Detection specificity studies of bacteriophage adhesin-coated long-period grating-based biosensor

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ABSTRACT

In this work, we present a label-free detection specificity study of an optical fiber long-period grating (LPG) biosensor working near the dispersion turning point of higher order cladding modes. The LPG sensor functionalized with bacteriophage adhesin is tested with specific and non-specific bacteria dry weight. We show that such biosensor is able to selectively bind, thus recognize different bacteria. We use bacteria dry weights of E. coli B as positive test and E. coli K12 and Salmonella enterica as negative tests. The resonance wavelength shift induced by E. coli B reaches over 90 nm, while for E. coli K12 and Salmonella enterica approximately 40 and 20 nm, respectively.

Keywords: optical fiber sensors, long-period gratings, label-free sensing, surface functionalization, biosensors, bacteriophage adhesin, bacteria detection

1. INTRODUCTION

In recent years, scientific community interested in sensing focused its attention towards application of optical fiber sensors for detection of various bio-molecules [1-4]. One of the main trends is to apply label-free methods, where kinetics of reactions taking places on the sensor’s surface can be monitored using devices sensitive to small changes of external refractive index (RI) or to an increase of thickness of the aggregating bio-film [4]. Among many other label-free optical fiber sensors, an interesting platform is the one based on long-period gratings (LPG). The LPG is a periodic modulation of refractive index within a core of an optical fiber [5]. The modulation induces coupling of the core mode with a series of cladding modes and results in appearing of resonances in LPG transmission spectrum that correspond to the coupling effect. Thanks to interactions between the cladding modes and the external medium, the LPGs are sensitive to variations in surrounding RI or formation of thin, high-RI overlay on its surface. The sensitivity is typically defined as a resonance wavelength shift per RI unit (RIU) of the external medium. It has been shown that the highest sensitivity of the LPG is at the dispersion turning point (DTP), where two resonances separate from each other with an increase of RI or a formation of the high-RI overlay [6]. There has been a number of biosensors shown, where the LPGs were applied for nucleic acids [2,7], bacteria [3] or bacteria parts [8], protein [4,9], or antigen [10] detection. Each of them requires functionalization of the LPG surface with the bio-molecules showing affinity to the measured molecules. In our previous works we have shown highly sensitive bacteria or bacterial endotoxin sensor based on the LPG working near the DTP and coated with bacteriophages [3] or their adhesins [8,11]. The binding of bacteria to bacteriophages or its adhesins is very specific, thus it is possible to receive both sensitive and selective sensing.

In this work, we use binding mechanism reported in [8,11] to study the specificity of highly sensitive label-free biosensor based on LPGs. Presented study considers bacteriophage T4 adhesin, which is specific to E. coli B bacteria strain. The advantage of biosensor functionalization with adhesins is an ability for low-cost biosensitive molecule exchange and for sensor’s surface regeneration [11]. The LPG-based sensing structure allows for monitoring of the binding phenomenon.

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in real time and with a good accuracy. In the presented study we investigate Salmonella enterica, E. coli K12 as non-specific, and E. coli B as highly specific strains.

2. EXPERIMENTAL DETAILS

The long period gratings were written in hydrogen-loaded standard Corning SMF28 fiber using Pulse Master 840 high-power KrF excimer laser (λ = 248 nm). A pulse repetition rate was set to 100 Hz, pulse duration to 12 ns, and peak pulse energy was about 10 mJ. The UV exposure has been done through an amplitude chromium mask (Δ = 226.8 µm) for about 7 minutes. In order to stabilize the LPGs optical properties, the gratings were annealed after exposure at 150 °C for approx. 4 h to release the excess of hydrogen. Further, to enhance the RI sensitivity towards DTP, the gratings were etched in HF acid for about 3 h. The etching procedure resulted in the sensor operation at the vicinity of the dual resonance regime. The sensors are 4 cm long, and the sensitivity of the structures in considered spectral range before functionalization were approx. 3000 nm/RIU [11].

After preparation of the highly RI sensitive LPG, its surface was bio-functionalized using N-(5-amino-1-carboxypentyl) iminodiacetic acid (AB-NTA), NiCl₂ reagents and recombinant phage protein with histidine-tag to obtain well-defined orientation of the adhesin on the fiber’s surface. This procedure comprised of chemical preparation of the fiber’s surface and then of adhesin binding. At first, the fiber was immersed in the 0.01 % acetic acid and 2 % (3-Glycidyloxypropyl) trimethoxysilane solution in T = 90 °C for about 3 h, then it was left for about 16 h at T = 20 °C in the 0.01M NaHCO₃ buffer (pH = 10) with 20 mM N-(5-amino-1-carboxypentyl) iminodiacetic acid solution. Next, the fiber was bathed in 10 mM NiCl₂ and 5 mM Glycine solution for about 2 h in room temperature. Finally, after washing in NaHCO₃ buffer the fiber sensor was immersed in adhesin solution for about 1 h [12]. As bacteria specific layer we used E. coli B bacteriophage g37 adhesin.

The optical transmission of the LPG in the range of λ = 1550-1750 nm was monitored using a NKT Photonics SuperK COMPACT supercontinuum white light laser source and Yokogawa AQ6375 optical spectrum analyzer. The ambient temperature (T) during the measurements was set to 22 °C and monitored with HP 34970A Data Acquisition Unit equipped with a thermocouple. The tension of the LPG was held constant throughout the experiment.

3. RESULTS AND DISCUSSION

For the purpose of this experiment we used three LPG-based structures, each showing a similar high RI sensitivity. The LPG-based sensing structures, i.e., LPGs with functionalized sensing region, were washed several times in phosphate buffered saline (PBS) after attachment of bacteriophage adhesin. Then, the sensors were immersed in bacterial dry weights for about 1.5 h, and finally washed again in PBS. The concentrations of all bacteria solutions were equal to 10⁷ cfu/mL. Examples of the spectra obtained at each stage of the experiment are shown in Figures 1a, 2a, and 3a for Salmonella enterica, E. coli K12, and E. coli B, respectively.

![Figure 1. a) Example of the measurement spectra, and b) measured resonance wavelength difference before, during and after Salmonella enterica application.](image-url)
The measured resonance wavelength change for these bacterial dry weights are depicted in Figures 1b, 2b, and 3b, respectively. In the figures, blue crosses represent the values of resonance wavelength from the spectrum measurement, while the straight lines show the mean value between the points (in terms of mean square error). In both figures we compare the shift of the resonance wavelength for PBS solution before and after introducing the specific bacteria solution.

The results in Figures 1 and 2 show resonance shifts caused by the non-specific bacterial dry weights. These shifts are 21.3 and 43.2 nm for *Salmonella enterica* and *E. coli* K12, respectively. During the bacteria incubation process the wavelength shifts grow significantly, which is in general caused by bacteria aggregation on fiber surface and to relatively smaller extent by increase of RI. The final PBS washing process greatly reduces the shift just by washing out the physically attached bacteria, especially in case of *Salmonella enterica*. However, the overall results show changed values of resonance wavelengths; this indicates some non-specific bonding, which most probably is induced by accumulation of bacteria dry weight on the fiber surface and mixing with adhesin. Greater shift for *E. coli* K12 indicates some specificity in terms of biological affinity within the same species with *E. coli* B.

Although the results for *E. coli* K12 and *Salmonella enterica* show some binding, the outcome for specific *E. coli* B is extensively greater (Figure 3). In this case, after dry weight attachment the PBS washing only removes residual bacteria, which were not able to attach to the fiber surface functionalized with adhesin. The absence of signal change after final PBS washing may indicate sensor’s saturation.
4. CONCLUSIONS

In this work, we have shown the study of bacteriophage adhesin application for bacteria dry weight sensing. We demonstrate that the LPG-based biosensor is capable of specific bacteria strain detection (here E. coli B), giving strong and well distinguishable signal. The phage adhesin seems to be a better recognition element for biosensors than a whole bacteriophage; it is more stable, free of bacterial contamination and can be immobilized on the sensor surface in well-defined orientation. Although presented study shows its vulnerability in case of analyzing whole bacterial cells, it does not exclude these molecules as good candidates for biosensing structures. Moreover, presented study proves that the adhesins are promising for application in biosensing and worth further examinations.

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