Long Period Grating based Biosensing Technology for the Detection of Vitamin D3

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Abstract: The label-free detection of vitamin D by employing fiber optic biosensor is reported in this work. Here, the long period grating (LPG) which is inscribed into a specialty fiber i.e., double cladding fiber (DCF) having W-type refractive index profile, is basically employed as a foundation for biosensing. The wet-chemical etching is performed to the outer cladding of the fiber to induce mode transition phenomenon which resulted in an enhanced sensitivity of -1400 nm/RIU towards surrounding medium along with significant visibility (> 15 dB) of grating's resonance band. The sensing surface of an LPG device is then coated with a graphene oxide (GO) layer of nanometric thickness providing carboxylic functional groups for grafting of 25-OH-D3 specific antibody for vitamin D3. Finally, the detection of vitamin D3 was tested using concentrations in buffer solutions within the clinical range of 1-100 ng/mL resulting a limit of detection (LOD) lower than 1 ng/mL.

1 INTRODUCTION

Vitamins are considered as an active component that are vital for metabolism and acts as a biocatalyst that regulates physico-chemical reactions in the body. They plays an important role in protein, fat and carbohydrate metabolism, immunity against infections, digestive functions, and healthy development of an overall body (Adela et al., 2016). The bioavailability of vitamins can be affected by biochemical changes that occur during the handling of foods (Al-Ani, 2006). Various factors such as oxygen, sunlight, interaction with metals, air pollution, temperature variation, etc. could be a probable source for the disruption of vitamins structure. Therefore, when vitamins intake is deficient inside the body, disorders occur in metabolism that in turn affects the overall body condition.

The deficiency of Vitamin D being considered as a global health concern posing a several serious health impact such as bone disorder, osteoarthritis, hypertension, diabetes (Dalgard, Petersen, Weihe, & Grandjean, 2011), tuberculosis, cardiovascular diseases (Judd & Tangpricha, 2009), infectious diseases, risk of cancers, and neuropsychiatric disorders (F Ozbakir & A Sambade, 2016). Recent research has also correlated a vitamin D deficiency with patients affected by COVID-19 (Jain et al., 2020). The recent work also showed that peoples having lower level of vitamin D, developed more inflammatory markers (i.e., ferritin and D-dimer) that are linked to poor COVID-19 outcomes.

Out of the available two major forms of Vitamin D i.e., Vit-D2 and Vit-D3, the most preferred one is Vit-D3 form (Armas, Hollis, & Heaney, 2004) whose one of the analytical forms i.e., 25-OH-D3 is regarded as the best parameter to indicate the level of Vitamin D. Since 25-OH-D3 has a higher serum concentration

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(~ 99%) and a longer half-life (about three weeks), hence it is measured during clinical diagnosis of Vitamin-D (Rezayi et al., 2018). Therefore, human plasma/serum 25-OH-D3 is widely used as a biomarker (Chauhan & Solanki, 2019; Pilz et al., 2019). The standard physiological range of Vit-D is 30-70 ng/mL, and a value < 30 ng/mL indicates a deficiency of Vitamin D. Hence, regular quantitative assessment of levels of Vitamin D in the body fluid can help avoiding nutritional deficiencies and prevents irreversible damage caused to vital organs.

Due to the high hydrophobicity nature of 25-OH-D3, the direct quantification of it in a serum sample is a quite complicated task and hence it requires dissociation from its carriers to be available for the recognition by means of specific bio-receptors. The techniques available for detecting and monitoring the Vitamin D concentration are namely mass spectrometry, high pressure liquid chromatography (HPLC), radioimmunoassay (RIA), enzyme linked immunosorbent assay (ELISA), and non-isotopic automated analyzer (Enko, Kriegshäuser, Stolba, Worf, & Halwachs-Baumann, 2015; Jafri et al., 2011; Snellman et al., 2010; Yin, Yang, Wu, Li, & Sun, 2019) which can detect in the range of sub nM. The main drawbacks of these techniques are non-economical, requires a highly qualified technician, longer analysis time, resource consuming (He, Gleeson, & Fraser, 2013), and hence does not fit to be employed as a point-of-care testing (PoCT) device.

In recent years, researchers have been paying their attention on the development of optical biosensors for the detection of Vitamin D and trying to make it suitable to be employed as a point-of-care testing platform (Zhang, Palit, Liu, Vaziri, & Sun, 2020). There are few research studies available regarding the type of sensors for the detection of Vitamin D. Recently, a photodetector-based vitamin D3 detection using Ag nanoparticles covered Er-doped TiO_2 nanowires (Dhar Dwivedi et al., 2020) was developed with a possibility to detect a concentration of 30 pg/mL of vitamin D3 in ethanol. A plasmonic biosensor for the detection of Vitamin D3 was also developed based on surface-enhanced infrared absorption spectroscopy which made them to detect the concentration as low as 86 pM (34 pg/mL), (Di Meo et al., 2020). Also, recently an all-optical based SPR sensor platform was developed for smartphones that utilizes a planar-optical waveguide structure integrated into a polymer chip (Walter, Alwis, Roth, & Bremer, 2020) wherein the detection down to 25 nM (10 ng/mL) was found in samples containing 10% of Vitamin D3 depleted human serum using an AuNP-enhanced aptamer-based assay.

This work is reported by considering an important class of an optical biosensor that is based on fiber optics technology specifically long period gratings (LPG). LPGs are in-fiber devices that are inscribed through a periodic period perturbation (typically, 100s of μm) of the refractive index, and/or geometry of an optical fiber. This results into the appearance of a series of discrete attenuation bands in the fiber transmitted spectrum, each one of which is associated to the power coupling between the fundamental propagating core mode and forward co-propagating different cladding modes. These resonance wavelengths are characterized by an intrinsic sensitivity to the surrounding refractive index of the medium by interacting with the cladding mode's evanescent waves (Esposito, 2021; Esposito, Srivastava, et al., 2021). This aspect of an LPG sensitivity is being utilized as a biosensing device e.g., DNA (Gonçalves et al., 2016), viruses (Janczuk-Richter et al., 2020), bacteria (Kaushik et al., 2019), immunoglobulins (Chiavaioli et al., 2015), and other biomarkers.

This work reports about the label-free detection of vitamin D3 by employing fiber optic biosensor specifically long period grating that is inscribed into double cladding fiber (DCF) having W-type refractive index (RI) profile. In general, focusing on higher sensitivity along with maintaining the grating's band visibility, stability and repeatability is the biggest concern amongst scientific communities (Dey, Biswas, Basumallick, & Bandyopadhyay, 2021). We addressed this concern by proposing the above-mentioned configuration of DCF based LPG wherein the proposed device presents high sensitivity, by tuning the working point towards the mode transition region by performing wet-chemical etching to the outer cladding of the fiber, which finally resulted in an enhanced sensitivity of -1400 nm/RIU along with significant visibility (> 15 dB) of grating's resonance band. The better band visibility and stability could be attributed to the non-use of additional layer of coating as the transition is achieved without any additional overlay due to the DCF fiber properties itself. Later-on, the LPG device is then coated with a nanosized layer of graphene oxide (GO) providing carboxylic functional groups for grafting of 25-OH-D3 specific antibody for vitamin D3. Finally, the detection of vitamin D3 was tested using concentrations in buffer solutions within the clinical range of 1-100 ng/mL resulting a limit of detection (LOD) lower than 1 ng/mL.

2 DEVELOPMENT OF FIBER BIOSENSOR

The development of fiber optic biosensor presented here involves an LPG inscribed in a DCF fiber having W-shaped RI profile. The sensing surface of the device is then coated with graphene oxide having a layer of nanometric thickness for the covalent immobilization of the capture antibody for Vitamin D3, as shown in Figure 1. The further sub-sections will be dealing with the sensor inscription and optimization of the working point towards the mode transition region, as well as the deposition of GO onto the same.

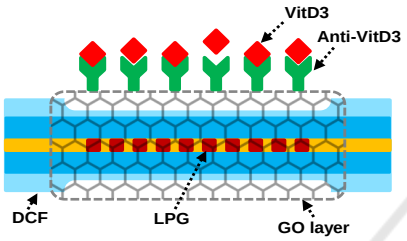

Figure 1: Schematic of DCF-LPG based biosensor coated with GO for the detection of vitamin D3 (not on scale).

2.1 Sensor Fabrication

The long period grating sensor was developed by employing a specialty fiber i.e., Nufern S1310 fiber (USA) which is a single-mode DCF with W-shaped RI profile having pure silica core and outer cladding whose diameters are similar with those of standard SMF28 fiber i.e., 8 µm and 125 µm, respectively. It also consists of a fluorine-doped inner cladding having diameter of about 95 µm. The other specifications of this fiber are Core NA = 0.12 and MFD = 10.4 ± 0.8 µm at wavelength of 1.55 µm.

A platform based on electric arc discharge technique (EAD) was employed to inscribe the LPGs whose details can be referred to our previous researches (Esposito, Ranjan, Campopiano, & Iadicicco, 2018; Esposito et al., 2019). Specifically, the LPG fabrication was done based on the design discussed in (Esposito, Sansone, Srivastava, Baldini, et al., 2021). The grating period Λ of 400 µm was used to inscribe the grating which created the power coupling with 6th and 5th order cladding modes in the wavelength range of 1200-1650 nm. The transmission spectrum of the fabricated LPG is shown in Figure 2(a) with orange line, where a deep attenuation band around resonance wavelength of 1375 nm (due to 6th mode) and a smaller one at resonance wavelength of 1225 nm (5th) can be clearly observed. The total length for this device is shorter than 20 mm.

Here, the mode transition phenomenon were induced in DCF-LPGs to significantly enhance the surrounding refractive index sensitivity of the device through wet chemical etching of the outer cladding (Cusano et al., 2006; Esposito et al., 2020). During the etching process, the fiber outer cladding was slightly thinned down to a diameter of about 107 µm, by using a 24% v/v hydrofluoric acid (HF) solution. The real time acquisition of an LPG transmission spectra during etching process were performed which are shown in Figure 2(b), wherein it is clearly evident that the working point of our transducer was tuned to a mode transition region, i.e., the area with the maximum slope (when in water-like environment).

The transmission spectrum of the LPG obtained after etching procedure is shown in Figure 2(a) represented by yellow line, which exhibits a resonance wavelength around 1560 nm with a transmission depth of > 15 dB. The surrounding refractive index (SRI) sensitivity of the LPG under test near water RI (i.e., biological type solutions) was measured to check the effectiveness of the etching process, which was found to be at -1400 nm/RIU. The sensitivity which we found here is increased in comparison to our previous research (Esposito, Sansone, Srivastava, Baldini, et al., 2021) which also shows the improved efficiency of our etching protocol.

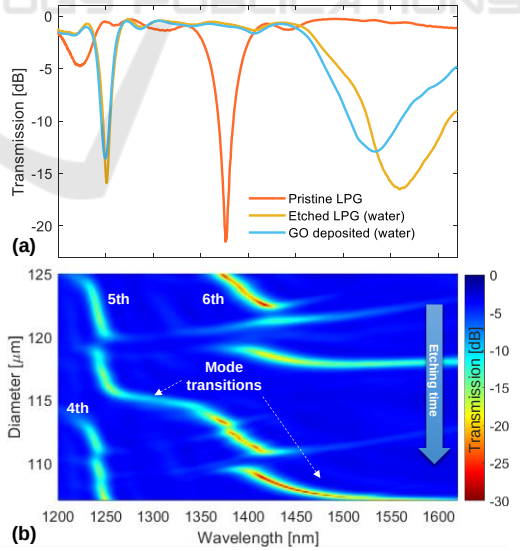

Figure 2: (a) A plot of transmission spectra of LPG (Λ = 400 µm): after inscription (Orange), after etching (Yellow) in water, after GO deposition (Blue); (b) Evolution of resonance wavelengths with respect to the fiber diameter during etching process.

2.2 Layering of GO

In recent years, graphene oxide has been gaining wider interests amongst scientific communities, specifically in biological domains due to its exciting features in terms of density, chemical inertness, and very high hydrophilicity. It also has excellent surface functionality, amphiphilicity, aqueous solubility, and fluorescence quenching ability.

Hummer's technique was employed to perform the synthesis of graphene oxide involving the process of oxidation of graphite by mixing the solution of $KMnO_4$, H_2SO_4 and graphite, as discussed in our previous research (Esposito, Sansone, Srivastava, Baldini, et al., 2021). A GO dispersion of about 4 mg/mL in water was coated around the sensor surface by a multi-dip coating procedure involving 12 dips.

The resulting transmission spectrum of the LPG after the GO deposition, as shown in Figure 2(a) represented by blue line, acquired in water. The resultant resonance band after acquired in water was blue shifted to the wavelengths of about 25 nm while its transmission depth was reduced to about 13 dB, due to the high RI of graphene oxide (1.8–2.0, as from ellipsometry measurement using VASE, J. A. Woollam, USA) and considerable value of extinction coefficient (Esposito, Sansone, Srivastava, Baldini, et al., 2021; Esposito, Sansone, Srivastava, Cusano, et al., 2021). We also observed the morphology of the material by employing SEM (Scanning Electron Microscope) images of the GO that is deposited around the etched DCF-LPG at the magnification of 5000x, taken by means of Quanta 200 FEG (FEI, USA), as shown in Figure 3. It confirms the homogeneity of the deposition around the fiber surface.

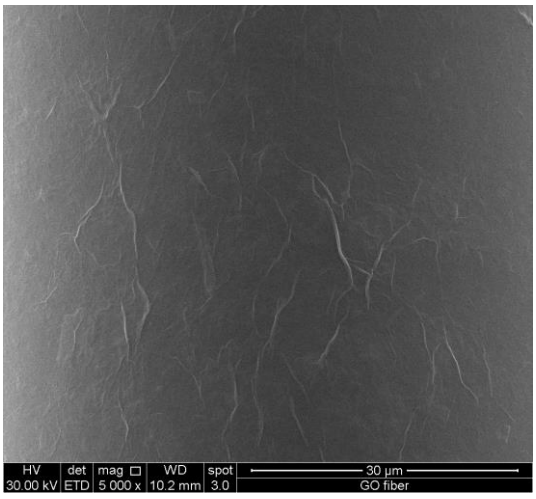

Figure 3: SEM image of GO-coated DCF-LPG.

So, finally based on the obtained spectra, we can remark that the final sensor is achieved with a high sensitivity due to efficient processing of mode transition and better visibility of the resonance band due to the all-silica structure of DCF.

3 EXPERIMENTAL SETUP FOR BIOSENSING

The experimental setup employed for the testing of biosensor involves the conjugation of two system, i.e., optoelectronic unit and microfluidic unit, whose schematic representation is shown in Figure 4.

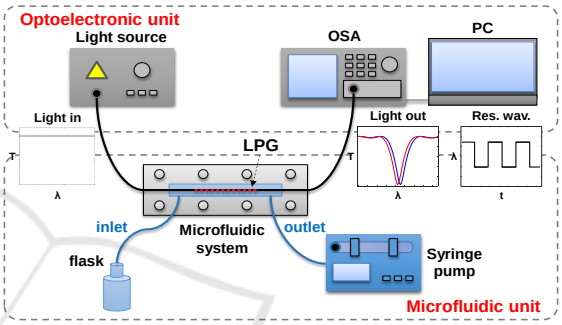

Figure 4: Schematic of biosensing test setup.

Here, the LPG under test was positioned into the microfluidic system which consists of a metallic base with 35 mm long channel with a cross-sectional area of 1.5 mm^2 (i.e., roughly 80 µL volume). A PMMA cover and wax film was used to firmly seal the case by fixing it through series of screws. There is provided with a cover with two holes for inserting the pipe meant for in-letting and out-letting the different biological solutions into the system by using a syringe pump (Pump 11 Elite, Harvard Apparatus, USA) for effective withdrawal procedure. The biosensing tests were performed under controlled temperature conditions i.e., a fiber Bragg grating sensor was used to monitor the temperature inside the system while a K-type thermocouple was placed outside the system to monitor the temperature outside.

Regarding the optoelectronic unit, a SLED broadband light source (1200-1650 nm) and an optical spectrum analyzer (OSA AQ6370B, Yokogawa JP) were used to monitor the real time acquisition of LPG spectra and later-on these raw spectral data were processed to retrieve the variations in resonance wavelength of the grating.

4 EXPERIMENTAL TEST

It involves the experimental procedure for the testing of the DCF-LPG based biosensor which includes the results and discussion of the biofunctionalization procedure for the covalent immobilization of the capture antibody, anti-vitamin D3, and finally the detection of the analyte, vitamin D3.

4.1 Biofunctionalization Procedure

Graphene oxide is very much suitable candidate to be employed as an excellent platform for the immobilization of biomolecules since it is consisted with carbon atoms that is arranged in a 2-Dim honeycomb lattice structure and exhibits functional groups that can be effectively used for the immobilization of biomolecules (Loh, Bao, Eda, & Chhowalla, 2010). These above-mentioned groups can be interactive enough towards ionic, electrostatic or covalent nature of the molecules, which shows a better extraction efficiency of biomolecules per unit area (Loh et al., 2010).

Here, for our biofunctionalization purpose, we activated the carboxylic groups of GO by immersing a 400/200 mM EDC/NHS solution in sodium acetate buffer (NaAc, 20 mM pH 5.5) at a flux of 30 µL/min rate for 15 minutes (Vashist, 2012). Later-on, the covalent immobilization of the biological recognition element (BRE) was performed with antiVit-D3 having concentration of 15 µg/mL in NaAc (monoclonal antibody VitD3.2F4, Bioventix UK) for 1.5 h by maintaining a flux of 10 µL/min. The sensor response curve of variation in resonance wavelength with respect to time during biofunctionalization procedure confirms an effective antibody immobilization as shown in Figure 5, wherein a noticeable blue shift of a few nm was clearly evident.

Finally, the passivation step was performed by utilizing a 3% bovine serum albumin (BSA) solution in phosphate buffered saline (PBS, 10 mM, pH 7.4) for the duration of 15 minutes at the flux of 30 µL/min to minimize non-specific adsorption (Vashist, 2012). We performed a washing after each step in the corresponding buffer solution at the flux of 100 µL/min to remove or minimize any type of unattached elements.

So, at the end, we can remark that when the sensor was rinsed in PBS no significant change was noticed, as the passivation was not effective due to the presence of very few non-specific absorption sites, i.e., the sensor surface was mostly covered with the antibodies.

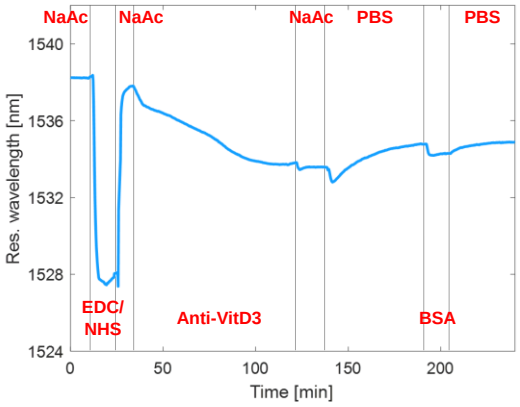

Figure 5: Sensor response curve of variation in resonance wavelength during antibody immobilization.

4.2 Detection of Vitamin-D3

To our aim of the detection of vitamin D3, different concentrations of it was used ranging from 1-100 ng/mL in PBS. The flux rate for each concentration test was set to 10 µL/min for the duration of 40 minutes. The washing procedure in PBS was performed after every concentration test at flux rate of 100 µL/min to remove any leftover or detached vitamin. The sensor signal was then monitored in the same conditions (in PBS) for all the concentrations under consideration, to remove any contribution related to the different RI of the testing solutions.

After measuring the sensor signal which was immersed in PBS solution corresponding to zero concentration as a reference, the different vitamin-D3 concentrations were then injected into the microfluidic channel and we then observed a blue shift in the resonance wavelength whose magnitude was dependent upon the concentration of vitamin-D3, and was found in agreement with the LPG sensing principle (James & Tatam, 2003). After getting rinsed with PBS and measured the LPG signal, the vitamin D3 concentration response curve of the biosensor was obtained and shown here in Figure 6 where each experimental point is analyzed and expressed in terms of mean value and 3σ (error bars) within twenty acquisitions. Resonance wavelength shifts comprised in 0.07-0.61 nm (negative values) were measured for concentrations ranging between 1-100 ng/mL and a limit of detection (LOD) (according to (Chiavaioli, Gouveia, Jorge, & Baldini, 2017)) found to be lower than 1 ng/mL, which is among the lowest achieved so far by any optical biosensors for the label free detection of vitamin D (Carlucci et al., 2013). Negative control test was also performed by using an LPG sensor without antibody and maximum resonance wavelength shift was only around –0.03 in

this case, demonstrating that the results reported in Figure 6 are due to selective detection of Vit-D3 by means of antiVit-D3 (Esposito, Sansone, Srivastava, Cusano, et al., 2021).

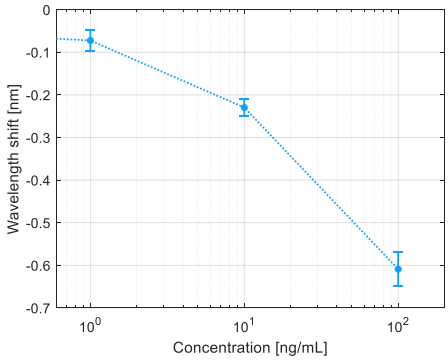

Figure 6: Biosensor response curve in terms of resonance wavelength shift with respect to vitamin D3 concentration.

5 CONCLUSIONS

A fiber optic biosensor platform is developed for the label free detection of 25-OH-D3 form of vitamin D3 based on DCF based LPG technology. The LPG is inscribed into a specialty DCF fiber whose outer cladding diameter is subjected to wet chemical etching, to make the sensing device working towards mode transition region in turn significantly enhance the sensitivity which is found to be about -1400 nm/RIU while maintaining a good visibility (> 15 dB) of the resonance band. The fiber sensing surface was then covered with few nm GO layers providing -COOH functional groups which were activated by EDC/NHS cross-linking chemistry and allowed the covalent immobilization of the 25-OH-D3 specific antibody. The final device was then tested by employing a microfluidic setup, wherein vitamin D3 concentrations in PBS within the clinically relevant range of 1-100 ng/mL were injected into the microfluidic channel, and a very low LOD below 1.0 ng/mL was achieved that can be the future consideration for medical domain.

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