Fiber optic biosensor for inflammatory markers based on long period grating

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***Abstract*—Here, we have reported the development and testing of a label-free fiber optic biosensor, for the real-time detection in serum of an inflammatory marker, the C-reactive protein (CRP). It is based on a novel configuration involving a long period grating (LPG) in double cladding fiber, with specific refractive index profile. Optimization of the sensitivity by means of mode transition was obtained through slight chemical etching of the fiber surface. A thin layer of graphene oxide was deposited around the LPG portion, to provide functional groups for the covalent immobilization of the biological recognition element. A large working range of CRP concentrations of clinical relevance was covered and a low limit of detection of 1.1 ng/mL was obtained in serum. The development of biosensors able to guarantee simple and rapid in-situ monitoring approach is quite an actual topic: an example is the SARS-CoV-2 infection.**

***Keywords*—Biosensors; Fiber gratings; Optical fiber sensors**

I. Introduction

Biosensor development is driven by the continuous need for simple and rapid in-situ monitoring techniques in a broad range of areas, e.g. medical, environmental, food quality, and so on. The coronavirus (SARS-CoV-2) pandemic is one of the most significant examples, due to the necessity of an effective identification of infected patients for timely treatment [1]. However, given the limited availability of the nucleic acid test and long waiting time for both the test and radiographic examination, the quarantine, or therapeutic decisions for many mixed patients were often not timely [2].

In this context, medical research has been focusing on the identification of simple and readily available laboratory biomarkers for more effective triage. Current studies are aiming at the identification of specific biomarker sets which can be related to the infection from SARS-CoV-2 [3], despite a single biomarker cannot be univocally related to it. Among them, for example, blood concentrations of interleukin-6, D-dimer, procalcitonin, and C-reactive protein (CRP) have been considered [3]–[5].

In this work, the attention is focused on the detection of CRP concentrations in serum. CRP is an important biomarker for infections or inflammations in general, which is mainly produced by the liver, but also by macrophages, endothelial cells, lymphocytes, smooth muscle cells and adipocytes [6], and can be related to different diseases. Typical levels of CRP in human serum are estimated to be around 0.8 µg/mL, which can be subject to significant increases in response to an inflammation process. For example, the levels in COVID-19 patients are tens of µg/mL [4]. Moreover, stable high concentrations of CRP have been associated, for example, to cardiovascular diseases, type 2 diabetes mellitus, hemorrhagic stroke, Alzheimer's and Parkinson's diseases [6].

Therefore, it is evident that having a reliable, affordable, and sensitive device for CRP quantification is of great importance. The gold-standard system is the enzyme-linked immunosorbent assay (ELISA) method, having a limit of detection (LOD) of 0.2 µg/mL [7]. In addition, different fiber optic biosensors have been lately reported for the CRP detection [8]. For example, a surface plasmon resonance device based on plastic optical fiber demonstrated a LOD of 9 ng/mL in serum [9], whereas the lossy mode resonance sensor based on indium tin oxide-coated D-shaped fiber from [10] attained a LOD of 62.5 ng/mL in tri-buffer solution. More recently, a 0.82 fg/mL limit of detection was obtained in buffer solution using an etched fiber Bragg grating [11].

An alternative technological platform, successfully employed for the development of biosensors, involves in-fiber long period gratings (LPG) [12]. Here, an unconventional LPG fabricated in a double cladding fiber (DCF) with W-shaped refractive index profile is proposed, and designed in order to induce the mode transition phenomenon in an all-silica structure [13]. The grating was coated with a thin layer of graphene oxide (GO) exhibiting functional groups for the covalent immobilization of the capture antibody. The final device was tested towards the detection of CRP concentrations in human serum within a range of clinical relevance.

II. Sensor fabrication

The sensor proposed herein consists of an LPG written in DCF with enhanced sensitivity, and subsequently coated with a GO thin layer providing functional groups for the biological recognition element (BRE) grafting process.

A. Graphene oxide

GO is a flexible material for biosensing, composed by graphite sheets modified with oxygen-containing functional groups. Here, it was prepared by means of the Hummers' method, involving the chemical attack of graphite (Asbury Carbons, USA) as detailed in [14]. The resulting 2 mg/mL GO water dispersion was deposited on the fiber surface by dip coating at velocity of 100 mm/min, to have a nanometric film.

B. Fiber transducer

As a specialty fiber, we selected single-mode DCF with pure-silica core (with d_{co} = 8 µm), fluorine doped inner cladding ($d_{in,cl}$ ~ 95 µm), and pure-silica outer cladding ($d_{out,cl}$

= 125 µm). The fiber has a W-shaped refractive index profile with core NA = 0.12. For writing the LPG in this particular fiber, the electric arc discharge (EAD) technique was selected; additional details about the fabrication platform are widely discussed in [15]–[17].

For the aim of the work, an LPG was fabricated with a period Λ = 400 µm to exhibit the coupling with 6-th order cladding mode below 1400 nm, as reported in Fig. 1 (black line). Due to the specific refractive index profile of the fiber, modal transition could be induced by following the novel approach reported in [13], i.e. through chemical etching of fiber outer cladding by means of 24% (v/v) HF acid solution. The final diameter of the fiber was $d_{out,cl}$ = 108 µm at the end of the process, corresponding to a working point of the device in transition region when immersed in water environment. Therefore, the bulk refractive index sensitivity of the device increased to −606 nm/RIU, that is eleven-fold the value of the pristine LPG. By looking at the spectrum reported in Fig. 1 (blue dashed line), after the etching the position of the attenuation band, when the grating is in air, is close to the limit of the interrogation system; anyway, when the grating is put in water the same shifted to 1530 nm with depth increased nearly to −15 dB (orange dashed line). Finally, the sensor fabrication is completed with GO deposition, resulting in a slight decrease in resonance depth and blue shift of the same, as from Fig. 1 (blue and orange solid lines, respectively). It is worth remarking that this approach combines a high visibility of the resonance dip with high sensitivity of mode transition, due to an all-silica structure without any additional lossy overlay coating on the fiber.

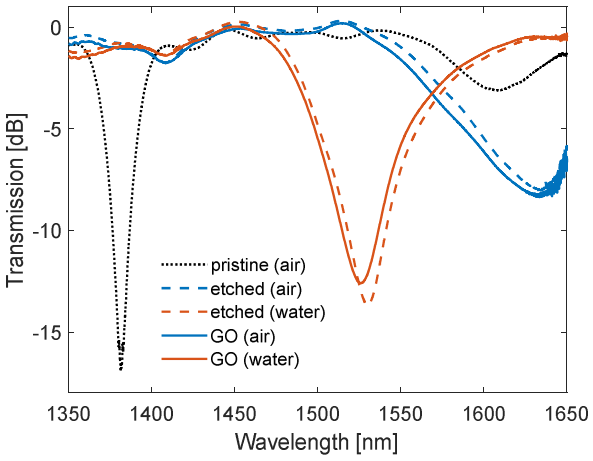

Fig. 1. Spectra of the LPG in DCF in correspondence of the different steps: fabrication (black dotted), cladding etching in air (blue dashed) and water (orange dashed), GO deposition in air (blue solid) and in water (orange solid).

III. Testing and Results

This section provides the details about the testing setup, the sensor functionalization procedure, as well as the results achieved in the detection of CRP in serum samples.

A. Setup

During the experimentation, the grating was housed in a custom designed thermally-stabilized microfluidic system, whose details can be found in [18], [19], supplied by a peristaltic pump to precisely manage the flux of the solutions injected into the cell. The grating was kept in the same strain conditions during the testing and always immersed in the solutions. Concerning the optoelectronic interrogation system, a broadband light source emitting in the wavelength range 1300-1700 nm was used, whereas Anritsu MS9030A-MS9701B optical spectrum analyzer (OSA) was utilized to measure the light at the grating output. Finally, a real-time monitoring of the resonance wavelength shift was performed by custom NI LabWindows™/CVI software, achieving resolution and accuracy of 0.01 nm and 0.007 nm, respectively [20]. A schematic picture of the whole setup is reported in Fig. 2.

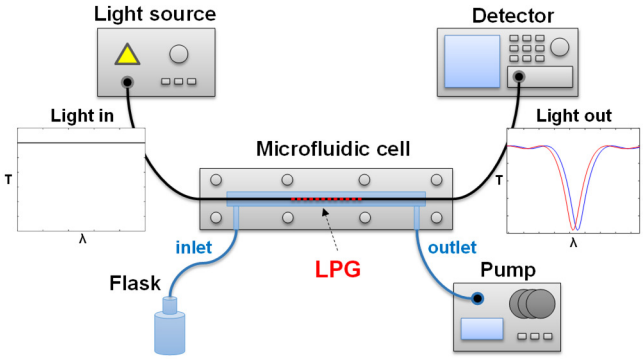

Fig. 2. Schematic of the experimental setup consisting of the microfluidic system and the optoelectronic systems for the LPG biosensor readout.

B. Functionalization and receptor immobilization

The GO nanometric layer deposited onto the fiber surface aimed to provide the -COOH functional groups for the immobilization of the BRE. These groups were activated by standard cross-linking chemistry with 200 mM/50 mM EDC/NHS for 30 minutes. The covalent immobilization of the receptor (monoclonal antibody clone C5, 500 µg/mL), anti-CRP, was performed at a very slow flow rate of 15 µL/min for about 40 minutes. The unattached antibodies were removed by washing in phosphate-buffered saline (PBS, 40 mM at pH 7.4) for 10 minutes at a faster flow rate of 150 µL/min. The surface passivation was obtained with 1% (w/v) of bovine serum albumin (BSA) in PBS, by treating the surface for 20 minutes and then washing for 10 minutes in PBS buffer to remove the BSA excess The passivation step blocked the remaining activated carboxylic groups, so as to prevent non-specific adsorption onto the surface [21], [22].

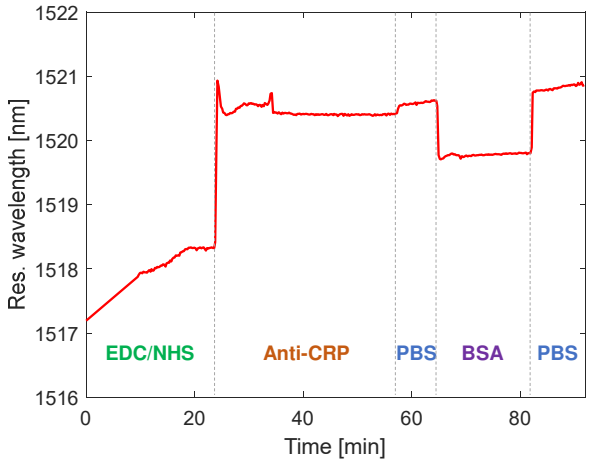

Fig. 3. Response of the LPG during the immobilization of the biological receptor, anti-CRP.

The sensorgram reporting the response of the sensor during these steps, in terms of resonance wavelength as

function of time, is reported in Fig. 3. An important information can be inferred from these results: the signal in PBS after the surface passivation with BSA did not change much, meaning that the available surface for the immobilization of the receptor was completely covered, potentially resulting in a low degree of non-specific binding.

C. *Sensing of CRP*

The detection was verified by performing analyte binding steps, with increasing concentrations of CRP in the range 0.01-100 µg/mL in real samples of human serum (diluted 1:10 (v/v) in PBS) for 20-30 minutes at a slow flow rate of 25 µL/min, followed by a washing step with PBS for 10-15 minutes. The LPG resonance wavelength during the process is reported in Fig. 4(a). The first step refers to injecting a solution of diluted free-CRP human serum used as a specificity test (blank measurement), for which a negligible change of the signal before and after the PBS washing was observed. Subsequently, the residual wavelength shift measured in PBS after rinsing accounts for the volume of bound CRP, as a consequence of increasing concentrations of the same injected into the flow cell. As one can observe, the resonance wavelength of the grating shifted monotonically towards shorter wavelengths, with increasing analyte concentration, according to the sensing mechanism of the LPG [12], [23].

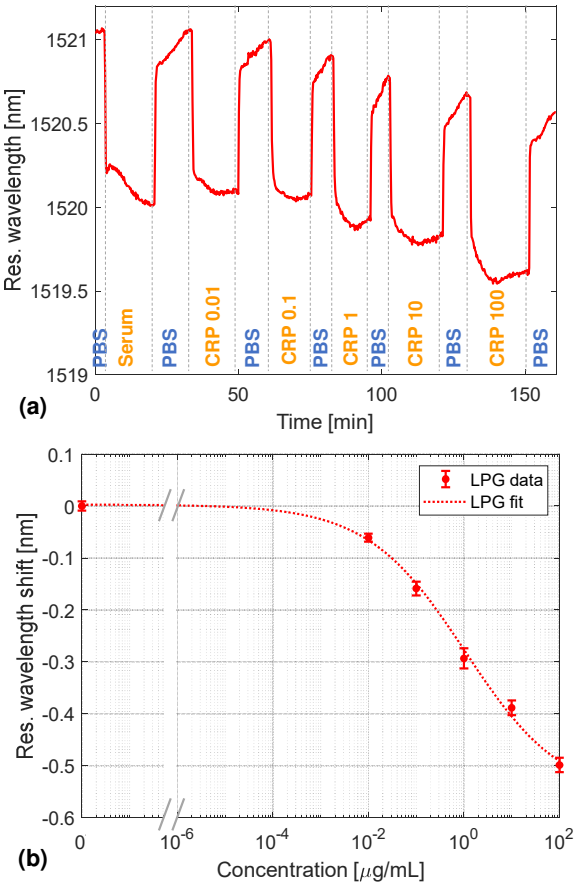

Fig. 4. (a) Real-time response of the LPG during the injection of CRP at increasing concentrations (reported as µg/mL); (b) Semi-log calibration curve of the LPG. The dotted curve represents the fitting of the experimental data by using the Logistic function.

The calibration curve of the biosensor is reported in Fig. 4(b) using semi-log scale. The resonance wavelength values have been scaled down with respect to the initial value of the baseline (i.e. the residual shift). The experimental data are reported in terms of the mean value and the corresponding standard deviation for 20 subsequent measurements (error bars). They were also fitted by using the Logistic function, reported as dotted line in Fig. 4(b). It was extrapolated from the Hill equation, which is a well-accepted model to consider the degree of interaction between ligand binding sites [24], as also detailed in [25].

The performance of the biosensor can be thus inferred from these results. The overall wavelength shift for CRP concentrations in the range considered was equal to −0.49 nm. Moreover, the LOD was equal to 1.1 ng/mL, defined as the sensor response related to the concentration zero of the analyte (blank measurement) plus three times its standard deviation [20]. Such value is one of the lowest reported so far concerning fiber biosensors [8]. Finally, it is worth highlighting that the proposed device can cover a large working range (2 ng/mL - 100 µg/mL) of clinical relevance [6].

IV. Conclusions

We have reported the results concerning the development of a label-free fiber-based biosensor for the real-time measurement of C-reactive protein in human serum. The device is based on long period grating fabricated in a W-type double cladding optical fiber. To provide proper sensitivity, the diameter of the fiber (i.e. outer cladding diameter) was modified, based on a design inducing the mode transition phenomenon when the device is in aqueous environment. Such technique also resulted in good visibility of the grating spectral features after the whole treatment, meaning a high SNR. Moreover, the absence of additional coatings onto the fiber surface is beneficial in terms of stability and long-time use. Afterwards, the so-prepared optical transducer was coated with a thin layer of graphene oxide providing oxygen-containing functional groups, which were used for the covalent immobilization of the capture antibody, anti-CRP. The biosensor was finally tested using a state-of-the-art microfluidic system, towards the detection of CRP concentrations in serum within clinical ranges. The device could cover a wide working range, with a LOD of about 1.1 ng/mL, which is one of the lowest reported so far concerning fiber optic biosensors.

REFERENCES

[1] J. Zhang, L. Zhou, Y. Yang, W. Peng, W. Wang, and X. Chen, "Therapeutic and triage strategies for 2019 novel coronavirus disease in fever clinics," *Lancet Respir. Med.*, vol. 8, no. 3, pp. e11–e12, Mar. 2020, doi: 10.1016/S2213-2600(20)30071-0.

[2] Q. Li *et al.*, "Eosinopenia and elevated C-reactive protein facilitate triage of COVID-19 patients in fever clinic: A retrospective case-control study," *EClinicalMedicine*, vol. 23, p. 100375, Jun. 2020, doi: 10.1016/j.eclinm.2020.100375.

[3] F. Liu *et al.*, "Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19," *J. Clin. Virol.*, vol. 127, p. 104370, Jun. 2020, doi: 10.1016/j.jcv.2020.104370.

[4] Y. Gao *et al.*, "Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID-19," *J. Med. Virol.*, vol. 92, no. 7, pp. 791–796, Jul. 2020, doi: 10.1002/jmv.25770.

[5] L. Wang, "C-reactive protein levels in the early stage of COVID-19," *Médecine Mal. Infect.*, vol. 50, no. 4, pp. 332–334, Jun. 2020, doi: 10.1016/j.medmal.2020.03.007.

[6] N. R. Sproston and J. J. Ashworth, "Role of C-Reactive Protein at Sites of Inflammation and Infection," *Front. Immunol.*, vol. 9, Apr. 2018, doi: 10.3389/fimmu.2018.00754.

[7] J. L. Clarke *et al.*, "Comparison of differing C-reactive protein assay methods and their impact on cardiovascular risk assessment," *Am. J. Cardiol.*, vol. 95, no. 1, pp. 155–158, Jan. 2005, doi: 10.1016/j.amjcard.2004.08.087.

[8] P. Salvo *et al.*, "Sensors and Biosensors for C-Reactive Protein, Temperature and pH, and Their Applications for Monitoring Wound Healing: A Review," *Sensors*, vol. 17, no. 12, p. 2952, Dec. 2017, doi: 10.3390/s17122952.

[9] A. Aray *et al.*, "SPR-based plastic optical fibre biosensor for the detection of C-reactive protein in serum," *J. Biophotonics*, vol. 9, no. 10, pp. 1077–1084, Oct. 2016, doi: 10.1002/jbio.201500315.

[10] P. Zubiate, C. R. Zamarreño, P. Sánchez, I. R. Matias, and F. J. Arregui, "High sensitive and selective C-reactive protein detection by means of lossy mode resonance based optical fiber devices," *Biosens. Bioelectron.*, vol. 93, pp. 176–181, Jul. 2017, doi: 10.1016/j.bios.2016.09.020.

[11] S. Schulze, M. Wehrhold, and C. Hille, "Femtosecond-Pulsed Laser Written and Etched Fiber Bragg Gratings for Fiber-Optical Biosensing," *Sensors*, vol. 18, no. 9, p. 2844, Aug. 2018, doi: 10.3390/s18092844.

[12] F. Chiavaioli, F. Baldini, S. Tombelli, C. Trono, and A. Giannetti, "Biosensing with optical fiber gratings," *Nanophotonics*, vol. 6, no. 4, pp. 663–679, Jan. 2017, doi: 10.1515/nanoph-2016-0178.

[13] F. Esposito, A. Srivastava, L. Sansone, M. Giordano, S. Campopiano, and A. Iadicicco, "Sensitivity Enhancement in Long Period Gratings by Mode Transition in Uncoated Double Cladding Fibers," *IEEE Sens. J.*, vol. 20, no. 1, pp. 234–241, Jan. 2020, doi: 10.1109/JSEN.2019.2942639.

[14] F. Esposito, L. Sansone, C. Taddei, S. Campopiano, M. Giordano, and A. Iadicicco, "Ultrasensitive biosensor based on long period grating coated with polycarbonate-graphene oxide multilayer," *Sensors Actuators B Chem.*, vol. 274, pp. 517–526, Nov. 2018, doi: 10.1016/j.snb.2018.08.002.

[15] F. Esposito, R. Ranjan, S. Campopiano, and A. Iadicicco, "Arc-Induced Long Period Gratings from Standard to Polarization-Maintaining and Photonic Crystal Fibers," *Sensors*, vol. 18, no. 3, p. 918, 2018, doi: 10.3390/s18030918.

[16] F. Esposito, S. Campopiano, and A. Iadicicco, "Arc-Induced Long Period Gratings in Erbium-Doped Fiber," *IEEE Photonics J.*, vol. 11, no. 1, pp. 1–8, Feb. 2019, doi: 10.1109/JPHOT.2019.2894300.

[17] F. Esposito, A. Srivastava, A. Iadicicco, and S. Campopiano, "Multi-parameter sensor based on single Long Period Grating in Panda fiber for the simultaneous measurement of SRI, temperature and strain," *Opt. Laser Technol.*, vol. 113, pp. 198–203, May 2019, doi: 10.1016/j.optlastec.2018.12.022.

[18] F. Chiavaioli *et al.*, "Sol–Gel-Based Titania–Silica Thin Film Overlay for Long Period Fiber Grating-Based Biosensors," *Anal. Chem.*, vol. 87, no. 24, pp. 12024–12031, Dec. 2015, doi: 10.1021/acs.analchem.5b01841.

[19] F. Chiavaioli *et al.*, "Femtomolar Detection by Nanocoated Fiber Label-Free Biosensors," *ACS Sensors*, vol. 3, no. 5, pp. 936–943, May 2018, doi: 10.1021/acssensors.7b00918.

[20] F. Chiavaioli, C. Gouveia, P. Jorge, and F. Baldini, "Towards a Uniform Metrological Assessment of Grating-Based Optical Fiber Sensors: From Refractometers to Biosensors," *Biosensors*, vol. 7, no. 4, p. 23, Jun. 2017, doi: 10.3390/bios7020023.

[21] P. Zubiate *et al.*, "Fiber-based early diagnosis of venous thromboembolic disease by label-free D-dimer detection," *Biosens. Bioelectron. X*, vol. 2, p. 100026, Oct. 2019, doi: 10.1016/j.biosx.2019.100026.

[22] A. Giannetti, C. Trono, G. Porro, C. Domenici, M. Puntoni, and F. Baldini, "Towards an Integrated System as Point-of-Care Device for the Optical Detection of Sepsis Biomarkers," *Chemosensors*, vol. 8, no. 1, p. 12, Feb. 2020, doi: 10.3390/chemosensors8010012.

[23] X. Shu, L. Zhang, and I. Bennion, "Sensitivity characteristics of long-period fiber gratings," *J. Light. Technol.*, vol. 20, no. 2, pp. 255–266, 2002, doi: 10.1109/50.983240.

[24] R. A. Dudley *et al.*, "Guidelines for immunoassay data processing.," *Clin. Chem.*, vol. 31, no. 8, pp. 1264–1271, Aug. 1985, doi: 10.1093/clinchem/31.8.1264.

[25] Chiavaioli, C. Trono, A. Giannetti, M. Brenci, and F. Baldini, "Characterisation of a label-free biosensor based on long period grating," *J. Biophotonics*, vol. 7, no. 5, pp. 312–322, May 2014, doi: 10.1002/jbio.201200135.