Direct Functionalization of TiO_2/PSS Sensing Layer for an LMR-Based Optical Fiber Reusable Biosensor

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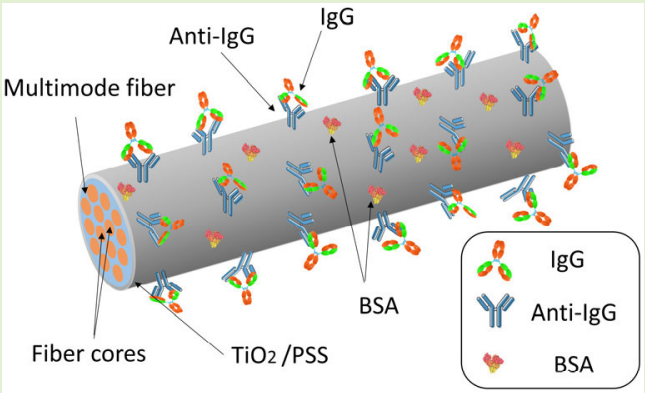
***Abstract*—Functionalization plays a crucial role in the development of biosensors. In this study, bioreceptors were directly immobilized onto the surface of a sensing layer after physical activation, avoiding the need for longer and more complex functionalization systems. This direct immobilization was applied to an optical sensing platform based on lossy mode resonances (LMRs) generated by a thin film of titanium (IV) dioxide/poly(sodium 4-styrenesulfonate) (TiO_2/PSS). To generate the LMR, a 200-μm bare optical fiber was coated with TiO_2/PSS using the layer-by-layer self-assembly technique. The PSS of the sensing layer was then physically activated using either UV-ozone or plasma to immobilize antirabbit immunoglobulin G (IgG) bioreceptors. This enabled specific and label-free detection of rabbit IgG concentrations ranging from 0.002 to 2 mg/mL. The results presented in this work include the real-time detection of rabbit IgG, a comparison between the two activation techniques (UV-ozone and plasma), and an analysis of the biosensor's reusability over four consecutive cycles, which demonstrates the promising potential of the TiO_2/PSS sensing layer for biosensing applications.**



***Index Terms*— Biosensor, layer-by-layer (LbL), lossy mode resonance (LMR), optical fiber, titanium (IV) dioxide (TiO_2) thin film.**

I. Introduction

OPTICAL fiber sensing is a technology that has experienced much progress thanks to decades of research, especially in the domain of chemical sensors and biosensors [1], [2]. This is possible because it is cost-effective and biocompatible [3] and because the optical fiber is flexible and small in size and weight [4], which allows monitoring physical variables within the organism with a catheter [5], for instance. Moreover, fiber optics has demonstrated the ability to perform measurements in hazardous environments such as nuclear facilities [6] and in strong magnetic fields due to its immunity to electromagnetic interference [2].

The deposition of thin films onto the sensitive area of the optical fiber leads to the generation of resonances whose spectral shift is employed to detect changes in either the thin film itself or in the subsequent deposition of additional layers [2]. In the generation of resonances, it is necessary to take into account the properties of the material used for the thin film. In the case of lossy mode resonances (LMRs), the real part of the material permittivity must be positive and higher in magnitude than both its own imaginary part and the real part of the permittivity of the material surrounding the thin film [7]. That is why LMRs are generated mainly with either metal oxides or polymer coatings [8], [9].

One of these materials is titanium (IV) dioxide (TiO_2) [10], which has been widely used in biomedical applications due to its biocompatibility and corrosion resistance, making it an excellent choice for implants and prosthetics [11]. Moreover, the properties of TiO_2 make it a metal oxide with a promis-

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ing future in biosensing applications. The influence of the surface properties of titanium on the biological response and the effect of different nanoscale surface modifications have been described previously [12]. Also, the photoelectrochemical biosensing field has been greatly benefited from the use of various TiO_2 nanostructures, including nanotubes and nanowires [13]. TiO_2 also offers physical and chemical resistance, which can extend the lifespan of a device. However, inducing a precise etching of TiO_2 thin films to achieve a particular profile is still a challenging task, especially on the nanoscale [14].

In the design and optimization of biosensors, the functionalization is a critical stage, as it ensures that the sensor can interact with the analyte and produce a detectable signal in response to its presence. The term "functionalization" refers to the process where the sensing surface is modified to allow the immobilization of the bioreceptors. Nowadays, several surface modification strategies are available in the literature. Choosing one of them depends on several aspects, such as the transducing mechanism of the sensor, the substrate, and the bioreceptor to be immobilized [16].

In this sense, direct physical adsorption is the simplest immobilization method, in which the biomolecules are directly attached to the surface through weak bonds such as van der Waals forces, hydrogen bonding, or hydrophobic interactions. Frequently, surface functionalization methods include self-assembled monolayers (SAMs), deposition of polymers, nanomaterials, and metal-organic frameworks (MOFs) to improve the efficiency and applicability of the sensors. There are different kinds of SAMs reported, which involve carboxylate SAMs on the oxide surfaces, silane SAMs on glass/silicon surfaces, and alkane thiol SAMs on noble metals [17].

Regarding optical fiber sensors, the focus of this work, it is well-described the subsequent activation of the previous mentioned thin films by means of crosslinkers, such as glutaraldehyde for silane SAMs [18], [19], [20] or N-ethyl-N′ -(3-(dimethylamino) propyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) for alkane thiol SAMs and polymers [21], [22], [23], [24]. In the proposed biosensor, the generation of LMRs based on TiO_2 thin films is obtained by using the layer-by-layer (LbL) technique, including the presence of the polymer poly(sodium 4-styrenesulfonate) (PSS) in the sensing surface available for its direct activation, thus avoiding subsequent deposition steps, as it will be shown.

Additionally, surface modification techniques such as plasma or UV-ozone have been extensively used as a way to change the properties of the polymer surfaces. This treatment can activate chemically inactive groups that can be used in the functionalization of biosensors [25], [26], [27], [28]. This reduces significantly the number of steps and the time invested during the manufacturing process, as well as it guarantees the absence of any residual chemical solvents or contaminants [29].

Regarding bioreceptors, immunoglobulin G (IgG) is the most abundant human antibody and is regarded as a significant physiological indicator of the immune response. The detection of IgG allows wide-ranging applications in disease diagnosis, prognosis, and therapeutic monitoring [30]. Its measurement provides valuable insights to facilitate early detection and effective management of various infectious, autoimmune, and inflammatory diseases [31]. A traditional method for IgG detection is enzyme-linked immunosorbent assay (ELISA), which presents the disadvantages of labeled methods: high cost, multistep processing, and long detection time [30]. Recently, various optical fiber biosensors have been developed for the detection of IgGs based on surface plasmon resonances (SPRs) [21], [22], [23], [32], [33], interferometers [18], [34], and Bragg gratings [20]. These strategies promote the development of label-free biosensors, leading the path to an actual and cost-effective alternative to the traditional clinical techniques.

In this article, the aim was to test and apply the physical activation techniques to optical fiber sensors as a time-effective functionalization method for the detection of biomolecules. To this purpose, a label-free LMR-based biosensing platform composed of a TiO_2/PSS-coated multimode optical fiber was developed for the real-time monitoring of rabbit IgG detection. The developed biosensor contains TiO_2 nanoparticles on its surface, which enable the generation of LMRs, thanks to the high refractive index of this material in combination with PSS polymer, which can be activated physically. In this sense, a characterization of the immobilization and the detection process of this specific sensor will be described. This study also includes a comparison between two simple functionalization techniques based on the physical surface activation by both UV-ozone and plasma treatments. In addition, the reusability of the biosensor activated by plasma will be tested. Finally, some conclusions will be presented on the applicability of biosensing platforms based on TiO_2/PSS thin films.

II. Methods and Materials

A. Optical and Chemical Materials

Multimode optical fiber FT200EMT, with 200/225-μm core/cladding diameter, was purchased from Thorlabs Inc., Bergkirchen, Germany, and UV optical adhesive from Norland Products Inc., East Windsor, NJ, USA. Titanium (IV) oxide (TiO_2) nanoparticles of 21-nm-diameter, polymer PSS (Mw $\sim$ 70 000), phosphate buffered solution (PBS, 0.1 M) pH 7.2, bovine serum albumin (BSA), potassium hydroxide (KOH) 1 M, IgG from rabbit serum (essentially salt-free, lyophilized powder), and its specific polyclonal antibody antirabbit IgG produced in goat (lyophilized powder) were purchased from MERCK, Madrid, Spain. Both optical and chemical materials were used to fabricate the biosensors and to develop the bioassays.

B. LMR Generation by Means of TiO_2/PSS Thin Films

In the fabrication of the biosensor, first, the cladding of a 2-cm segment of the optical fiber was chemically removed and cleaned with acetone and acid piranha solution. Then, the LMR was obtained by coating the core exposed region with TiO_2/PSS employing the LbL assembly technique, as explained in [10]. Briefly, the PSS water solution was used as the polyanionic solution, whereas the TiO_2 nanoparticles in ultrapure water were the cationic solution. The pH of both the polymer and the nanoparticle solutions was adjusted to 2.0. In each LbL cycle, the optical fiber was dipped for 2 min in the TiO_2 solution, washed out for 1 min in acid water, then dipped for 2 min in PSS solution, and finally washed out for 1 min in water (pH 2). These cycles were repeated to obtain the second LMR centered in a wavelength range from 600 to 750 nm, in order to track the wavelength shift of the LMR at every stage during the process.

C. Instrumentation and Optical Setup

The transmission spectra were acquired using the optical setup and the microfluidic system detailed in Fig. 1(a). The

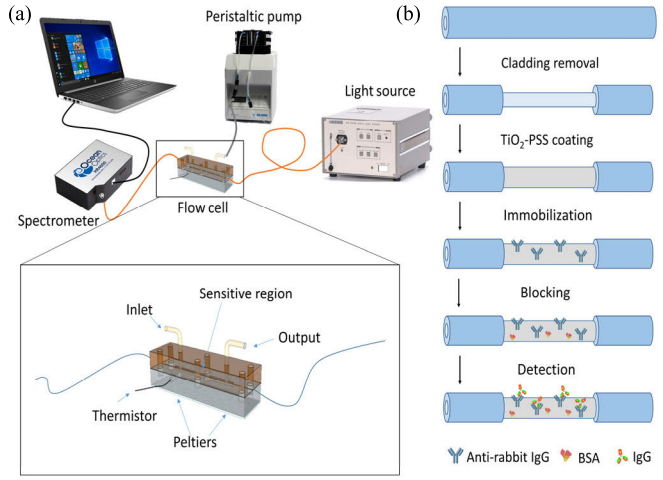

Fig. 1. (a) Complete experimental setup to check over in real time all the steps of the assay, the functionalization, and the detection of the rabbit IgG. The light goes through the optical fiber represented by an orange line from the light source to the spectrometer. The black line shows the cable that connects the spectrometer to the computer and collects the transmission spectral data. The inset shows the thermo-stabilized system in detail. (b) Sketch of the steps carried out to fabricate the proposed optical fiber biosensor.

sensitive region of the fiber was placed in the thermo-stabilized microfluidic system as described in [35]. Both ends of the fiber were connected to a halogen white light (Ando AQ-4303B) and to an HR4000 spectrometer (OceanInsight Inc., Ostfildern, Germany), respectively. The output wavelength range of the halogen white light was set to 400–1800 nm, and the HR4000 spectrometer allowed to measure in a range from 200 to 1100 nm. During the experimental assays, the transmission spectra data were sent from the spectrometer to the computer and were real time processed by Spectra Suite[1] (OceanInsight Inc., Ostfildern, Germany) and MATLAB[1] (The MathWorks, Inc., Natick, MA, USA) software.

As previously described in [35], the microfluidic system includes two equal-size parts (23 mm wide, 10 mm deep, and 100 mm long) made of polyetherimide (ULTEM resin) and stainless steel. A microchannel is fabricated on both the polyetherimide and in the stainless-steal parts, with the size of 1 × 1 × 50 mm, thus achieving a volume of 50 μL. The size of the microchannel allows handling small fluid quantities. Two Peltier cells (23 × 25 mm) located below the stainless-steel part of the flow cell heat the system up, while a thermistor inside a lateral hole in the same stainless-steel part controls the temperature. Hence, the temperature during the experimental process is kept stable at 30 °C ± 0.05 °C by the thermostabilized microfluidic chamber. In addition, during the bioassays, the solutions are passed through the microfluidic cell with accurate flow rates, thanks to a peristaltic pump, which circulates the fluids using thermoplastic elastomer tubes.

D. Bioassay's Protocol

After the generation of an LMR with the TiO_2/PSS thin film, the first step for the development of the biosensing assays was surface activation. Two different activation systems were tested, UV-ozone (Ossila Ltd, Sheffield, U.K.) and plasma (ACE1 Gala Instruments). The activation with UV-ozone required 30-min treatment, whereas the plasma treatment needed just 5 min at 50 W. The fiber with its jacket was glued to the opposite ends of the flow cell system using the UV optical flexible adhesive indicated in Section II-A. After that, different solutions were pumped through it during the immobilization and detection process. As shown in Fig. 1(b), during the immobilization, the bioreceptor was attached to the surface and then the unreacted active functional groups were blocked. During the experiments, a solution of 100 μg/mL of antirabbit IgG was flowed for an hour to attach the antibodies to the sensor surface. After that, PBS was flowed for 10 min, in order to rinse and remove the unbounded antibodies. The immobilization concluded by flowing during 10 min a blocking solution, BSA 0.1 mg/mL in PBS, and with a further PBS washing step. In the detection process, increasing concentrations of the analyte rabbit IgG were flowed for 20 min and the sensor surface was washed after each step with PBS until signal stabilization was achieved. The rabbit IgG-detected concentrations were 0.002, 0.02, 0.05, 0.2, 0.5, and 2 mg/mL diluted in PBS. All the steps of the experimental assays were performed at a flow rate of 45 μL/min.

III. Results and Discussion

The biosensing platform is based on monitoring the LMR generated by the deposition of TiO_2/PSS with the LbL method. The LMR wavelength shift is tracked in real time during every assay, allowing the detection and recording of the optical changes influencing the sensing layer behavior.

A. LMR Formation

As previously mentioned, the thin film is formed by depositing alternating layers of TiO_2 and PSS materials with washing steps in between. Fig. 2(a) shows the progress of the transmission peak, while the last bilayers of the coating are being deposited. The process is stopped when the second LMR is located between 600 and 750 nm. In addition, Fig. 2(b) presents a scanning electron microscope (SEM) image of the optical fiber coated by a TiO_2/PSS thin film. As can be seen, the average thickness of the coating in the SEM measurements is estimated between 250 and 300 nm. These results are consistent with the theoretical and experimental results obtained by Del Villar et al. [36]. According to this, the obtained resonance corresponds to the second LMR, something that is confirmed by the 850-nm/Refractive Index Unit (RIU) sensitivity attained by the sensor in the refractive index range from 1.333 to 1.351 RIU (see Figs. S1 and S2 in the Supporting document).

It is important to emphasize that the optical fiber that has been used is standard 200-μm multimode fiber. The use of a standard optical fiber as well as the position of the LMR in the visible instead of the infrared wavelength range reduces significantly the cost of the setup and brings it closer to commercialization.

B. Experimental Bioassays

During the experimental bioassays, the transmission spectrum is monitored in real time while the LMR wavelength shift is recorded. Fig. 3 shows the sensorgram of the LMR shift for a complete experimental bioassay. In this case, the optical fiber biosensor is previously activated with plasma. According to this, Fig. 3(a) and (b) represents the immobilization and detection process, respectively. The bioassay starts with the immobilization, which plays an important role concerning the development of the biosensors. In this section, the surface is

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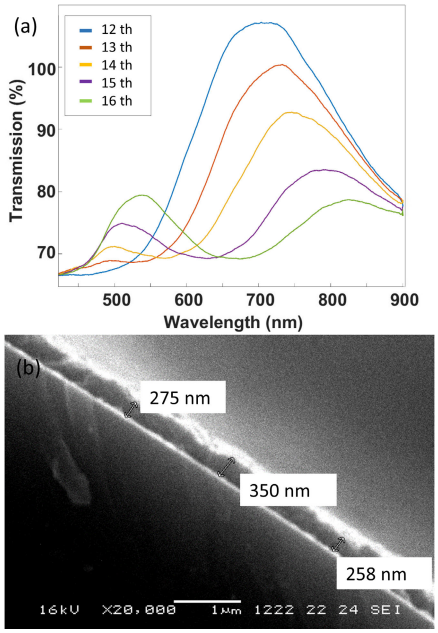

Fig. 2. (a) Experimental transmission spectra achieved in the last five layers of the TiO_2/PSS LbL deposition process. (b) SEM image of the optical fiber coated by the TiO_2/PSS layer.

covered with the antirabbit IgG bioreceptor. The antirabbit IgG is attached to the active groups of the PSS, obtaining a 13-nm wavelength shift. Then, the surface is rinsed with PBS as a way to remove the nonattached antirabbit IgG biomolecules. As it is noted in the sensorgram, the unreacted functional groups are blocked with BSA to avoid unspecific binding. The immobilization concludes with a new rinsing step in PBS.

The use of a specific bioreceptor for the analyte and the blocking of the surface permits the selective detection of rabbit IgG. The surface blocking prevents nonspecific interactions and interference, allowing the sensor to focus exclusively on the intended analyte. This selective approach enhances the accuracy and reliability of the detection process.

The sensorgram in Fig. 3(b) displays the signal change, compared to the baseline, of the rabbit IgG detection stage. During the detection, increasing concentrations of rabbit IgG are injected in a range between 0.002 and 2 mg/mL. After each analyte concentration, a washing step with PBS is carried out for the purpose of removing all the unbound rabbit IgG. The wavelength shift after the washing step corresponds to the amount of rabbit IgG bound to the specific bioreceptor and can be directly related to the increasing rabbit IgG concentration. The response of the sensor at the end of the washing steps remains stable, which demonstrates a consistent binding of the analyte.

C. Plasma Versus UV-Ozone Activation Methods

Three complete independent bioassays ($n = 3$) were developed with optical fibers activated by UV-ozone, and the other three complete independent bioassays ($n = 3$) were developed after the functionalization with plasma treatment. The results obtained in the detection process after each activation treatment are shown in Fig. 4. The LMR wavelength shift is represented as a function of the rabbit IgG concentration. Here, it must be pointed out that the error bars correspond to the data of three independent biosensing assays ($n = 3$), to assess the

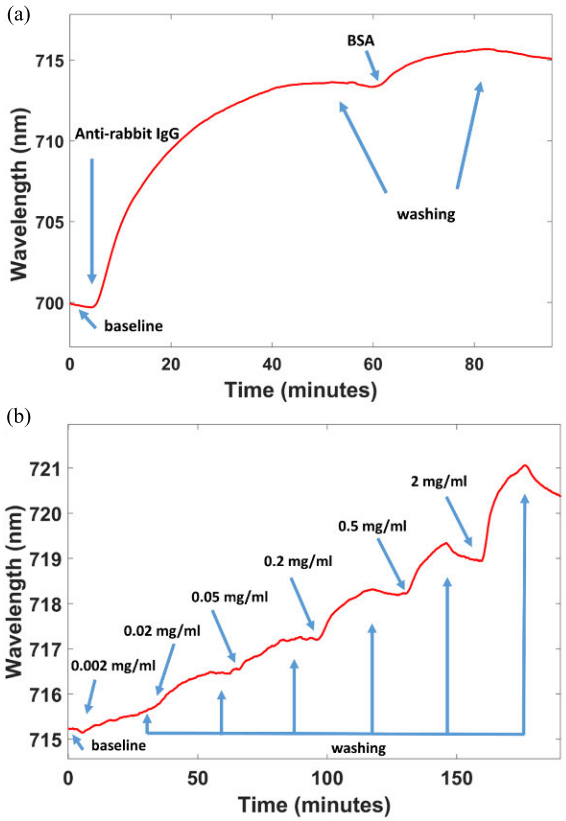

Fig. 3. (a) Sensorgram of the immobilization part of the experimental bioassays, comprising the antirabbit IgG attachment and the blocking step with BSA. (b) Sensorgram of the proposed biosensor during the detection of increasing rabbit IgG concentrations (range: 0.002–2 mg/mL).

repeatability of the present biosensor. The size of the error bars indicates certain variability on the data that can be attributed to different wavelength locations of the LMR in each experiment since it is well-known that it plays a role in the sensitivity of the LMR. In order to mitigate this problem, it will be necessary to control more precisely the parameters during the deposition of the LbL structure, i.e., pH of the solution, temperature, etc.

In Fig. 4, the experimental points are fit by the Hill function with an R^2 regression coefficient of 0.9998 in the case of UV-ozone and 0.9989 in the case of plasma treatment. The Hill equation is a well-known mathematical model to quantify the degree of interaction between ligand binding sites [37]. Based on the recommendations made by the International Union of Pure and Applied Chemistry (IUPAC), it also permits to obtain the theoretical limit of detection (LOD) from the calibration curve of the biosensor. Specifically, the LOD is calculated as the average value of the blank signal plus three times the maximum standard deviation obtained among all the measurements, as reported in [38] and [39]. The theoretical LOD achieved from the UV-ozone treatment curve is 0.0195 mg/mL of rabbit IgG and 0.0312 mg/mL for the plasma treatment, which demonstrates that the results of both UV-ozone and plasma treatments are in the same order of magnitude.

According to these results, both UV-ozone and plasma functionalization systems are rapid and simple methods that are suitable for being used in optical fiber biosensor fabrication. UV-ozone functionalization takes 30 min, while plasma

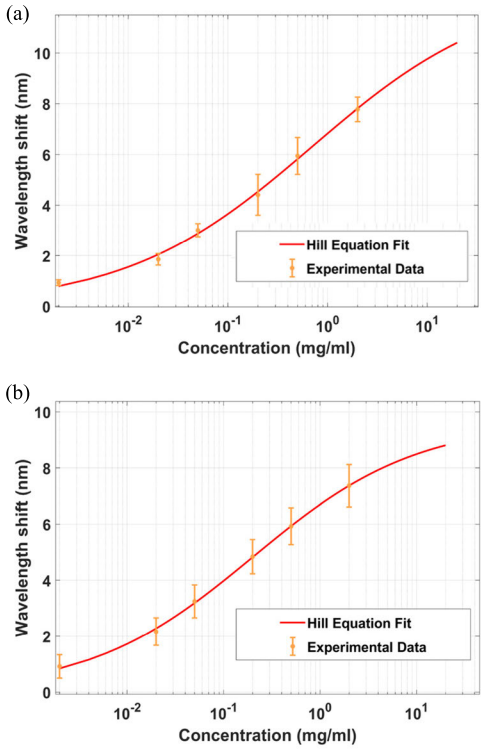

Fig. 4. Hill fitting curve of the experimental points obtained in the detection of different concentrations of rabbit IgG: (a) biosensors activated by plasma and (b) biosensors activated by UV-ozone. Both calibration curves are calculated from the results of three identical and independent biosensors ($n = 3$). The error bars indicate the standard deviation of the experimental points for the three experiments.

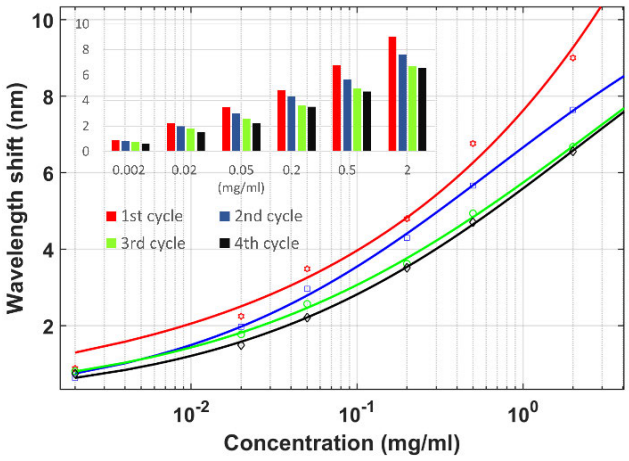

Fig. 5. Calibration curve of four experiments made in consecutive days with the same biosensor to test the reusability of the sensor. The fitting of the curves has been calculated by the Hill equation.

treatment takes just 5 min, and then, the sensor is ready for the immobilization of the bioreceptor.

In contrast, as it is described in Table I, most of the functionalization methods present in the literature require more steps and longer processing times. Specifically, a functionalization based on a silanization process requires initial surface activation, silane attachment, drying, and silane group activation for bioreceptor attachment [18], [19], [20]. As an alternative, the functionalization technique based on an alkane thiol monolayer of mercaptoundecanoic acid (MUA) is frequently used for sensors based on gold thin films [17]. However, a longer period of time is needed for the adequate deposition of MUA onto the sensing surface, which is usually made overnight [21], [22], [23], [32], [33].

The next aspect to analyze is the sensitivity. It must be noted that other structures have already attained higher values than 850 nm/RIU, the sensitivity obtained by the proposed sensor. For instance, a sensor based on a single-mode optical fiber (SMF) core mismatch interferometer has presented a 14 000-nm/RIU sensitivity in [18]. In addition, the sensitivity attained with gold thin-film-coated photonic crystal fibers using gold nanoparticles is 3951 nm/RIU, something that has been overcome by using gold nanoparticles on an localized surface plasmon resonance (LSPR)-based sensor, reaching values between 2054 and 3980 nm/RIU [20].

However, in spite of being more sensitive, there is not a considerable difference achieved in the range of detected concentration among different strategies presented in Table I. The lowest detected concentration is attained using the described high-sensitivity interferometer [18]: 0.5 and 0.1 μg/mL with the nonreported sensitivity fiber bragg grating (FBG) sensor based on cladding mode resonance [20]. Those values are just an order of magnitude lower than 2 μg/mL, reported as the lowest detected concentration in this work. The same minimum concentration has been achieved with a gold nanoparticle-based LSPR sensor (2 μg/mL) [33]. Even higher concentrations have been detected using an interferometric sensor constructed by a long thin-core single-mode fiber sandwiched between two SMFs (100–1000 μg/mL) [19] and a different LSPR sensor using nanorods (1–100 μg/mL) [23].

Given this, it is worth recognizing that the LOD parameters in the literature are excellent. According to Table I, the LOD reported with interferometers is 47 ng/mL [18], the lowest LOD for IgG detection with SPR is 37 ng/mL [40] and 0.8 nM (0.12 μg/mL) with gold nanorots-based LSPR sensor [33], while the lowest LOD is achieved with FBG based on cladding mode resonance coated with graphene oxide: 32 pM (4.8 ng/mL) [20]. The LOD reported in this work is 19.5 μg/mL with plasma activation and 31.2 μg/mL with UV-ozone activation. Contrary to most of the previous works here these values have been calculated from the calibration curve with the average value of three experiments, reflecting the deviation of the data among these three experiments.

Despite the previous considerations, there are undeniable advantages in favor of the presented functionalization in terms of reducing steps, which results in a consequent reduction of costs in both time and materials. In addition, given that the value of human IgG in normal adults relies within the range of 6–16 mg/mL [34], the sensor proposed in this work has proven its useful value for the detection of IgG molecules.

Since the plasma treatment resulted in a shorter functionalization time and yielded comparable results, the reusability of these sensors was tested using the plasma treatment.

D. Reusability

The physical and chemical resistances of TiO_2/PSS coating make it an ideal material to create reusable biosensors. In this study, the position of the LMRs has been checked during all the experimental period. In this sense, the transmission spectrum of the fiber employed during the experiment in Fig. 3 was registered after a year and it was included in Fig. S3 of the supporting document. In the same document, Fig. S4 shows

TABLE I
COMPARATIVE ANALYSIS OF DIFFERENT OPTICAL FIBER SENSORS EMPLOYED FOR THE DETECTION OF IgG BIOMOLECULES

Sensor configuration	Transduction method	Functionalization		Activation		Sensitivity (nm/RIU)	LOD	Concentration range	Ref.
		Technique	Time	System	Time				
SMF (core mismatch)	Mach Zehnder interferometer	Piranha + APTES	1 h + 1 h	Glutaraldehyde + Protein A	2 h 1 h	~14,000	47 ng/ml	0.5 - 50 μg/ml	[18]
SMF - thin core - SMF	Mach Zehnder interferometer	Piranha + APTES	30 min + 24 h	Glutaraldehyde	1 h	82.7	-	100 - 1000 μg/ml	[19]
Au film coated PCF + AuNPs	SPR + LSPR	MUA	12 h	EDC + NHS + Protein A	20 min 1 h	3,915	37 ng/ml	1 - 30 μg/ml	[32]
SMF- PMF - SMF + Au film	SPR	MUA	12 h	EDC + NHS	30 min	1,625	3.4 μg/ml	10 - 100 μg/ml	[21]
SPR image sensor	SPR	MUA	overnight	EDC + NHS	15 min	-	47.4 nM (7.1 μg/ml)	67 nM - 1 μM (10-150 μg/ml)	[22]
LSPR (AuNPs)	LSPR	PDA / MUA	30 min / 12 h	EDC + NHS	30 min	2,054 / 3,980	2 μg/ml / 6.02 μg/ml	2 - 100 μg/ml / 10 - 100 μg/ml	[23]
LSPR (Au nanorods)	LSPR	MUA	12 h	EDC + NHS	15 min	753	0.8 nM (0.12 μg/ml)	6.7 - 667 nM (1-100 μg/ml)	[33]
FBG based on cladding mode resonance (/GO coating)	FBG	Piranha + APTES / Piraña + APTES + GO	30 min + 30 min / 30 min + 30 min + overnight	Glutaraldehyde / EDC + NHS	30 min / 20 min	-	306 pM (46 ng/ml) / 32 pM (4.8 ng/ml)	0.667 nM - 670 nM (0.1 - 100 μg/ml)	[20]
LMR TiO_2/PSS	LMR	-	-	Plasma / UV-ozone	5 min / 30 min	850	19.5 μg/ml / 31.2 μg/ml	2 - 200 μg/ml	This work

A vertical slash ("/") indicates that the article compares the results obtained by two different sensors, with different functionalizations.

the results of the experimental bioassay developed a year after the fabrication of the LMR.

In addition, the reusability of an individual biosensor was analyzed in Fig. 5 by performing the complete bioassays for four consecutive days using the same LMR. The calibration curves are obtained with the Hill equation. The value of the R^2 regression coefficient exceeds 0.99 in all cases. As noted in Fig. 5, even if the sensitivity of the biosensor decreases in each cycle of detection, it still detects all the concentrations of the target molecule after four cycles. As an example, the highest concentration, 2 mg/mL, leads to a 9-nm wavelength shift in the first cycle, 7.64 nm in the second cycle, 6.68 nm in the third cycle, and 6.56 nm in the last cycle. In terms of percentages, this means a reduction of 15.8% between the first and second cycles, 10.7% between the second and third cycles, and just 1.3% between the third and fourth cycles. After four cycles, the biosensor keeps 72.9% of the initial detection capacity, as can be observed in the inset of Fig. 5.

IV. CONCLUSION

In this work, an easy-to-handle, fast, and robust real-time LMR biosensor has been developed based on the properties of the TiO_2/PSS coating. The TiO_2/PSS LMR is generated by the well-known LbL technique. Once the LMR is generated, the optical fiber is functionalized by either UV-ozone or plasma treatments. After that, the effectiveness of both plasma and ozone activation methods for the functionalization of the PSS was verified.

One of the characteristic highlights of the proposed biosensor is that it requires a short and easy functionalization protocol for the bioreceptor attachment, which is based on a physical activation of the TiO_2/PSS coating. This promotes the direct attachment of the rabbit IgG to the sensing layer surface. After that, the biosensor allows the specific and label-free detection of biomolecules. In this case, a concentration range from 0.002 to 2 mg/mL of rabbit IgG can be successfully detected. In addition, due to the chemical and physical resistance of the TiO_2/PSS coating, the biosensor presents a stable time response and reusability for at least four consecutive cycles. After the detection of rabbit IgG, a plasma activation cycle is enough to restart a complete bioassay.

The real-time detection, as well as label-free capabilities, entails a noticeable departure from the current methodology and significant cost reduction. Less time is necessary to obtain results and fewer materials are required. Furthermore, the ability to perform these detections in the visible spectrum, although reducing sensitivity, enables the use of simpler and more affordable optical equipment, thus paving the way for potential future commercialization.

Additionally, the functionalization proposed in this study could also be applied to other more sensitive optical structures and materials to achieve the detection of smaller biomolecules or molecules with a more demanding LOD.

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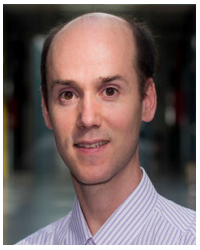

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