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Towards sensitive label-free immunosensing by means of turn-around point long period fiber gratings

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ABSTRACT

Long period fiber gratings have been effectively used in the field of biochemical sensing since a few years. Compared to other well-known label-free optical approaches, long period gratings (LPGs) take advantage of the typical peculiarity of optical fibers. Coupling the propagating core mode with a high-order cladding mode near its turn-around point (TAP) was the strategy adopted to achieve good performances without additional coatings, except for the sensing and selective biolayer deposited on the fiber. Both the modeling and manufacturing of TAP LPGs were discussed. After the functionalization of the fiber surface with the deposition of a Eudragit L100 copolymer layer followed by immunoglobulin G (IgG) covalent immobilization, an IgG/anti-IgG bioassay was implemented along the grating region and the kinetics of antibody/antigen interaction was analyzed. A quantitative comparison between a TAP LPG and a non-TAP LPG was carried out to highlight the improvement of the proposed immunosensor. The real effectiveness and feasibility of an LPG-based biosensor were demonstrated by using a complex matrix consisting of human serum, which also confirmed the specificity of the assay, and a limit of detection of 70 μg L^{-1} (460 pM) was achieved.

1. Introduction

Label-free optical biosensors are increasingly finding applications in fast, reliable and in situ measurements in the field of chemical, biochemical and biomedical sensing and nowadays are certainly among the most fascinating and groundbreaking fields of research (Wang and Wolfbeis, 2013). Based on the measurement of refractive index (RI) changes, induced by a chemical and/or a biochemical interaction with a sensing layer deposited on a suitable substrate, they are able not only to allow the quantitative measurement of the investigated analyte, but also to provide the unique chance to analyze the dynamic interactions. Among all the optical approaches providing a sensitive and accurate measurement of RI, those based on surface plasmon resonance (SPR) are the most widespread ones (Homola, 2008; Estevez et al., 2014; Voisin et al., 2014), with values of resolution down to 10^{-7} RI units (RIU) (Piliarik and Homola, 2009). Other interesting and competitive approaches are based on interferometric configurations, implemented on optical fibers and planar waveguides, and on resonating structures (Fan et al., 2008; Kindt and Bailey, 2013).

Optical fiber long period gratings (LPGs) constitute a novel promising class of label-free optical biosensors (Eftimov, 2010; Baldini et al., 2012). An LPG is characterized by a series of periodic RI changes of the order of hundreds of μm in the core of a single-mode optical fiber, capable to create a modulation pattern that runs along the fiber axis; this property provides the condition for a wavelength-dependent coupling between the propagating core mode and the cladding modes. Consequently, one or more attenuation bands characterize the transmission spectrum of an LPG, with the minimum of each band representing the coupling with a selective cladding mode that occurs at a well-defined wavelength, the resonance wavelength λ_{res} (Erdogan, 1997). Compared to the other well-known optical approaches, LPGs exploit the peculiarities of optical fibers, such as compactness, lightweight, intrinsic miniaturization, high compatibility with optoelectronic devices used in the telecommunication windows (i.e. 1.3 μm and 1.5 μm), as well as multiplexing and remote measurement capabilities since the signal is spectrally modulated. Therefore, LPG-based biosensors can become a real alternative to the more consolidated label-free approaches when the advantages of the use of optical fibers can play a fundamental role (e.g. in

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distributed sensing). The literature until now accounts for several examples of LPG-based chemical sensors and biosensors (Shevchenko et al., 2011; Chiavaioli et al., 2014; Deep et al., 2012; Korposh et al., 2012; Pilla et al., 2012a; Tripathi et al., 2012; Yang et al., 2012; Lepinay et al., 2014; Voisin et al., 2014).

When a chemical or biochemical sensing layer is deposited onto the grating region, the interaction with the investigated analyte gives rise to a modification of the RI. In this case, only a portion of the optical radiation, which comes out of the LPG, is affected, depending on both the thickness of the interaction region and the penetration depth of the evanescent field related to the coupled cladding mode (Baldini et al., 2012). One of the reasons that has prevented LPG-based biosensors from reaching competitive values of limit of detection is the lack of a reliable and effective mechanical and thermal stabilization in the measuring setup. In fact, an LPG sensor is also sensitive to strain and temperature and this dependence greatly affects the accuracy of the RI measurements. With this in mind, some examples of compensated RI measurements were proposed in the literature (Trono et al., 2011; Garg et al., 2013). Moreover, it should be noted that the best performance of these devices in terms of RI sensitivity is achieved for a surrounding RI close to the RI of fiber cladding (i.e. 1.44–1.46 RIU) (Patrick et al., 1998), far from the RI range of the aqueous samples (i.e. 1.33–1.34 RIU) which are generally used in biosensing applications. In order to overcome this problem, two different approaches were proposed. In the first approach, a thin overlayer of RI higher than the cladding RI is deposited on the fiber. By selecting a suitable value of both the RI and thickness of the overlayer, a great increase of the RI sensitivity was observed for measurements in aqueous solutions (Del Villar et al., 2005; Pilla et al., 2012b). Despite the outstanding novelty of this approach, the deposition of an overlayer makes the sensor manufacturing process longer and adds additional time-consuming chemical treatments onto the fiber. The second approach consists of coupling the propagating core mode with a high-order cladding mode near its turn-around point (TAP) (Ramachandran et al., 2002), without the application of any additional layer between the fiber and the surrounding medium. In this case, the relationship between the grating period and the resonance wavelengths, at which the coupling between the propagating core mode and the cladding modes takes place, is described by the phase-matching curves (PMCs). Typically, the slope of PMCs for higher order mode exhibits a change in sign from positive to negative and thus, for a specific grating period, the transmission spectrum of the LPG exhibits two resonant bands characterized by two resonance wavelengths. TAP is defined as the point in the PMC of a specific cladding mode of an LPG at which the two resonant bands of the same cladding mode merge into a broader resonant band. The TAP wavelength is different for each cladding mode and decreases with the increase of the mode order. It was proved that the highest RI sensitivity of LPGs is achieved around the TAP (Shu et al., 2002). Furthermore, it is possible to perform a differential measurement, i.e. the spectral difference between the minima of the two bands, which increases the total shift of λ_{res} during the RI measurements. Henceforth, the measured parameter becomes the spectral difference $\Delta\lambda_{res}$ between the λ_{res} at longer wavelengths (hereafter called red resonance wavelength λ_{RED}) and the λ_{res} at shorter wavelengths (hereafter called blue resonance wavelength λ_{BLUE}).

In the present work, a TAP LPG for sensitive label-free biosensing is proposed and fully characterized. After the functionalization of the fiber surface using Eudragit L100 copolymer, an IgG/anti-IgG immunoassay was performed in buffer and the kinetics of antibody/antigen interaction was analyzed and a quantitative comparison between the TAP LPG and a non-TAP LPG was carried out. Finally, in order to assess the real effectiveness and feasibility of an LPG-based biosensor, the calibration curve of the immunosensor was achieved, using a complex matrix such as human serum that also proved the specificity of the assay.

2. Materials and methods

2.1. Chemicals

Ethanol (EtOH), bovine serum albumin (BSA) and all the reagents for buffer preparation (phosphate-buffered saline (PBS), 40 mM, pH 7.4) were purchased from Sigma-Aldrich (Milan, Italy). Glycerol was purchased from RPE-ACS Carlo Erba Reagents (Milan, Italy). The methacrylic acid/methacrylate copolymer (Eudragit L100) was purchased from Evonik Degussa GmbH (Düsseldorf, Germany). Mouse IgG and goat anti-mouse-IgG were purchased from Zymed Laboratories, Invitrogen Immunodetection (Milan, Italy). 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were purchased from Pierce (Illinois, USA). A monoclonal antibody anti-PSA (prostate-specific antigen) was kindly provided by EXBIO (Praha, Czech Republic). Human serum (C Reactive Protein Free Serum) was purchased by HyTest Ltd. (Turku, Finland).

2.2. Manufacturing of the TAP LPGs

The TAP LPGs used in this experiment were inscribed in a standard single-mode fiber (SMF28e, Corning Inc.). The fiber was hydrogen loaded at a pressure of 1500 psi (10.34 MPa) and a temperature of 100 °C for 48 h in order to increase the fiber photosensitivity. A point-to-point technique was used for the manufacturing of LPGs (Hill and Meltz, 1997) by means of a KrF pulsed Excimer laser (Braggstar-500, Tuilaser, Germany) operating at a wavelength of 248 nm. LPGs with dual resonant bands were written and then the two resonance wavelengths were adjusted up to their conversion into a one broader resonant band (i.e. TAP) by reducing the diameter of the fiber cladding. The results of the theoretical modeling and of the final manufacturing of TAP LPGs are explained in Section 3.1.

2.3. The sensing layer and the bioassay protocol

The functionalization of the optical fiber in correspondence of the LPG was achieved by the deposition of a layer of Eudragit L100 consisting of approximately a 1:1 ratio of ester groups and free carboxyl functional groups (–COOH) useful for antibody immobilization. The Eudragit L100 was dissolved (0.04 w/v%) in ethanol and this solution was used for coating the fiber surface by immersion for 1 min and evaporation in air (15 min).

Once the TAP LPG was functionalized, the optical fiber was placed inside a temperature-stabilized closed flow cell with a total volume of 50 μL (Trono et al., 2011). All the steps for the implementation of the immunoassay were performed using the flow cell connected to a peristaltic pump and keeping the temperature of the flow cell at (23 ± 0.1) °C. The followed bioassay protocol was already described in a previous publication (Chiavaioli et al., 2014). The preparation of the biolayer consisted of the following steps: activation of –COOH groups by cross-linking chemistry (EDC and NHS), covalent immobilization of mouse IgG (1000 mg L^{-1} in PBS), washing with PBS for removing the un-reacted antibodies, and surface passivation with BSA (3% in PBS) in order to block the remaining activated carboxylic groups and to prevent non-specific adsorption onto the surface.

As preliminary characterization and for comparison purposes with a non-TAP LPG, the antigen binding step was performed in PBS. An anti-PSA antibody was used as negative control at a concentration of 1 mg L^{-1}. In order to validate the performance of the TAP LPG as biosensor, the assay was also performed in