed to obtain the D-shaped region [6]. Then, a Microposit S1813 photoresist layer was deposited on the exposed POF core by spin-coating and a gold film was deposited by sputtering process, using a Bal-Tec SCD 500 machine to obtain a 60 nm thick film. In particular, the sputtering process was repeated three times by applying a current of 60 mA, at 0.05 mbar of pressure, for 35 s (20 nm of gold per step). The realized D-shaped sensing region was about 10 mm long [6].

2.2. MIP for SARS-CoV-2 recognition

First, a self-assembled monolayer with a terminal allyl group was formed on the gold surface of the SPR platform by incubating a 10% v / v solution of allyl thiol in 80% v / v ethanol solution and 10% v / v water for 12h. Subsequently, the platform was washed with Milli-Q water (flushing 3mL 5 times).

Then, the polymeric receptor specific for SARS-CoV-2 was synthetized by using functional monomers able to interact with the S1 subunit of SARS-CoV-2 spike protein, as explained in [7].

A small volume of 50 μL of the pre-polymeric mixture was dropped over the planar D-shaped sensing region and let polymerize for 15 min at room temperature and then the surface was washed with Milli-Q water to stop the reticulation process. The template removal was obtained by incubating trypsin 4.2×10^{-8} M for 2 h at room temperature on the sensor surface and then by washing with an SDS 5% (w/v) solution.

2.3 Experimental setup

In order to test the developed sensor a very simple and low-cost equipment was used, including a halogen lamp as white light source at the input and a spectrometer at the output. The halogen lamp (HL-2000-LL, manufactured by Ocean Optics, Dunedin, FL, USA) had an emission range from 360 nm to 1700 nm, whereas the spectrometer (FLAME-S-VIS-NIR-ES, manufactured by Ocean Optics, Dunedin, FL, USA) had a detection range from 350 nm to 1023 nm. Two SMA connectors connected the POF sensor to the light source and to the spectrometer. A software provided by Ocean Optics was used to display on the computer screen and save the transmission spectra, along with data values, setting the integration time at 1000 μs and the averaging of the scans at 150. The SPR transmission spectra were normalized to a reference spectrum, achieved with air as surrounding medium, using the Matlab software (MathWorks, Natick, MA, USA).

2.4 Experimental protocol

All experiments were carried out by dropping about 50 μl of the sample (spiked or real) over the sensing region of the D-shaped POF SPR sensor and incubating at room temperature for ten minutes to let the interaction between the MIP sites and analyte occur. At the end of this incubation, a washing step with Milli-Q water was performed and subsequently the spectrum was recorded. By adopting this protocol, only the shift of the resonance wavelength determined by the specific analyte-receptor binding was measured, eliminating shifts due to bulk changes or non-specific interactions.

Before the analysis on real samples, a preliminary test was performed by incubating buffer solutions of SARS-CoV-2 Spike S1 subunit at increasing concentrations.

Subsequently real swab samples in both UTM and physiological solution were tested. Serial dilutions performed with physiological solution were prepared for each sample and a dose-response curve was obtained by incubating from the most diluted to the whole sample [7].

3. Experimental Results

3.1. Preliminary characterization of the sensor

A preliminary characterization of the sensor was performed by testing buffer solutions of SARS-CoV-2 S1 Spike subunit at different concentration. Figure 1 shows the transmission spectra normalized to the reference spectrum (spectrum achieved with air as the surrounding medium), obtained with the different concentrations of the SARS-CoV-2 S1 subunit Spike protein.

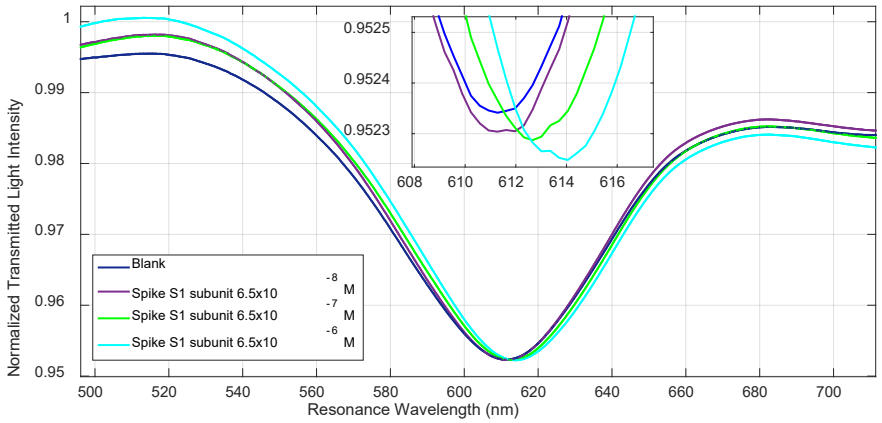


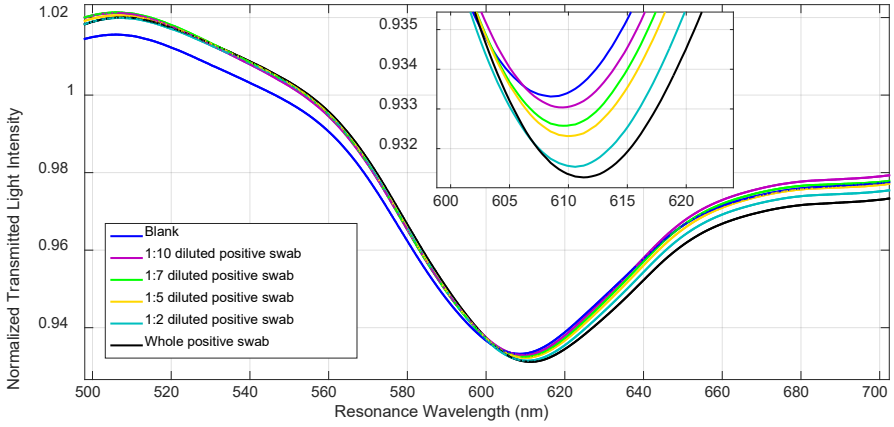
Figure 1. Response curves of Sars-Cov-2 Spike S1 subunit - MIP at different concentrations of protein.

1.1. Detection of SARS-CoV-2 in real samples

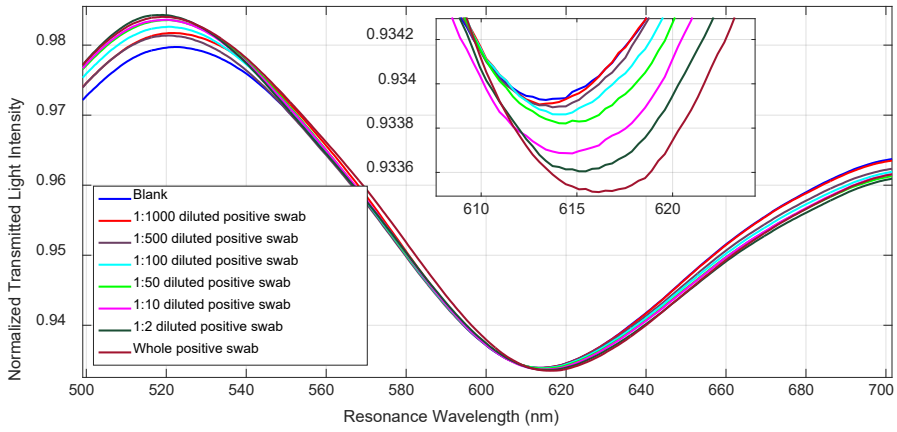
After the preliminary analysis on the detection of the SARS-CoV-2 Spike S1 subunit protein, SARS-CoV-2 real samples were tested in two different matrices (UTM and physiological solution). The samples were collected from a patient, previously diagnosed as Covid-19 positive. Before testing, serial dilutions of the samples with physiological solution were prepared to perform a dose-response curve.

Figures 2(a) and 2(b) report the SPR curves of the different dilutions of NP swabs collected in UTM (universal transport medium) and physiological solution (0.9% NaCl), respectively.

For NP swab in UTM, no resonance shifts are observed when the dilutions are higher than 1:10. The SARS-CoV-2 sensor sensitivity is higher in physiological solution, probably due to the complexity of the UTM formulation.



(**a**)



(**b**)

Figure 2. (**a**) Response curves of different dilutions of a SARS-CoV-2 Positive swab (36th RT-PCR cycle) collected in UTM, tested with SARS-CoV-2 MIP-sensor. Physiological solution was used to make dilutions; (**b**) Response curves of different dilutions of a SARS-CoV-2 Positive swab (36th RT-PCR cycle) collected in physiological medium, tested with SARS-CoV-2 MIP-sensor. Physiological solution was used to make dilutions.

4. Conclusions

An optical chemosensor based on D-shaped POF and MIP for SARS-CoV-2 selective detection has been reported. After a preliminary characterization of the proposed sensor with spiked solutions of SARS-CoV-2 S1 subunit, tests on real samples were reported. In particular, nasopharyngeal swabs were tested in two different matrices (UTM and physiological solution), in order to investigate the sensitivity in both media.

These preliminary results demonstrated the effectiveness of the proposed sensing approach in detecting the virus in real samples within few minutes, making this kind of sensor a promising tool for a more rapid detection system.

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Conflicts of Interest: The authors declare no conflict of interest.

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