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Invited Article

(INVITED)Quantitative detection of SARS-CoV-2 virions in aqueous mediums by IoT optical fiber sensors

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ABSTRACT

The COVID-19 pandemic has emphasized the need for portable, small-size, low-cost, simple to use, and highly sensitive sensors able to measure a specific substance, with the capability of the transmission over the Internet of statistical data, such as in this specific case on the spread of the SARS-CoV-2 virions. Moreover, to resolve the COVID-19 emergency, the possibility of making selective SARS-CoV-2 measurements in different aqueous matrices could be advantageous. Thus, the realization of rapid and innovative point-of-care diagnostics tests has become a global priority. In response to the current need for rapid, highly sensitive and on-site detection of the SARS-CoV-2 virions in different aqueous solutions, two different nanolayer biorecognition systems separately combined with an adaptable optical fiber sensor have been reported in this work. More specifically, two SARS-CoV-2 sensors have been developed and tested by exploiting a plasmonic plastic optical fiber (POF) sensor coupled with two different receptors, both designed for the specific recognition of the SARS-CoV-2 Spike protein; one is aptamer-based and the other one Molecular Imprinted Polymer-based. The preliminary tests on SARS-CoV-2 virions, performed on samples of nasopharyngeal (NP) swabs in universal transport medium (UTM), were compared with data obtained using reverse-transcription polymerase chain reaction (RT-PCR). According to these preliminary experimental results obtained exploiting both receptors, the sensitivity of the proposed SARS-CoV-2 optical fiber sensors proved to be high enough to detect virions. Furthermore, a relatively fast response time (a few minutes) to detect virions was obtained without additional reagents, with the capability to transmit the data via the Internet automatically.

1. Introduction

The Coronavirus Disease 2019 (COVID-19) pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pathogen has generated an international public health emergency from the first months of 2020. This COVID-19 emergency has produced difficulties in social and economic systems, educational and health fields, and the human species seems to have lost all certainties (Guo et al., 2020; Chan et al., 2020; Di Marzo et al., 2020).

Reverse Transcription Polymerase Chain Reaction (RT-PCR) real-time analysis is the reference diagnostic technique for the detection of SARS-CoV-2 virions, even if several pre-analysis tools have been proposed and used (Shen et al., 2020). However, RT-PCR analysis requires specialized equipment, reagents and facilities and typically 3–4 h are necessary to perform the analysis. Moreover, the transmission over the Internet of statistical data on the spread of the SARS-COV-2 virions, with the possibility of making selective SARS-CoV-2 measurements both in biological fluids and in aqueous matrices, cannot be carried out...
automatically; in fact, today the people collect, analyze, and transmit the data without automatic procedures.

The development of automated techniques for monitoring water and air quality has been growing rapidly in recent years. This, together with the development of sensors connected to the Internet - IoT (Internet of Things), is fueling various areas, such as the birth of “Smart cities”, automated medical diagnostics, big data, and Industry 4.0. For instance, using this approach in the SARS-Cov-2 detection, the governments could propose efficient solutions to exit from this COVID-19 emergency because statistical data on the spread of the SARS-Cov-2 virions could be made rapidly available. In this context, novel sensor systems will be required to analyze biological fluids in real-time and transmit the results over the Internet, exploiting an IoT approach. In fact, the necessity to use, in the specific detection of substances, innovative point-of-care diagnostics has become a global priority (Tang et al., 2020; Moraz et al., 2020). However, many efforts have been made to realize more rapid diagnostic tools, primarily based on the determination of antibodies in blood (Xiang et al., 2020; Djaileb et al., 2020) and few concerning sensing devices for detecting the virions in other biological samples.

Irvani S. has proposed an interesting analysis about the challenges and opportunities in the SARS-Cov-2 virions detection exploiting alternative nano- and biosensor-based diagnostics approaches compared to conventional methods (Irvani, 2020). See G. et al. realized a graphene-based Field-Effect Transistor (FET) biosensor for detecting SARS-Cov-2 in clinical specimens (See et al., 2020); using an antibody as the recognition element. This kind of SARS-Cov-2 sensor is interesting because it can be realized by coupling a specific receptor with an electronic general-purpose sensing platform able to monitor the recognition event. Similarly, optical and mechanical platforms could be used to monitor a molecular recognition element (MRE). In particular, a surface plasmon resonance (SPR) sensing platform is technologically suitable to monitor receptors (natural or synthetic) in optical fibers with several advantages. Surface plasmon resonance is widely exploited as a highly sensitive optical detection method for monitoring interactions between an analyte in solution, and an MRE immobilized on the sensing area of the SPR sensor (Homola, 2003). Several SPR sensors have been realized to date, from the classic prism-based configurations to the latest fiber-optic-based ones (Nguyen et al., 2015; Homola, 2008; Gandhi et al., 2019).

In our recent works, we have developed plastic optical fiber (POF)-based SPR sensors in different configurations, coupled with either biological or chemical receptors, such as aptamers, antibodies, and molecularly imprinted polymers (MIPs) for environmental, industrial, and medical applications (Cennamo et al., 2021). In particular, to obtain a general-purpose plasmonic sensor suitable for different kinds of receptor layers several years ago, we designed and realized an SPR sensor in D-shaped POFs (Cennamo et al., 2011) with the following specifications: a planar sensing region simple to use by the dropping of the aqueous solutions; an SPR sensor with a wide refractive index range to work in different matrices and with different kinds of receptors; a sensor that could be used with a low-cost and small-size experimental setup connected to the Internet (Cennamo et al., 2020); a plastic optical fiber sensor, with remote sensing capability, based on a highly flexible, durable, easy to manufacture and to use optical fibers.

In response to the current need for simple and fast approaches to detect SARS-CoV-2 virions, we have proposed two different SPR-POF sensors. Both the SARS-CoV-2 SPR-POF sensors can be monitored exploiting the same experimental setup (Cennamo et al., 2021). These sensors have been realized by covering the same gold nano-film of the SPR-POF platform with two different kinds of receptors specific to detect the SARS-CoV-2 spike protein: an aptamer (Cennamo et al., 2021) and an MIP (Cennamo et al., 2021). This SARS-CoV-2 application can also be useful to demonstrate the capability of the proposed general-purpose POF platform system in monitoring several kinds of MREs.

Hence, the large-scale production of low-cost POF sensors could be used to detect the SARS-CoV-2 virions in aqueous matrices exploiting an IoT sensor system based on this general-purpose optical fiber platform combined with a specific biomimetic or biological receptor for the SARS-CoV-2 spike protein. Furthermore, this diagnostic approach, based on a general-purpose sensor platform that monitors reprogrammable MREs (e.g. MIPs), will be highly desirable to face a possible next pandemic crisis or any other analysis problems.

2. Materials and methods

2.1. A general-purpose IoT SPR-POF sensor system

The SPR POF sensor chips (Mod. A1002) used in this work were provided by Moresense srl (Milan, Italy), as well as the setup used to carry out all the measurements (Spectra 340, Moresense srl, Milan, Italy), as shown in Fig. 1 (a).

Data acquisition and processing were performed by using a developed software tool (Moresense Capture Spectrum Data ver. 2.3), which can also be connected to the Internet and acquire data automatically (Cennamo et al., 2020). Moreover, the instrument provided by Moresense srl can also be used with a Raspberry PI connected to the Internet instead of an expensive laptop, allowing the realization of a low-cost and small-size IoT sensor system (Cennamo et al., 2020).

Fig. 1b shows an outline of the proposed IoT universal sensor system, which can be used in different biochemical applications by covering the gold sensing area with a specific receptor film, as the aptamer and the MIP films used for the SARS-CoV-2 detection here reported.

2.2. Materials to realize MIP nano-films

Reagents: Acrylamide (Aam) (CAS 79–06-1), N-tert-butylacrylamide (TBAm) (CAS 107–58-4), N,N'-methylene bisacrylamide (BIS) (CAS 110–26–9), 2-hydroxymethyl methacrylate (HEMA) (CAS 868–77-9), N,N',N'-tetramethylethylenediamine (TEMED) (CAS 110–18–9), ammonium persulfate (APS) (CAS 7727–54-0), sodium dodecyl sulfate (SDS) (CAS 151–21–3), phosphate buffer solution 1.0 M were from Sigma-Aldrich (Darmstadt, Germany) and used without any further purification. All other chemicals were of analytical reagent grade. The solvent was Milli-Q water.

Trypsin (CAS 9002–07-7) was from Sigma-Aldrich (Darmstadt, Germany), while SARS-CoV-2 (2019-nCoV) Spike protein (S1 subunit, His-Tag) was from Sino Biological.

2.3. Materials to realize aptamer nano-films

SARS-CoV-2 (2019-nCoV) Spike S1 + S2 ECD-His Recombinant Protein was purchased from Sino Biological Inc. (China). DNA-aptamer sequence (5′-/5BiotinTEG/CAG CAC CGA CCT TGT GCT TTG GGA GTG CTG TGC CAA GGG GTT TAA TGG ACA-3′) was purchased from IDT Integrated DNA technologies (Leuven, Belgium) with HPLC purification. Streptavidin from Streptomyces avidinii (SA) and all powders for buffers were purchased from Sigma-Aldrich s.r.l. (Milan, Italy). M–dPEG®₈- Thiol and Biotin-dPEG₈-Lipoamide for the PEG self-assembled mono-layer (SAM) were purchased from Stratech (United Kingdom).

2.4. Experiment procedure

All experiments were performed by dropping about 50 µl of the sample (spiked or real) over the planar sensing region of the SPR-POF sensor (see Fig. 1a), which was incubated at room temperature for ten minutes to let the interaction between the receptor sites and analyte occur. At the end of this incubation, a washing step with buffer 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.

3. Receptor nano-films to detect SARS-CoV-2 virions

3.1. The aptamer nano-layer specific to SARS-CoV-2 spike protein

To immobilize the aptamer specific for the SARS-CoV-2 spike protein on the sensing film, the gold derivatization on the SPR-POF platform was achieved through a mixed PEG-based SAM, followed by SA immobilization and biotin-aptamer binding, as previously reported (Cennamo et al., 2021).

SAM formation was obtained first cleaning the surface with an Argon plasma at 6.8 W of RF power for two minutes, then immersing it in a 0.2 mM of a PEGthiol and BiotinPEGlipo mixture at 8:2 M ratio (in MilliQ water) at room temperature for an overnight incubation, followed by a washing step in ultrapure water and drying with nitrogen. A 5 μg/ml SA solution in phosphate buffer (10 mM phosphate, 138 mM NaCl, 2.7 mM KCl, pH 7.4) for one hour was applied. Finally, after washing in buffer, 10 μM of biotin-aptamer solution (previously heated at 95 °C for one minute and quickly cooled on ice to unfold the sequence) for three hours in the same buffer was applied, followed by washing in a buffer.

The interface outline of the biosensing layer deposited on the POF’s gold film is reported in Fig. 2. More details about the characterization of the realized surface are reported in (Cennamo et al., 2021).

Fig. 1. (a) Pictures of the sensors (mod. A1002) and instrument (Spectra 340), produced by Moresense srl (Milan, Italy), where the sensor chip can be modified with specific receptors (e.g. receptors for the SARS-CoV-2 detection). (b) Outline of the IoT SPR-POF sensor system.
3.2. The MIP nano-layer specific to SARS-CoV-2 spike protein

A specific MIP layer for the recognition of S1 subunit of SARS-CoV-2 spike protein has been prepared as extensively described in (Cennamo et al., 2021) and summarized below for the sake of completeness.

First, the transducer surface was cleaned by an Argon plasma and then was modified by dropping a 10% v/v solution of allyl thiol in 80% v/v ethanol solution and 10% v/v water on the gold surface and left for 12 h. Subsequently, the platform was washed with Milli-Q water (flushing 3 mL 5 times). Through this process a self-assembled monolayer (SAM) with a terminal allyl group is formed.

The pre-polymeric mixture was prepared and dispersed by sonication (sonic bath model VWR USC200T) for 10 min and bubbled with N2 for 30 min at room temperature.

Acrylamide (Aam), N-t-butylacrylamide (TBAm), 2-hydroxyethyl methacrylate (HEMA) were added at 1:0.5:0.6 M ratio, in 15 mM phosphate buffer (PB) pH 7.4. The final concentration of N,N’-methylene bisacrylamide (BIS) in the monomeric mix was 0.19 M. The template (S1 subunit SARS-CoV-2 Spike protein) was added to the pre-polymeric mixture to the final concentration of 1 μM. Then APS (0.08% w/v) and TEMED (0.06% w/v) were added.

After the realization of SAM, about 50 μL of the pre-polymeric mixture were dropped over the D-shaped sensing region and let polymerize for 15 min at room temperature, after which the reticulation process was stopped by washing the sensor surface with Milli-Q water.

Finally, the template was removed by incubating trypsin 4.2x10⁻⁸ M for 2 h at room temperature on the sensor surface and then by washing with an SDS 5% (w/v) solution and Milli-Q water. Fig. 3 shows an outline of the cross-section sensing region.

4. Results and discussion

In this section, the experimental results obtained by both the modified SPR-POF probes, by using specific aptamer and MIP receptors for SARS-CoV-2 spike protein, have been reported. In particular, the dose–response curves obtained by the SARS-CoV-2 Spike protein in buffer solution are reported together with the Langmuir fitting of the data. These results obtained by the SARS-CoV-2 Spike protein can be used to test the capability of the sensor systems in terms of the limit of detection (LOD). After this analysis, preliminary results obtained detecting the
SARS-CoV-2 virions in samples of nasopharyngeal (NP) swabs in UTM (universal transport medium) are reported for both the plasmonic surfaces modified with the receptor nano-layers. All the spectra reported here have been acquired using the experimental procedure explained in the Experiment procedure Section (Section 2.4).

4.1. Results relative to SARS-CoV-2 SPR-POF-MIP sensor

To test the capability of this MIP receptor, imprinted for the SARS-CoV-2 Spike protein S1 subunit, several results have been presented in (Cennamo et al., 2021), where a selectivity analysis has been reported together with other tests.

Fig. 4a shows characteristic SPR spectra collected using several commercial S1 subunit solutions in buffer. The SPR spectra reported in Fig. 4a can be obtained exploiting a developed custom software tool that makes the sensor system easy to use (Cennamo et al., 2021). Fig. 4b reports the relative dose–response curve where the experimental values are plotted together with the Langmuir fitting of the data and the error bars that represent the highest measured standard deviation (equal to 0.2 nm). From the Langmuir fitting parameters, the limit of detection (LOD) relative to the SARS-CoV-2 Spike protein S1 subunit in the buffer can be approximated to about 60 nM, as already reported in (Cennamo et al., 2021).

Finally, the SARS-CoV-2 positive samples in UTM are here reported. The samples were collected from a patient previously diagnosed as Covid-19 positive and analyzed in parallel with RT-PCR technique (36th RT-PCR cycle), in a similar way to (Cennamo et al., 2021). In particular, Fig. 5 reports the experimental results obtained by the SPR-POF-MIP sensor with a real SARS-CoV-2 positive sample of nasopharyngeal (NP) swabs in UTM with different dilutions in physiological solution.

![SPR curves](image-a.png)

**Fig. 4.** (a) SPR curves of the SPR-POF-MIP sensor at different concentrations of SARS-CoV-2 Spike S1 subunit protein. (b) Dose-response curve in a semi-log scale with the Langmuir fitting of the experimental values and error bars.
Dilutions are reported in order to investigate the detection sensitivity in the tested media (UTM). Less significant resonance shifts in UTM are observed for this NP swab when the dilutions are more than 1:10.

Moreover, in (Cennamo et al., 2021) similar dilutions have also been carried out when the SARS-CoV-2 positive sample of nasopharyngeal (NP) swabs is in physiological solution. More specifically, the sensor sensitivity is higher in physiological solutions, probably due to the complexity of the UTM formulation (Cennamo et al., 2021).

Furthermore, it is important to stress that the SARS-CoV-2 measurement lasts approximately 10 min in both matrices.

4.2. Results relative to SARS-CoV-2 SPR-POF aptasensor

Spectra acquired exploiting the proposed SPR-POF aptasensor at different SARS-CoV-2 spike protein concentrations in buffer (PBS, pH = 7.4, including 136.8 mM NaCl, 10.1 mM, NaHPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄, 0.55 mM MgCl₂) are reported in Fig. 6a, confirming data report in (Cennamo et al., 2021). Fig. 6b shows the resonance wavelength variation at different SARS-CoV-2 spike protein concentrations, fitted by the Langmuir equation, with the error bars. From the Langmuir fitting parameters, we can estimate for the SARS-CoV-2 spike protein detection in the buffer a limit of detection of about 10 nM, a value better than that obtained exploiting the MIP receptor, anyway in the same nanoM range of it, confirming the higher affinity of aptamer-based sensor.

Concerning the SARS-CoV-2 virions detection in real samples, we have reported in Fig. 7 the SPR spectra obtained by the aptasensor with a real SARS-CoV-2 positive sample of nasopharyngeal (NP) swab in UTM with a 1:2 dilution in physiological solution (0.9% NaCl). The sample has been collected from a patient diagnosed as Covid-19 positive and has been diluted with a physiological solution (0.9% NaCl). The sample has been analyzed by RT-PCR technique (27th RT-PCR cycle). As shown in Fig. 7, when a SARS-CoV-2 positive sample in UTM 1:2 diluted is incubated for 10 min, the resonance wavelength variation is equal to about 1.8 nm, in a similar way to the that obtained by the MIP-based sensing approach (see Fig. 5).

4.3. Discussion

From the results here reported in terms of the SARS-CoV-2 Spike protein and SARS-CoV-2 virions detection, the obtained LODs of the aptamer-based and MIP-based sensing approaches are very promising.

According to the tests performed with the proteins, aptamers resulted more sensitive in terms of LOD. This sensitivity value could be due to the use of SARS-CoV-2 (2019-nCoV) Spike S1 + S2 ECD, which has a higher molecular weight compared to SARS-CoV-2 (2019-nCoV) Spike S1 subunit, used with MIP receptor.

Anyway, in the SARS-CoV-2 spike protein detection both the proposed sensors have shown LODs in a nanoM range. Similarly, the tests on real nasopharyngeal samples demonstrated a very high sensitivity of both the proposed sensors (aptamer-based and MIP-based). For instance, Fig. 5 shows just how an MIP-based sensor can recognize the virion in a 10-fold diluted sample. In a previous work, the same MIP receptor demonstrated higher sensitivity in a sample from a Covid-19 positive patient but collected in physiological solution instead of UTM, with an estimated amount of 10 viral copies/mL (Cennamo et al., 2021).

In other words, the described experimental results show how a bio-receptor or a biomimetic-receptor nano-layer can be combined with a plasmonic POF platform to perform quantitative measurements of the SARS-CoV-2 virions in aqueous solutions. Table 1 summarizes some aspects of these two kinds of receptors, indicating their similarities and differences.

More specifically, the aptamers are quite expensive and present the capability to realize nanometric bio-receptor films with a very high specificity.Anyway, their cost could be eventually shot down on a large scale production. As a final remark, we report in the following, a comparison of this approach with that based on MIPs, to investigate the appropriate path for the reproducibility of SARS-CoV-2 sensor on an industrial scale, considering the differences of both approaches.

Biomimetic receptors, such as molecularly imprinted polymers, are synthetic molecular recognition systems, and the capabilities of the designer can modify their properties. The synthesis typically involves the use of low-cost functional monomers readily available on the market. Once crosslinked with a suitable crosslinker, they strongly maintain the originally intended structure. The stability over time as well as the resistance to bases, acids, solvents or relatively high temperatures, make them suitable materials for the development and realization of synthetic biomimetic receptors that, apart from the presented SARS-CoV2 detection, can exploited to realize a variety of chemical and biochemical sensors. Given the simplicity of synthesis, a large-scale industrial application of this type of technology is also conceivable. The only aspect to be considered in depth, in view of an industrial production, concerns the supply of the template molecule to be used and its commercial availability.

On the other side, the aptamers are very small molecules with a high affinity for their target. Being synthetic molecules, a high batch to batch reproducibility is obtained and it is possible to introduce functional groups useful for their immobilization on solid substrate. However, a long-term stability of aptamer-based sensing surfaces is certainly lower.
Fig. 6. (a) SPR curves of the aptasensor at different concentrations of SARS-CoV-2 (2019-nCoV) Spike S1 + S2 ECD in buffer. (b) Dose-response curve in a semi-log scale with the Langmuir fitting of the experimental values and error bars.

Fig. 7. SPR response of the aptasensor at a SARS-CoV-2 positive swab in UTM 1:2 diluted with a physiological solution (0.9% NaCl).
than MIPs’ one, and also the high cost could initially discourage their employment.

These two worlds can however be combined too. In 2013 a pioneering work proposed by Bai and coworkers suggested the possibility to imprint aptamers (Bai et al., 2013). This novel approach opens the way to an hybrid system with higher performances: the role of the MIP is the increment in the aptamer stability. Starting from these works, others have been published even recently (Tan et al., 2018; Sullivan et al., 2021; Shen et al., 2021; Roushani et al., 2021; Chen et al., 2021; Poma et al., 2021) paving the way to a new highly stable and performing hybrid recognition system.

5. Conclusions

The capability to realize simple to use, low-cost, and highly sensitive optical fibre sensors for the SARS-CoV-2 detection in aqueous solutions have been reported. The work has shown that both MRSs, combined with the SPR-POF platform, exhibit good performances in the SARS-CoV-2 detection, on purified spike protein and virions, as well. Both sensors resulted in a nanomolar range of detection of spike protein and an attractive ability in the measures of the SARS-CoV-2 virion, indicating the capabilities of these POF sensors to carry out quantitative measurements of NP swab samples. Furthermore, by considering the high sensitivity of the SARS-CoV-2 sensors, the proposed sensing approach offers the opportunity to test the positivity just analysing the cumulative swabs from a group of people (e.g. a class of students, or enterprises, etc.) because even high dilutions do not prevent the detection of the virion presence. In this case, an individual test must be carried out only when the test on a group of people is positive. Furthermore, this approach could open up the possibility to perform fast analysis directly on the swab centre or on-site, automatically storing the results on a cloud-based data centre, also for the statistical data analysis, in this specific case on the spread of the SARS-CoV-2 virions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.