Reusable Bacteriophage Adhesin-Coated Long-Period Grating Sensor for Bacterial Lipopolysaccharide Recognition

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***Abstract*—In this study, we report an application of the reusable optical fiber long-period grating (LPG) biosensor working near the dispersion turning point of higher order cladding modes. The presented device is capable of bacterial lipopolysaccharide (LPS) recognition. We show that the LPG sensor biofunctionalized with bacteriophage adhesin can be regenerated and successfully reused for very specific LPS detection. Thus, we show three series of label-free binding effect for specific to the adhesin *E. coli* B LPS and nonspecific *H. alvei* 1189 LPS (as a reference test) on a single LPG-based sensor. The resonance wavelength shift induced by the specific tests is significantly higher than that induced by the reference test. The LPG-based sensing structure allows for monitoring of the binding phenomenon in real time and with good accuracy.**

***Index Terms*—Bacterial lipopolysaccharide, bacteriophage adhesin, biosensors, label-free sensing, long-period gratings, optical fiber sensors, proteins, surface functionalization.**

I. Introduction

In recent years there has been a significant attention of the scientific community focused on application of optical fiber sensors for detection of various bio-molecules [1]–[5]. The well visible trend is to apply label-free methods, where kinetics of reactions taking place 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 bio-film aggregating there [5]. Among other label-free optical fiber sensors, an interesting platform is the one based on long-period gratings (LPGs). The LPG is a periodic modulation of RI within a core of an optical fiber [6]. The modulation induces coupling of the core mode with a series of cladding modes and results in appearance of resonances in LPG transmission spectrum. Thanks to interactions between the cladding modes and the surrounding medium, the LPGs are sensitive to variations of external RI or to formation of an overlay on their surface, even only nanometers thick. The RI sensitivity is typically defined as a resonance wavelength shift per RI unit (RIU) of the surrounding 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 increase of external RI or formation of the overlay [7]. There has been a number of biosensors shown, where the LPGs were applied for nucleic acids [3], [8], bacteria [4], [9], protein [5], [10], or antigen [11] detection. Each of them requires functionalization of the LPG surface with biomolecules showing affinity to the measured molecules. In our previous works we have shown a highly sensitive bacteria sensor based on the LPG coated with bacteriophages and working near the DTP [4], [9]. The binding of bacteria to bacteriophages is very specific, thus it is possible to accomplish both sensitive and selective sensing.

In this work a new binding mechanism is applied for developing highly sensitive label-free regenerable biosensors based on LPGs [12], [13]. The biosensor can be used for bacteria itself or for bacterial toxins detection thanks to the coating of the LPG surface with an adhesive protein (adhesin) extracted from bacteriophages. The recognition mechanism is based on the capability of the bacteriophage T4 to specifically bind lipopolysaccharide (LPS) existing on the surface of *E. coli* B bacteria strain. The protein (adhesin) responsible for binding is located on the long tail fiber of the phage particle. The advantage of the functionalization method where only the adhesins are applied instead of the whole phages, is the fact that the sensor's surface is coated with well-oriented biosensitive molecules, and that adhesins can be exchanged on the functionalized surface in a low-cost process. Moreover, in order to show high potential of this approach we demonstrate a sensing effect using only some small fractions of bacteria— LPSs—responsible for the interactions with the adhesins. Since both adhesins and LPSs are smaller than the bacteriophages or bacteria, the sensing effect occurs at a molecular level, and thus such label-free interactions indicate high sensitivity and flexibility in application of the LPG-based sensing devices. In our previous work [13], we have proved the specificity of the adhesin-LPS binding mechanism by using

Manuscript received August 11, 2014; revised October 6, 2014 and October 16, 2014; accepted October 17, 2014. Date of publication October 19, 2014; date of current version April 29, 2015. This work was supported by the Natural Sciences and Engineering Research Council of Canada, the Canada Research Chairs Program, the National Centre for Research and Development of Poland within LIDER/03/16/L-2/10 project, and the Polish National Science Centre within 2011/03/D/NZ7/02054 project.

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Digital Object Identifier 10.1109/JLT.2014.2364118

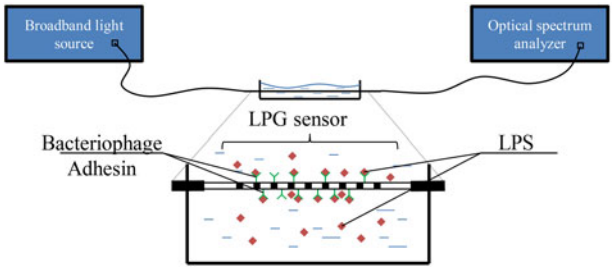

Fig. 1. Schematic representation of the measurement setup.

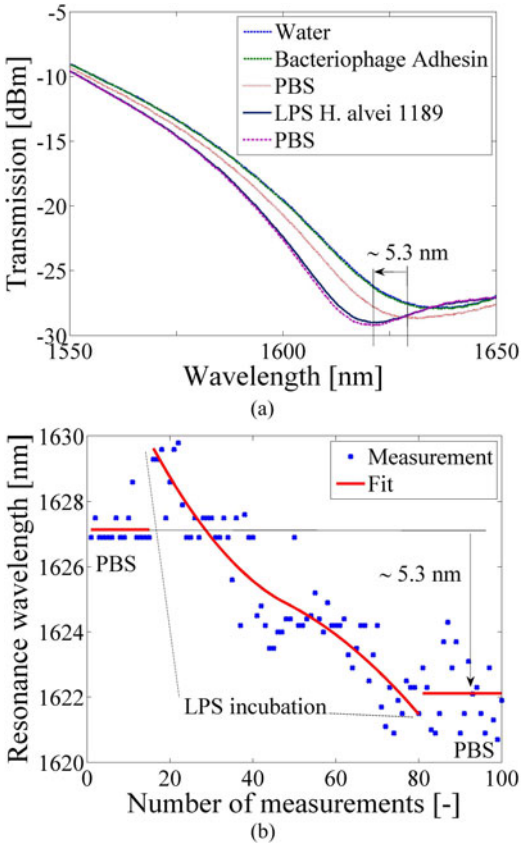

Fig. 2. Results of test no. 1 with introduced LPS–, where (a) shows selected spectra measured before, during and after the LPS application and (b) shows resonance wavelength at LPS incubation and washing experiment steps, similarly as in [13].

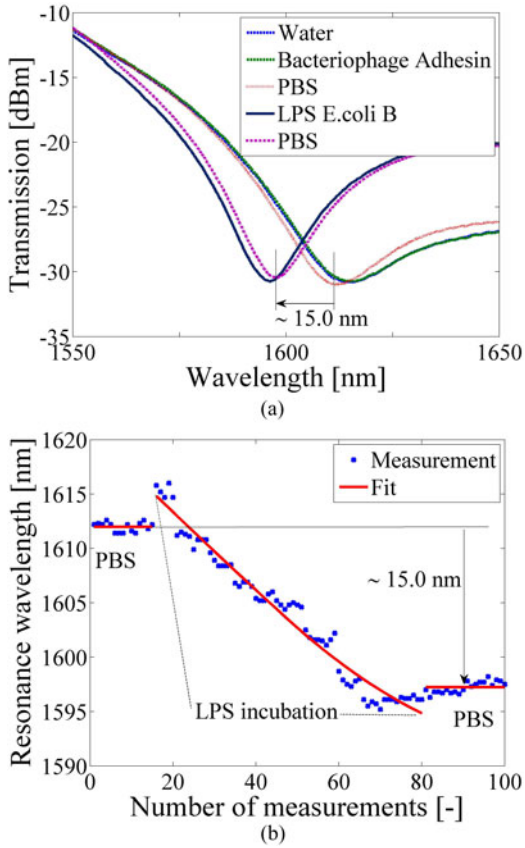

Fig. 3. Results of test no. 2 with introduced LPS+, where (a) shows selected spectra measured before, during and after the LPS application, and (b) shows resonance wavelength at LPS incubation and washing experiment steps, similarly as in [13].

biochemical tests, i.e., Immunoblotting, ELISA, Biacore, and finally using LPG-based optical sensor test. We compared there specific *E. coli* B and non-specific *H. alvei* 1189 LPSs. We further investigated the specificity of the binding process using the LPS of *E. coli* O56 which is classified in the same species as *E. coli* B, but it is a non-specific molecule [14].

For the purpose of this work the LPS of *E. coli* B strain recognized by T4 phage adhesin will be referred to as LPS+, while the other LPS of *H. alvei* 1189, which is not specific for the phage protein recognition, as LPS–. Moreover, in this work we focus on the fact that the applied surface preparation procedure enables successful regeneration and reusability of the sensor. It must be noted that all presented tests have been conducted on the same LPG structure.

II. Experimental Details

The LPGs were written in hydrogen-loaded Corning SMF28 fiber using Pulse Master 840 high-power KrF excimer laser ($\lambda = 248$ nm) from Light Machinery. 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 a chromium amplitude mask ($\Lambda = 226.8$ μm) for about 7 min. In order to stabilize the LPGs optical properties, they were annealed after exposure at 150 °C for ~4 h to release the excess of hydrogen. The LPGs fabricated this way were monitored in a spectral range from 1100 to 1700 nm, and had a single resonance wavelength (λ_R) near $\lambda = 1245$ nm. In order to enhance the RI sensitivity, the gratings were etched in low concentration HF acid for about 3 h. The etching procedure resulted in obtaining a sensing structure operating at the vicinity of the dual resonance regime. The optical transmission of the fiber was monitored during the LPG fabrication process in order to obtain the desired spectral attenuation notch. The sensor length is 4 cm, and the sensitivity of the structures before functionalization was from 2600 to 7000 nm/RIU depending on the RI range [13].

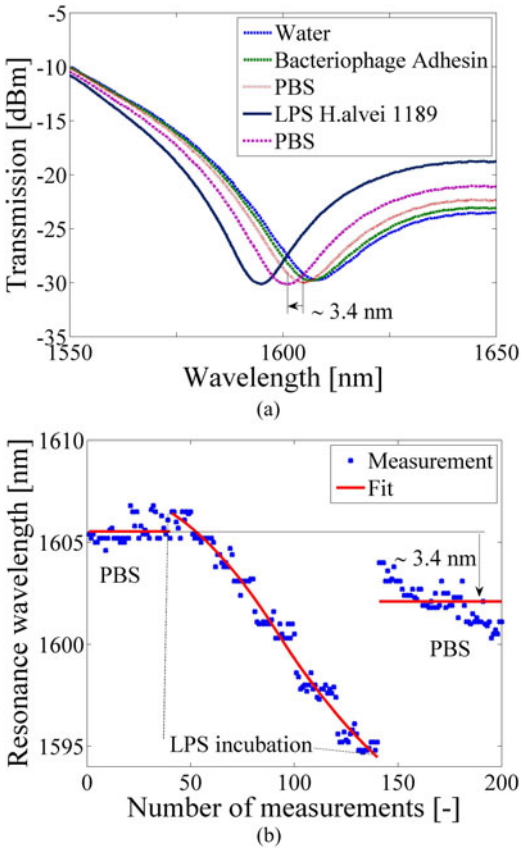

Fig. 4. Results of test no. 3 with introduced LPS–, where (a) shows selected spectra measured before, during and after the LPS application, and (b) shows resonance wavelength at LPS incubation and washing steps of the experiment.

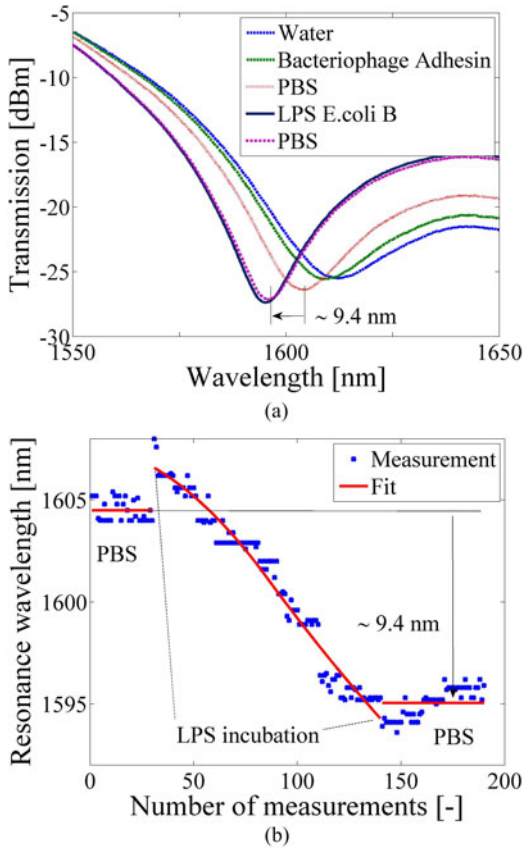

Fig. 5. Results of test no. 4 with introduced LPS+, where (a) shows selected spectra measured before, during and after the LPS application, and (b) shows resonance wavelength at LPS incubation and washing steps of the experiment.

After preparation of the highly RI sensitive LPG, its surface was bio-functionalized using N-(5-amino-1-carboxypentyl) iminodiacetic acid (AB-NTA), $NiCl_2$ reagents and recombinant phage protein with histidine-tag to obtain a well-defined orientation of the adhesin on the fiber's surface [12], [16]. The bacteriophage adhesin and bacterial LPSs of *E. coli* B (PCM1935) and *H. alvei* (PCM1189) were obtained according to the method described by Brzozowska *et al.* [13]. The procedure of chemical preparation of the fiber's surface and then adhesin bonding comprised of the following steps. 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 $T = 60$ °C in the 0.01M $NaHCO_3$ and 10% N-(5-amino-1-carboxypentyl) iminodiacetic acid solution. Next, the fiber was immersed in 10 mM $NiCl_2$ and 5 mM Glycine solution for about 2 h in room temperature. Finally, after washing in water the fiber sensor was immersed in the adhesin solution with concentration of 50 μg/ml for about 1 h.

To achieve the possibility of the sensor regeneration after each LPS measurement, its surface was immersed and extensively washed in water and then in 0.35 M Ethylenediaminetetraacetic acid. This caused the removal of any residual bio-coating and a layer created in the process of bathing the sensor in a solution of $NiCl_2$ with Glycine. In order to reuse the sensor after each test we repeated the bio-functionalization procedure starting from immersing the fiber in $NiCl_2$ and Glycine solutions for 2 h at room temperature.

The optical transmission of the LPG in the range of $\lambda = 1550-1650$ nm was monitored using a NKT Photonics SuperK COMPACT supercontinuum white light laser source and Yokogawa AQ6370C optical spectrum analyzer (see Fig. 1). 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.

For the purpose of this experiment we used the same LPG-based structure for six biosensing experiments. The structure, i.e., LPG with functionalized sensing region, was extensively washed in Phosphate Buffered Saline (PBS) after the attachment of adhesin. Then, the sensor was immersed in LPS− or LPS+ solution for about 2–3 h, and finally extensively washed in PBS. The concentrations of LPS was ∼1 mg/ml, and for the testing it was five-fold diluted. We conducted six consecutive tests, each ending with the sensor regeneration. The tests were divided into three groups, in this way each pair of experiments consisted of both LPS– and LPS+ trials. The breaks between the tests were about 12 h, except a break between the tests no. 2 and 1 where the break duration was about 36 h.

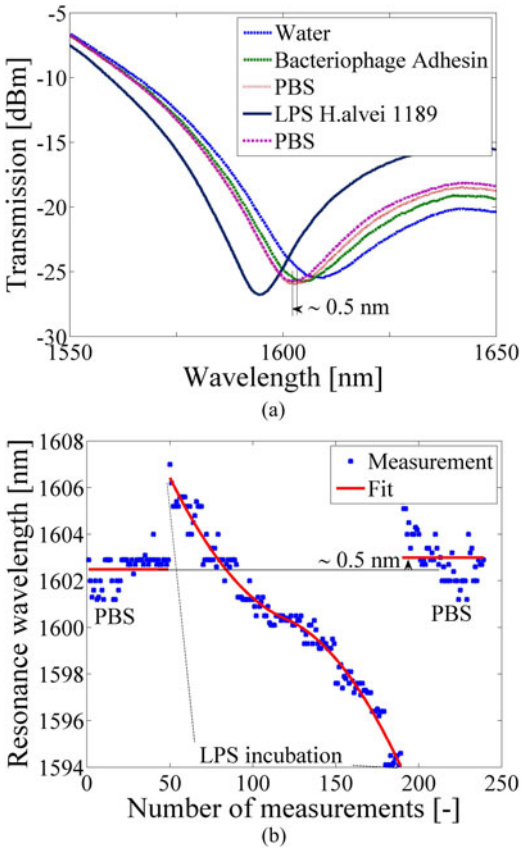

Fig. 6. Results of test no. 5 with introduced LPS–, where (a) shows selected spectra measured before, during and after the LPS application, and (b) shows resonance wavelength at LPS incubation and washing steps of the experiment.

III. Results and Discussion

There is a number of influences that can shift the resonance wavelengths (λ_{res}^{m}) of the LPG [15], [17]. The main relation describing wavelength-dependent coupling from the guided core mode (LP_{01}) to the *m*th cladding mode ($\text{LP}_{0\text{m}}$) is shown in (1), where (n_{eff}^{01}) is the effective RI of the propagating core mode, (n_{eff}^{0m}) is the effective RI of the *m*th cladding mode and (Λ) is the period of the LPG

$$\lambda_{\text{res}}^{m} = (n_{\text{eff}}^{01} - n_{\text{eff}}^{0m}) \cdot \Lambda. \tag{1}$$

A resonance wavelength shift can be induced by variation of either the period of the grating or the effective indices of the modes. As far as the influence of external RI is concerned, only n_{eff}^{0m} can be influenced by external medium, which in specific case can be a bio-overlay. According to (1) when the external RI increases or when the overlay is formed on the LPG surface, the λ_{res}^{m} shifts towards shorter wavelengths (experiences a blue shift).

Examples of the spectra obtained at each stage of the experiment are shown in Figs. 2(a), 3(a), 4(a), 5(a), 6(a), and 7(a) (even figure numbers for LPS– and odd for LPS+). It can be seen that each step of the applied procedure, except washing, induces the blue shift. The shift is induced by aggregation of the bio-material on the LPG surface. In turn, the washing steps with PBS induce a red shift, which is correlated to the removal of unbounded biomaterial. In all the cases binding of adhesin induces an insignificant shift due to the small size of the molecules. Washing in first PBS in reference to water results in a small blue shift. This effect is related to the slightly higher RI of PBS comparing to the one of water [4]. Next, when the LPG is immersed in LPS, the highest shift towards shorter wavelength can be recorded. This effect again is related to the highest RI of the LPS solution. From the sensing point of view, the last step of the procedure brings most of information about selectivity of the device. The change in resonance wavelength during the second washing in PBS was monitored during the whole measurement and it is shown for each case in the figures

TABLE I
Resonance Wavelength Shift Obtained as a Result of Each Test

Test No.	Bio-molecule Bio-molecule	Wavelength shift [nm]	Resonance wavelength in second PBS [nm]
1	LPS –	5.3	1627
2	**LPS +**	**15.0**	**1612**
3	LPS –	3.4	1605
4	**LPS +**	**9.4**	**1604**
5	LPS –	0.5	1602
6	**LPS +**	**6.6**	**1602**

The regeneration procedure has been applied between the tests.

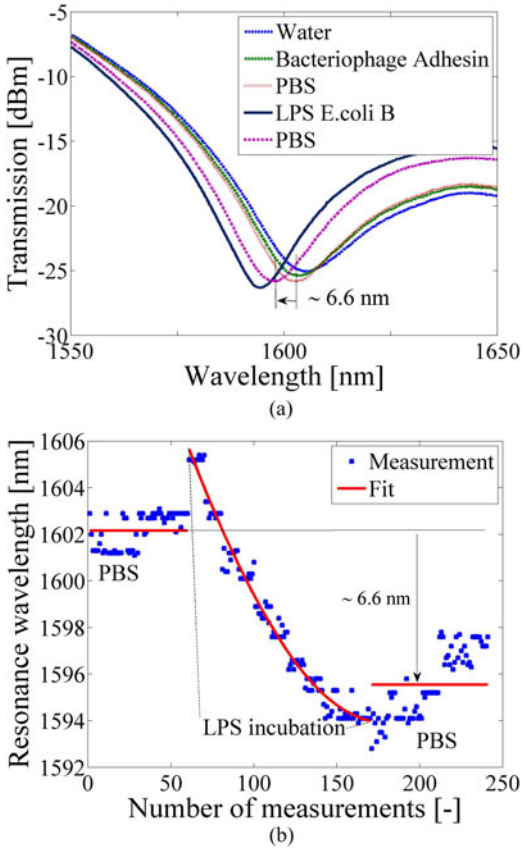

Fig. 7. Results of test no. 6 with introduced LPS+, where (a) shows selected spectra measured before, during and after the LPS application, and (b) shows resonance wavelength at LPS incubation and washing steps of the experiment.

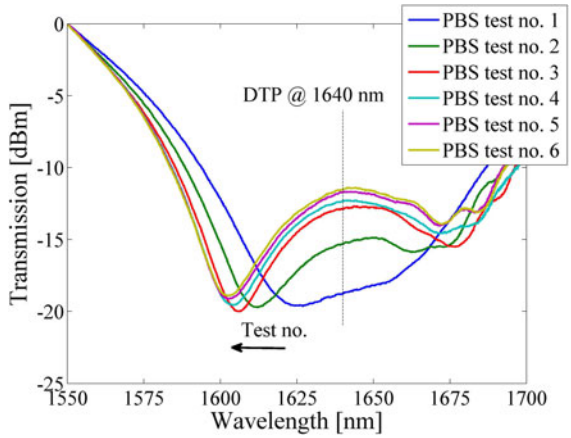

Fig. 8. Results of spectra measurements after PBS washing for all six tests.

'b'. In these figures, the points represent the values of resonance wavelength extracted from the spectrum measurement, while the straight lines show the mean value between the points (in terms of mean square error). A blue shift of the resonance with LPS incubation induced by aggregation of the bio-material on the sensor's surface and also by slight evaporation of water from the solution, by its densification and increase in its RI, can be seen there. In all the figures we compare the shift of the resonance wavelength for PBS solution before and after introducing the LPS, when the samples have been extensively washed to remove the unbounded LPS biomolecules. The summary of the obtained results, i.e., resonance wavelength shift induced by introduction of the LPSs, is given in Table I. A higher shift induced by LPS+ than by LPS– can clearly be seen there, which confirms specificity of the binding process.

In contrast to the results shown in Figs. 2, 4 and 6, in the respective Figs. 3, 5 and 7 where the LPS+ was introduced, a clear and significant shift in the resonant wavelength is observed for all the experiments. The shift is preserved even after tens of PBS washing steps. Bonding reactions between the adhesin and the LPS+ taking place on the surface of the LPGs lead to the increase of both external RI (change in density thus in optical properties of adhesin-LPS+ bio-layer) and the thickness of the bio-overlay. These reactions are monitored as shift of the LPG's resonance in the transmission spectrum, which reaches from almost 7 to about 15 nm depending on the order (number) of test. Two reasons of these differences can be pointed out: firstly, a slight difference in RI sensitivity of the LPG for each test, i.e., different resonance wavelength when the LPG is immersed in the first PBS (see Table I), and secondly the sensor's bio-functionalized surface aging with each test. When the sensitivity issue is concerned, it must be noted that LPGs have it highest in close proximity to the DTP, which for the investigated LPGs is at 1640 nm (see Fig. 8) and reaches about 7000 nm/RIU. With every test the starting conditions are slightly different and tend to shift towards the lower sensitivity range, where it reaches about 2600 nm/RIU at 1600 nm. The highest sensitivity has been obtained for the test no. 1 and 2, where shift induced by the binding reached 5.3 and 15 nm for the negative and positive tests, respectively. The lowest sensitivity among the tests was for the tests no. 5 and 6, where the shift induced by the binding reached 0.5 and 6.6 nm for the negative and positive test, respectively. The separation of the resonances occurring with each test also suggests that the bio-material is not entirely removed from the surface during the regeneration procedure.

The other reasons of different results obtained for each pair of the tests may also come from factors affecting stability of the chelating linkage, for example protein concentration, buffer flow rate, pH and NaCl concentration [18]. It should be noted that the precise protein immobilization level, and levels of pH and salt concentration compatible with stability varied slightly with different nitrilotriacetic acid-derivatized surfaces, and with the age of a particular surface [18]. However, to obtain the reusable biosensor, finding the optimum conditions such as the concentration of the chemicals and biomolecules, pH, type of buffers, flow rates and incubation times, requires further studies.

In general, the consecutive tests clearly show the possibility of successful LPG-based fiber sensor regeneration, and confirm occurrence specific adhesin and LPS bounding. Thus, the sensor not only is capable of sensing any bacteria specific LPS with compatible protein, but also can be effectively reused.

IV. Conclusion

In this work we have shown the possibility of regeneration of the bacteriophage adhesin bio-functionalized fiber sensor. We have demonstrated that the LPG-based biosensor is capable of specific LPS of *E. coli* B bacteria detection after several regeneration processes, giving strong and distinguishable response. Moreover, the presented label-free method can be applied to any specific LPS -protein pairs, and in general, the sensor can be used not only for detecting *E. coli* B bacteria, but also for detection of bacterial toxins. The toxins are released after bacterial cell lysis and are very dangerous for human and animal health.

The phage adhesin seems to be a better biomolecule for biosensors than a whole bacteriophage, because it is more stable, free of bacterial contamination and can be better immobilized on the sensor's surface with well-defined orientation. The presented biosensor is a very promising new type of device where different biomolecules (adhesins) are applied. Finally, since each regeneration process may end with a different adhesin coating, we confirmed that the described functionalization procedure offers ability for the sensor's regeneration and recoating with same or different types of adhesive proteins.

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