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Label-Free Anti-human IgG Biosensor based on Chemical Mod ification of a Long Period Fiber Grating Surface

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Abstract: This work introduces a method specially developed to produce a biorecognition element based on modified Stöber silica nanoparticles by the covalent immobilization of the human IgG. The sensing structure is based on long period fiber gratings (LPFG), specially developed to allow the interaction of the electromagnetic wave with the target analytes through its evanescent field. The surface was modified by the immobilization of the IgG-modified nanoparticles serving has recognition elements for specific target molecules. The resulting configuration was tested in the presence of anti-human IgG, recording the refractometric response of the modified LPFG in contact with different amounts of analyte. The selectivity of the sensor was also assessed.

Keywords: Optical fiber; long period gratings; evanescent field; chemical immobilization; biosensor

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1. Introduction

Biosensors are powerful allies for food safety, drug discovery, environmental monitoring, and clinical diagnosis [1–3]. The sensing methodology comprises a bioreceptor, a recognition element, and a transducer whose properties changes upon analyte binding [4]. Typically, the bioreceptor is immobilized on the surface of the transducer and the binding event can be, e.g., mechanically, electrically, or optically transduced[5,6] producing an increase in mass, a change in electrical resistivity or changes in the refractive index at the surface of the used material allowing to be measured. In recent years, optical biosensors are an active field of research worldwide, presenting rapid progress [7–9]. In this perspective, optical biosensors based on refractometric sensing schemes have been developed with great successes in the last decades [7]. Moreover, optical fibers (OF) based on evanescent wave sensing are an excellent platform to develop high-stability and high-sensitive optical biosensors [10]. The quantitative and/or qualitative measurements result from the interaction of the biorecognition element with the evanescent field of light at the fiber surface. It's good biocompatibility makes them appropriate for biochemical functionalization, creating very sensitive structures targeting viruses, drugs, and proteins [11]. In recent years, several authors have reported transduction scheme's using optical fibers for optical biosensing. *Chiavaioli* et al. reported a D-shaped single mode optical fiber (SMF) nanocoated with different metals for IgG/anti-IgG assays, reaching limit of detections (LOD) around to the femtomolar values [12]. Other authors showed a plasmon-assisted tilted fiber Bragg gratings (TFBGs) based biosensor for non-enzymatic D-glucose using polydopamine-immobilized concanavalin A [13]. More recently, *Liyanage* et al. developed a label-free sensitive tapered optical fiber plasmonic biosensor targeting microRNAs. The

sensing platform comprises different types of gold nanoparticles immobilized on the surface of the fiber to enhance the evanescent mode, followed by self-assembled ssDNA probes[14].

This work presents a method based on silica nanoparticles (prepared based on Stöber[15] method), immobilized in the surface of commercial SMF28 OF, serving has recognition elements for specific target molecules. The nanoparticles surface was functionalized by the introduction of an aminosilane (APTMS) followed by the covalent immobilization of the immunoglobulin G from human serum (human-IgG). The antibody was activated by the EDC/NHS protocol to allow the interaction of the amine exposed groups, located on the surface of the silica nanoparticles, with the activated carboxyl acid groups of the human-IgG molecules. The resulting template was immobilized onto the surface of an OF by electrostatic interactions between the negative charges of the fiber surface and the positively charged amine groups located in the IgG molecules. The sensing structure is based on long period fiber gratings (LPFGs), specially developed to allow the interaction of the electromagnetic wave with the target analytes through its evanescent field. The refractometric system comprises a Braggmetter unit (HBM, FiberSensing) working in a wavelength range from 1500 to 1600 nm, and a reference LPFG to correct possible false interactions. The resulting configuration was tested in the presence of anti-human IgG, recording the refractometric response of the modified LPFG in contact with different amounts of analyte.

2. Materials and Methods

2.1. Chemical Reagents

Silica nanoparticles were prepared following the *Stöber* method [16], using tetraethyl orthosilicate (TEOS; Sigma-Aldrich, ≥ 98%) and ammonium hydroxide solution (NH_4OH; Sigma-Aldrich, 28% m/m) as reagents. The functionalization of the nanoparticles surface was attained using the following reagents: (3-Aminopropyl)trimethoxysilane solution (Sigma Aldrich, 97%), anhydrous toluene (Sigma-Aldrich, 99.8%), phosphate buffered saline (PBS; pH 7.4, tablets, Sigma-Aldrich), 2-(N-Morpholino)ethanesulfonic acid (MES; Sigma-Aldrich), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC; Sigma Aldrich, ≥ 99%), N-Hydroxysuccinimide (NHS; Sigma Aldrich, ≥ 98%), and immunoglobulin G from human serum (human-IgG; Sigma-Aldrich, ≥ 95%). For the LPFG surface cleaning were used sodium hydroxide anhydrous (NaOH; Sigma-Aldrich, ≥ 98%) and hydrochloric acid (HCl; Sigma-Aldrich, 37%). For surface activation were used sulfuric acid (H_2SO_4; Sigma-Aldrich, 95% - 98%) and hydrogen peroxide solution (H_2O_2; Sigma-Aldrich, 30%) to perform piranha solution. For the affinity and selectivity assays were used the human IgG, the human serum albumin (HAS; Sigma-Aldrich, ≥ 98%), and the anti-human IgG (Fab specific; antibody produced in goat, Sigma-Aldrich). Ultra-pure water (type II-analytical grade, < 1 $\mu S.cm^{-1}$) and ethanol (Labchem, 96%) were also used.

2.2. Synthesis of the SiO_2 Nanoparticles

The SiO_2 nanoparticles were synthesized according with the *Stöber* method. Briefly, in a proper container the ethanol, the ultra-pure water and the TEOS, were mixed in that order. The mixture was sonicated for 20 min and was added, under stir, the ammonia hydroxide and the final mixture was stirred for 24h at room temperature. The resulted solution was centrifuged at 6000 rpm for 10 min and the beads were redispersed/centrifuged (five times) in deionized water and acetone. The resulted beads were dried at 40 °C for 12 h in the oven. The average size of the nanoparticles was determined by W130i Dynamic Light Scattering (DLS, AvidNano, UK), showing an average diameter ranging from 300-400 nm, and were evaluated by Attenuated total Reflectance (FTIR-ATR).

2.3. Immobilization of the Biorecongnition Molecule Onto the SiO_2 Surface

The dry beads were incubated in freshly prepared APTMS solution 2% (v/v) in anhydrous Toluene for 24h at room temperature in a closed container, using 10 mg/mL of beads concentration. After incubation, the nanoparticles were centrifuged at 6000 rpm for 10 min and redispersed/centrifuged for five times in acetone and ethanol. From this step resulted amino-functionalized silica nanoparticles that were verified by ATR-IR. The beads were redispersed in the PBS solution in a concentration of 5 mg/mL. A solution of the biorecognition element in MES buffer (pH 5.5) and the activation of the template was prepared by adding EDC (10x molar excess) to NHS (10x molar excess) and incubating for 30 min at room temperature. The previous prepared beads solution was added to the template solution and the pH was adjusted to 7.4-8.0. The incubation carried out for 4h at room temperature without stirring (just swirled the mixture every half-hour). The resulted modified nanoparticles were centrifuged at 6000 rpm for 10 min and redispersed/centrifuged in deionized water. The nanoparticles were assessed by FTIR-ATR.

2.4. Working Principle of the Evanescent Wave based Sensors, Long-Period Fiber Grating Fabrication and Surface Modification

In this work, a long-period fiber grating was microfabricated on the optical fiber surface. This grating works as a wavelength selective filter, displaying a spectra with several resonances resulting from the combination of the mode of the core and the different cladding modes[17]. Figure 1 shows a description of a LPFG on an optical fiber and the resultant transduced optical signal from the interaction between the recognition molecule and the target.

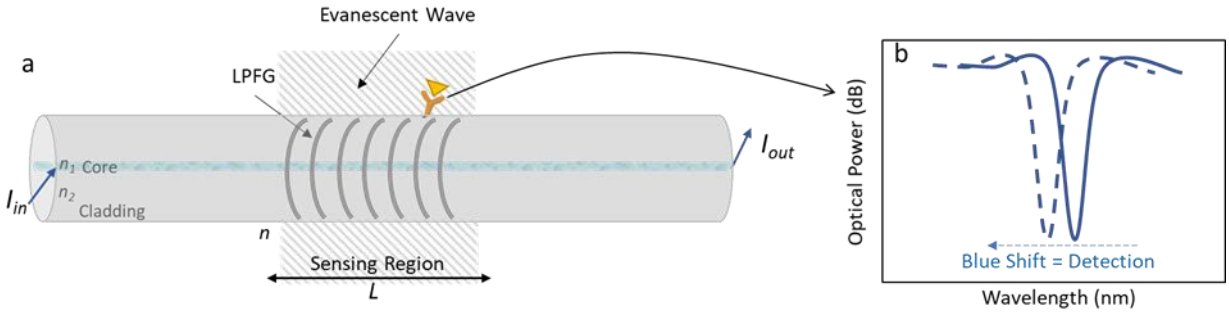


Figure 1. a) Schematic figure of a long period fiber grating; and **b**) the optical signal resultant from the biorecognition.

Moreover, the LPFGs were fabricated by the induced electric-arc technique following the protocol published by *Rego (2016)*[17] by creating a modulation in the propagating mode refractive index which, in this case, is achieved point-by-point through electric arc discharges with a current of 9 mA and a duration of 1 s along 30 to 50 mm with a period of 415 μm. Afterwards, the LPFG was chemically modified by the immobilization of the biorecognition molecule on the fiber surface. The sensitive section of the optical fiber was cleaned with a 2 M NaOH solution for 10 min followed by immersion in a 0.5 M HCl solution for 2 h. After washing with deionized water, the sensitive surface was activated with piranha solution (3:1 v/v) for 1h at 60 °C. Finally, the LPFG was washed with deionized water and kept in the oven for 10 min to completely dry and was cooled with pure nitrogen. The process is schematically presented in Figure 2.

2.5. Affinity and Selctivity Assays

The modified LPFGs were tested in the presence of the anti-human IgG in different concentrations ranging from 0.1 to 60 nM to attest the affinity of the sensing platform. To verify the selectivity of the modified optical fiber, the sensing scheme was exposed to a 60 nM solution of human IgG, a 60 nM solution of HSA and a 60 nM solution of anti-human IgG, in the same experimental conditions. In order to obtain the most trustable values,

was used a bare LPFG as a reference signal. All data will be presented as the *differential between sensing LPFG and reference LPFG*.

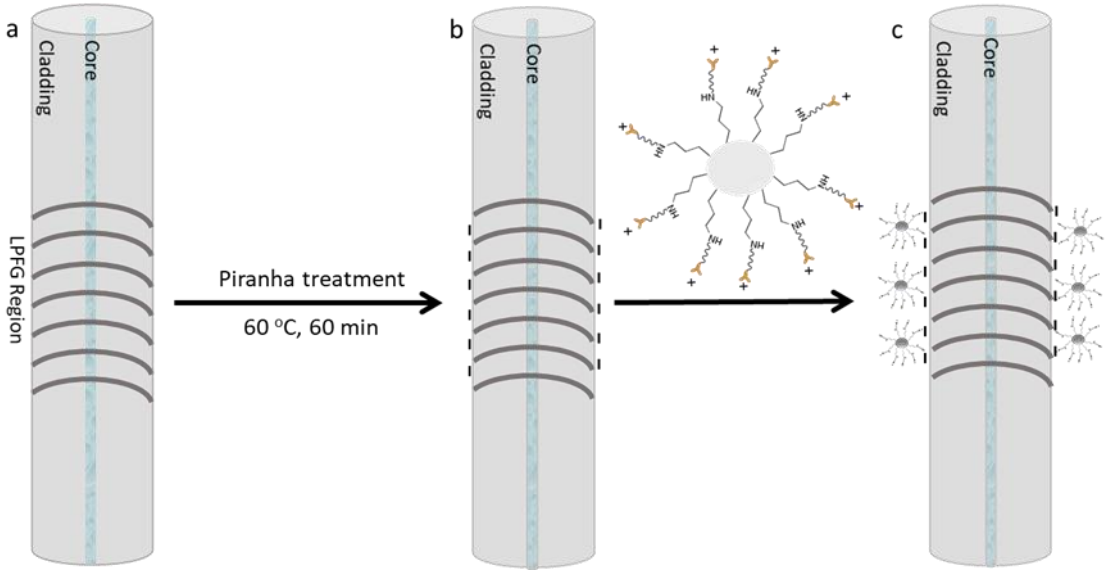


Figure 2. Schematic figure of the LPFG surface chemical modification: a) Bare LPFG; b) activated surface with negative electrical charges; and c) modified surface by the template immobilization.

3. Results and Discussion

3.1. SiO_2 Nanoparticles Bare, SiO_2-NH_2, and SiO_2-NH_2-IgG FTIR-ATR Spectra.

To confirm the introduction of the new functional groups after each step of the SiO_2 nanoparticles surface (nanoSiO_2) modification, were made FTIR-ATR analysis. Figure 3 shows the spectra of the bare SiO_2 (nanoSiO_2), the aminated SiO_2 (nanoSiO_2-NH_2), and the obtained nanospheres after the incubation of the IgG (nanoSiO_2-NH_2-IgG). In the main figure, the absorption peaks at 3000-3500 cm^{-1} are related to the stretching -OH bands, the absorption peaks at 1000-1150 cm^{-1} are assigned to the Si-O-Si asymmetric stretching bands, and the peaks at 800-950 cm^{-1} are appointed to the asymmetric bending of Si-OH. In the Figure 3a, is displayed the asymmetric deformation vibration of the -NH_2 at around 1550 cm^{-1}, suggesting that the amino groups were successfully fixed in the silica nanoparticle surface. Figure 3b show a peak at around 2900 cm^{-1} that are attributed to the presence of methyl groups of the APTMS structure. Finally, Figure 3c show the carboxylate peak at 1650 cm^{-1}, assigned to the presence of the IgG molecule. This evaluation is similar to the evaluation made by *Feifel* and his co-worker when the authors proved the possibility to create electro-active cytochrome C multilayers by using carboxyl-modified SiO_2 nanoparticles[18]. Moreover, *Hernandez-Leon* et al. also showed parallel spectra when the authors modified a core-shell SiO_2 nanobeads for capture low molecular weight proteins and peptides[19].

3.2. Affinity and Slectivity Assays

The SiO_2/IgG-modified LPFG probe was tested in the presence of different concentrations of anti-human IgG to attest the affinity of the sensing platform. The sensing LPFG (sensLPFG) was placed in an experimental chamber as well as the reference LPFG (refLPFG). Both gratings were exposed to a freshly prepared standard target solutions ranging from 0.1 to 60 nM in PBS. After 10 min of exposure time, the LPFGs were washed three times with fresh PBS and three times with deionized water. All the data was obtained measuring the LPFGs in deionized water at 22 °C. Figure 4a show the experimental data of the wavelength shift (sensLPFG – refLPFG) *versus* the anti-human IgG. Data is

reported as a mean value with standard deviation (n = 3). Other similar works were described recently such as the interferometric optical fiber biosensor for IgG/anti-IgG immunosensing presented by *Wang et al.*, reporting a limit of detection around of 50 ng/mL [20]. Other approach was demonstrated by *Han* et al. that combined a Bragg acoustic reflector with an Au electrode and an aluminum nitride piezoelectric thin film, to develop a biosensor for anti-human IgG detection by immobilization of the human IgG antibody onto the modified Au electrode. The sensing platform was able to detect anti-human IgG concentrations smaller than 0.4 mg/mL [21].

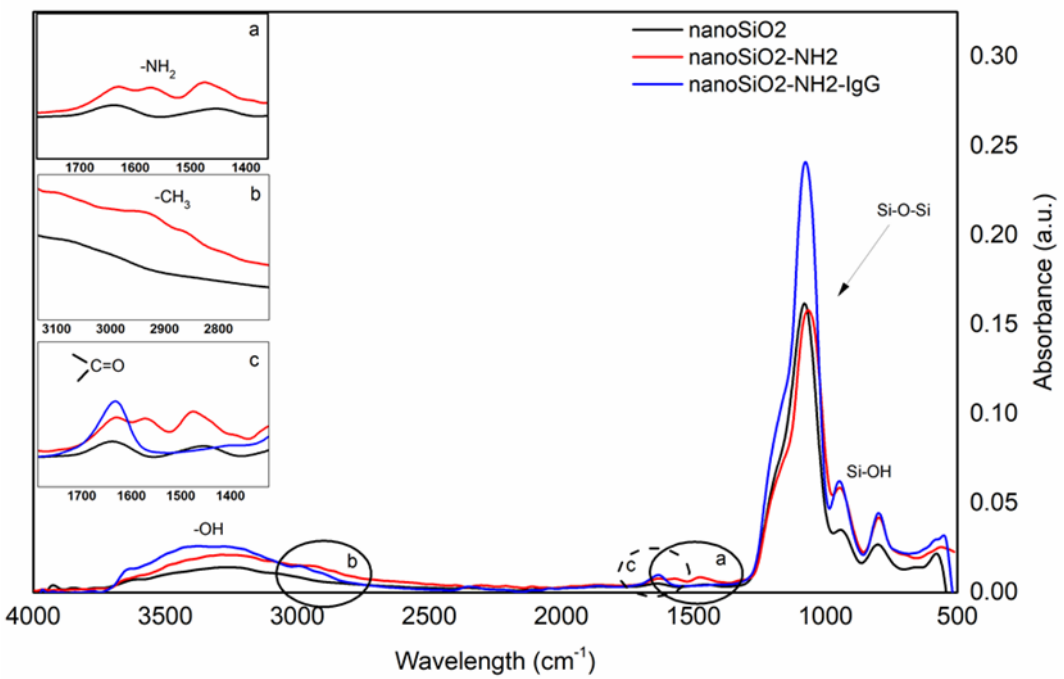
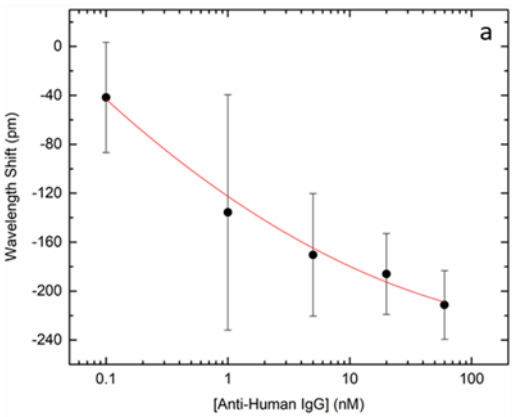


Figure 3. Obtained absorbance spectra from FTIR-ATR analysis of bare SiO_2 nanoparticles, after amino-functionalized SiO_2 surface, and after IgG immobilization.

To validate the specificity of the sensing platform, the same protocol was followed in the presence of human IgG antibody, the HSA protein, and finally the anti-human IgG. Figure 4b show the resulted data after 10 min of incubation time for each target in 60 nM (in PBS). The results are reported as a mean value with standard deviation (n = 3). These results showed the specificity of the built sensing platform to the proposed target, revealing a very relevant wavelength shift when exposed to it. By other side, the shifts showed by the LPFG in the presence of the other targets are not relevant.



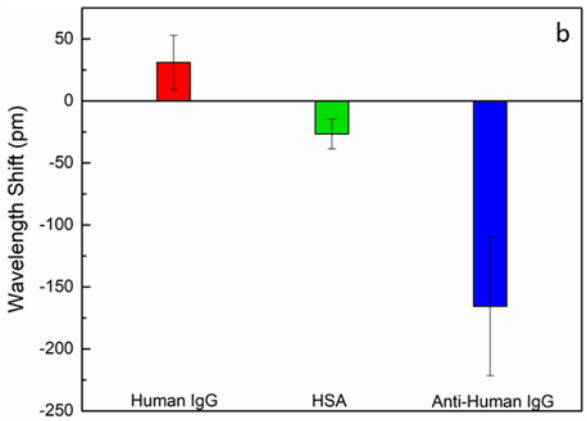


Figure 4. a) Resonance shift vs. the anti-human IgG concentration (0.1 to 60 nM). Data is reported as a mean value (n = 3) with standard deviation; b) Resonance shift obtained by 10 min incubation in 60 nM in human IgG, HSA protein, and anti-human IgG. Data is reported as a mean value (n = 3) with standard deviation.

4. Conclusion

In this work a sensing platform for the detection of the anti-human IgG antigen was developed by chemical modification of long period fiber grating surface. The sensing methodology is based on refractometric changes due to the interactions between the biorecognition molecule and the target. The surface of the optical fiber was changed by immobilization of IgG- modified silica nanoparticles. The FTIR-ATR spectra proved that the biorecognition molecule was successfully attached onto the SiO_2 nanoparticles surface, and specificity assays demonstrated the selectivity of the method. As next step, we aim to imprint molecularly the analogue synthetic molecule of this template. The goal is to produce highly sensitive and selective molecularly imprinted polymers using the template of this work as a target molecule carrier.

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

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