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ABSTRACT

In this work, a TiO_2 coated etched long-period grating (e-LPG) fiber optic biosensor is developed for the detection of *Escherichia coli* (*E. coli*) bacteria in food items. Label-free *Escherichia coli* bacteria monitoring is done over the detection range of 0 cfu/ml–50 cfu/ml using an advanced spectral interrogation mechanism. The thin film deposition of 40 nm TiO_2 over the e-LPG is confirmed by the microscopy method, such as scanning electron microscopy. In our proposed biosensor design, T4-bacteriophage is covalently immobilized over the TiO_2 coated fiber surface. This biosensor system has reached sensitivity at 2.55 nm/RIU. Our experiments confirm the resolution and the limit of detection ($3\sigma/S$) of 0.0039 RIU and 10.05 ppm, respectively. The proposed biosensor with enhanced sensitivity is suitable for monitoring harmful pathogens/infectious agents in various food products.

I. INTRODUCTION

E. coli is the most common bacteria found in food items and water whose consumption is harmful to human beings.[1–5] Earlier, the monitoring of such bacteria is done using antibodies,[2,3] nucleic acids,[4,5] bacteriophages,[6] etc. To detect such harmful pathogenic bacteria, various research groups have followed both label and label-free detection methods. Specific dyes are used in the label detection method,[6] making this method more costly and complicated. In the label-free sensing method,[1] changes in the sensor surface are monitored, such as the variation in the analyte refractive index ($n_{A\text{-}RI}$) or variation in thickness of the bio-layer coated on the fiber. These surface changes modify the optical properties of the optical sensor, mainly resulting in the resonating wavelength shift. Hence, a label-free sensor helps in the real-time monitoring of bacteria effectively.

In Ref. 7, Bragg grating, especially long-period grating (LPG), has been used for label-free biochemical sensing. The authors have fabricated two LPG based fiber optic sensors (LPG-A: grating period = 615 μm; length: 2.46 cm, and LPG-B: grating period = 370 μm; length: 2.035 cm) demonstrating limits of detection (LODs) ~ 7.6 mg l^{-1} and 0.5 mg l^{-1}, respectively. Here, bacteria interaction is monitored through an IgG/anti-IgG bioassay. Another interesting work is performed in Ref. 8, where *E. coli* bacteria detection is done with an optical deforming method. Here, a tapered optical fiber with dielectrophoretic (DEP) trapping is used for the rapid label-free detection of bacteria in water. The authors have achieved 95 200% RIU (refractive index unit) sensitivity and 5.2×10^{-6} RIU (at 0.5% intensity stability) detection limit, respectively.

In this paper, a TiO_2 coated e-LPG biosensor is developed using a single-mode optical fiber (SMF-28), as shown in Fig. 1. The metal-oxide coating is done over the e-LPG region to enhance the sensitivity of the suggested label-free fiber optic biosensor. Our real-time experiments confirmed the capability of our proposed coated e-LPG biosensor for monitoring significantly smaller index variations of pathogenic bacteria, such as *E. coli*, in food items and water.

II. MATERIALS AND METHODS

The schematic of the suggested TiO_2 coated e-LPG fiber optic biosensor is shown in Fig. 1. We have used an optical grating (long-period grating) with 3 cm length, 9 μm core radius (r), and 125 μm cladding radius, respectively. With this specification, the LPG is fabricated using a 248 nm Krypton Fluoride (KrF) excimer laser

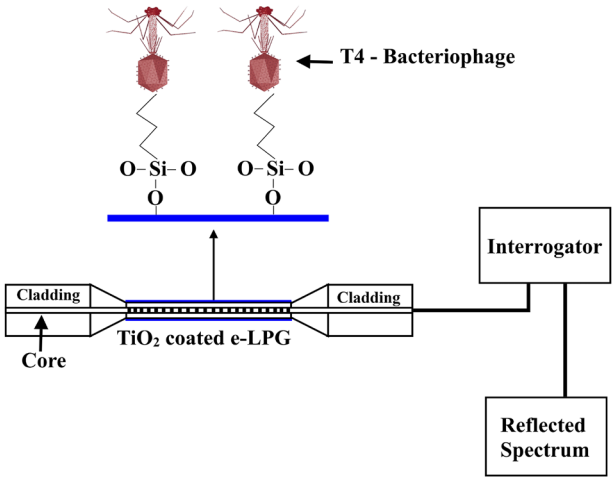

FIG. 1. Schematic of the proposed TiO_2 coated e-LPG biosensor with the experimental setup.

(Lumonics^{TM}). The long-period grating (LPG) then anneals at a temperature of 150 °C to release the excess hydrogen. Here, the LP_{01} mode propagates as cladding modes with suitable symmetry, i.e., (LP_{0m}, where m = 2, 3, . . .). In general, the filtering wavelength, λ_R, for the LPG is expressed as[9]

$$\lambda_R = \left(n_{01}^{eff} - n_{0m}^{eff}\right) \cdot \Lambda, \tag{1}$$

where Λ is the grating pitch and n_{01}^{eff} and n_{0m}^{eff} are the effective mode index of the LP_{01} and LP_{0m} modes, respectively.

For metal-oxide coating over the LPG region, cladding is partially removed from the fiber by dipping the LPG region vertically into 4% concentrated hydrogen fluoride (HF) solution for nearly 3 h at 20 °C. Due to etching, the cladding diameter changes from "d = 125 μm" to "$\overline{d}$ = 30 μm." In general, the filtering wavelength of the e-LPG is given by[10]

$$\Delta\lambda_R = \frac{\mu_\infty^2 \cdot \lambda_0^3 \Lambda}{8\pi^3 n_{cl} r^3}\left[\frac{1}{\sqrt{\left(n_{cl}^2 - n_{ex}^2\right)}} - \frac{1}{\sqrt{\left(n_{cl}^2 - \tilde{n}_{ex}^2\right)}}\right], \tag{2}$$

where n_{cl} is the cladding refractive index and n_{ex} is the external refractive index, respectively. A real-time monitoring system for etching the long-period grating using hydrofluoric (HF) acid solution is shown in Fig. 2(a). An optical interrogator (HBM-FS22DI) is used to observe the filtering wavelength during the etching process. The etching step results can be analyzed by monitoring the wavelength shift of LPG with respect to the etching time.

After the etching process, a thin coating of titanium oxide (TiO_2: 99.99% pure) over the sensing surface of the long-period grating sensing head is carried out. For this, an electron beam gun–cum–thermal evaporation system is used, which is purchased from HPVI (High Vacuum Products and Instruments), Bangalore, India. This instrument is working on both the modes: thermal evaporation and electron beam gun. In our experimental work, we have used the electron beam gun method rather than thermal evaporation to precisely evaporate titanium oxide. In addition, the wastage of material is less, and the purity of the material is substantially high. The deflection angle is set to 270°, and it is a thyristor-controlled system based on high voltage. In addition, its feed is done by Teflon insulation rated for 15 K V/100 Amps.

Moreover, we can control the X-direction and Y-direction beams with the help of beam knobs. With this knob, important parameters can be controlled, such as frequency, electron beam, and amplitude. Next, we have maintained the vacuum up to 10^{-6} mbar using this instrument. We have used a customized rotational holder that can hold optical grating to rotate at 360° angle. These rotations are performed at 30 rpm for the uniform TiO_2 deposition over a long-period optical grating sensing head. Figure 2(b) shows the complete coating process with a rotational mechanism. The coating thickness of TiO_2 over the sensing head is monitored by using a digital thickness monitor (DTM) employed in an evaporation chamber.

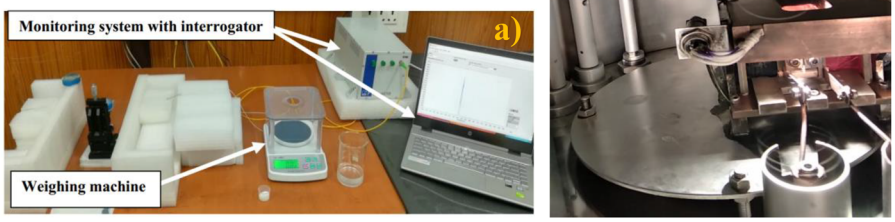

FIG. 2. (a) Etching process setup; (b) TiO_2 deposition over the etched long-period grating (e-LPG) using the rotational mechanism.

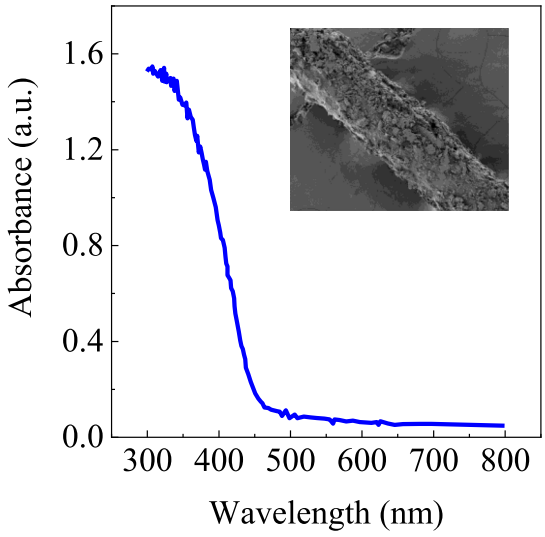

FIG. 3. Absorbance spectrum of the TiO_2 solution used for coating of the e-LPG biosensor. The inset shows the TiO_2 coated e-LPG biosensor for monitoring the *E. coli* bacteria.

Finally, a SiO_2 layer is deposited over TiO_2 for better binding and detection of *E. coli* bacteria.

Next, the etched region is neutralized with HF acid followed by washing with de-ionized water. Furthermore, TiO_2 solution is synthesized with the microwave-assisted hydrothermal method. In addition, the absorption spectrum of TiO_2 has been recorded with UV–Vis–NIR spectroscopy; see Fig. 3.

E. coli culturing is done by taking frozen pieces of *E. coli* bacteria to seed Luria-Bertani (LB) for 8 h–12 h. The dilution of *E. coli* culturing was plated on the LB agar for colony forming unit (cfu) measurements. Next, T4-bacteriophage solution is prepared by mixing 110 μl of *E. coli* culture with 5 ml cooled top agar. The mixture is incubated at 35 °C for 12 h. After incubation, macroplaque is developed over the lawn of bacteria. The content is passed through a 0.25 μm filter to obtain the phage stock for experiments. Here, the phage concentration is measured with plaque forming units (pfu).

III. EXPERIMENT AND RESULTS

The suggested TiO_2 coated e-LPG biosensor is 2.5 cm in length, and the experimental setup is shown in Fig. 1. A sensor head is prepared by depositing 40 nm TiO_2 over the e-LPG with 30 μm diameter. The biosensor is spliced to a 5 m long patch cord for interrogator connections. The presence of bends near the e-LPG biosensor may lead to measurement errors. The ends of coated e-LPG are fixed with the fiber holders to fix the sensor position.

Moreover, all experiments are conducted at constant room temperature to avoid temperature fluctuations. Light from the broadband light source (Ocean Optics LS1) is launched to the TiO_2 coated e-LPG sensor. For frequency response measurements, the proposed sensor is connected to the optical spectrum analyzer (Yokogawa AQ6370C) with 0.45 nm spectral resolution.

First, immobilization of T4-bacteriophages over the coated surface of LPG is carried out using the incubation process. Next, LPG samples are exposed to 3-aminopropyltris(trimethylsiloxy)silane for 2 h–4 h. As a result, the silane thin-film immobilizes with the amino function group on the top of the coated e-LPG surface, follow Fig. 1.[11] Next, these samples are placed in methanol solution and then de-ionized water for almost 30 min. These amino groups bind with T4-bacteriophages and help in the label-free detection of *E. coli* bacteria. Therefore, samples are finally put in the bacteriophage solution with 10^8 pfu/ml for nearly 3 h. The complete covalent binding of T4-bacteriophage is summarized in Fig. 1. Furthermore, analyte refractive index sensitivity ($n_{A\text{-}RI}$) measurements are performed with the Abbe refractometer (Atago DR-M2) at $\lambda = 1.55$ μm. The variation of the filtering wavelength (λ_B) with respect to $n_{A\text{-}RI}$ is shown in Fig. 4(a). The slope of λ_B in contrast to $n_{A\text{-}RI}$ confirms the sensitivity of 12.64 nm/RIU and 2.55 nm/RIU for the bare e-LPG and coated e-LPG, respectively.

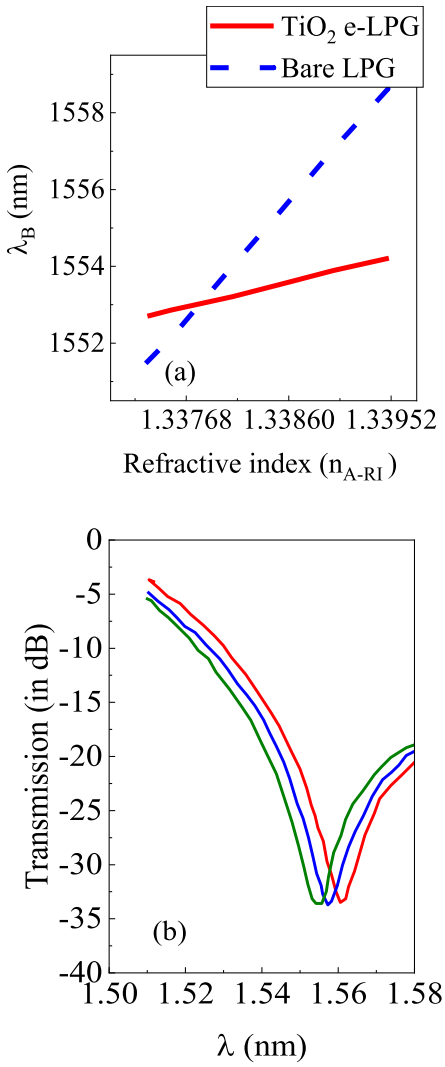

FIG. 4. (a) Plot of Bragg wavelength vs analyte refractive index ($n_{A\text{-}RI}$) for the developed biosensor and (b) its corresponding frequency response.

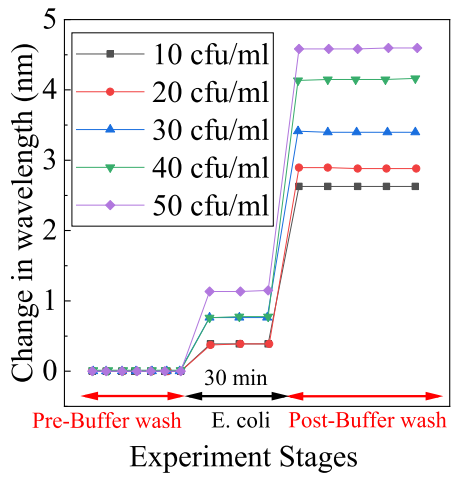

FIG. 5. Plot of the change in wavelength (spectral shift) with respect to various experiment stages.

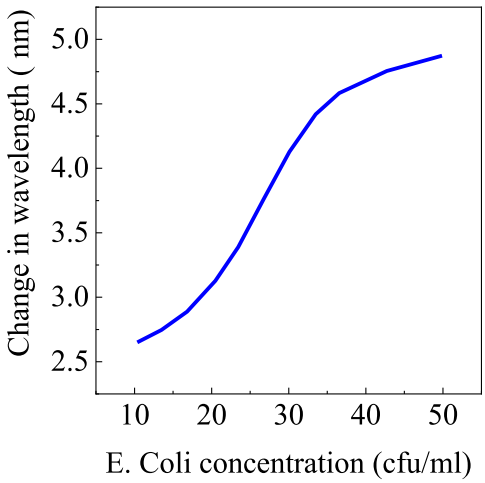

FIG. 6. Plot of the change in wavelength (spectral shift) with respect to the *E. coli* bacteria concentration (in cfu/ml).

The frequency response of the suggested metal-oxide coated e-LPG sensor is plotted in Fig. 4(b). The signal noise is calculated in terms of standard deviation of the reflected signal, and it is nearly 3.35 pm for our design. The resolution and the limit of detection

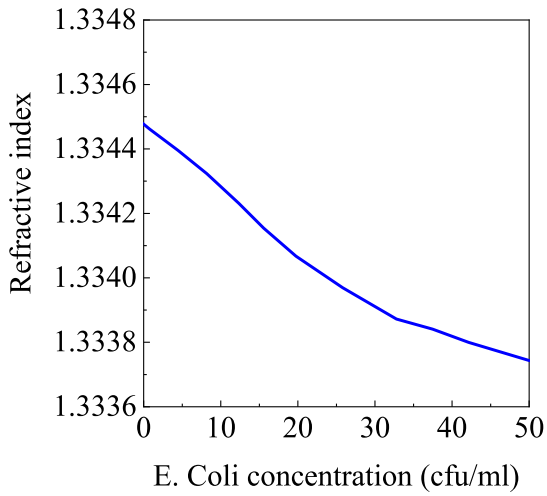

FIG. 7. Refractive index measurements with respect to the *E. coli* bacteria concentration.

(3σ/S) are 0.0039 RIU and 10.05 ppm, respectively.[12] Therefore, any filtering wavelength shift greater than 10.05 ppm is valid for the proposed fiber optic biochemical sensor. In addition, the wavelength shifts are calculated using Eq. (2). It has been observed that with adsorption measurements, *E. coli* bacteria wipe out from the fiber surface during buffer washes. However, with the proposed sensor design, *E. coli* bacteria bind to the coated TiO_2 surface due to immobilized T4-bacteriophages covalent bond.

Figure 5 shows the change in wavelength (spectral shift) vs various experiment stages, i.e., pre-buffer wash, *E. coli* incubation, and post-buffer wash. The response time of the suggested biosensor is nearly 30 min. In addition, the change in wavelength (see the y-axis) in the range 2.5 nm–4.5 nm after the bacteria incubation confirms the *E. coli* binding over the TiO_2 coated e-LPG with different concentrations. For this, we have plotted the change in wavelength (from pre-buffer wash to post-buffer wash) with respect to the *E. coli* concentration (in cfu/ml), see Fig. 6. It starts saturating after 45 cfu/ml, due to a decrease in the refractive index of bacteria. Similarly, the main reason for the low "change in wavelength" at lower bacteria concentrations is the less e-LPG surface covered by *E. coli* (Table I).

Finally, we have studied the effect of bacteria concentration on the refractive index behavior of the bacteria samples. We have again

TABLE I. Sensitivity comparison of etched-fiber based optical sensors.

Cladding diameter after the etching process (μm)	Coating material used	Sensitivity δλ/δn (nm/RIU)	Reference
32	...	7	13
18.4	...	1.3	14
30	Reduced graphene oxide	1.5395	15
30	TiO_2	2.55	This work

used an Abbe refractometer, maintaining the accuracy of 0.0001 RIU, see the y-axis of Fig. 7. The net refractive index variation of 7.4 $\times 10^{-4}$ RIU is observed for the *E. coli* concentration from 0 cfu/ml to 50 cfu/ml, as per Fig. 7. This means that the TiO_2 coated e-LPG with a sensitivity of 2.55 nm/RIU must demonstrate a wavelength shift of nearly 18.87 nm. In addition, this confirms the binding of *E. coli* bacteria over the TiO_2 coated surface of the proposed biosensor.

IV. CONCLUSION

In conclusion, we have reported a 40 nm TiO_2 coated e-LPG ultrafast, highly sensitive, label-free, and cost-effective biosensor for the monitoring of *E. coli* bacteria over the detection range of 0 cfu/ml–50 cfu/ml. The proposed biosensor has a device sensitivity of 2.55 nm/RIU. The resolution and the limit of detection (3σ/S) as per the experiments are 0.0039 RIU and 10.05 ppm, respectively. The refractive index variation of the TiO_2 solution may improve device sensitivity. With better stability, high durability, and label-free operation, the proposed optical grating-based sensor is suitable for various future integrated optical biosensor applications.

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DATA AVAILABILITY

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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