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Long period grating based biosensor for the detection of *Escherichia coli* bacteria

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ABSTRACT

In this paper we report a stable, label-free, bacteriophage-based detection of *Escherichia coli* (*E. coli*) using ultra sensitive long-period fiber gratings (LPFGs). Bacteriophage T4 was covalently immobilized on optical fiber surface and the *E. coli* binding was investigated using the highly accurate spectral interrogation mechanism. In contrast to the widely used surface plasmon resonance (SPR) based sensors, no moving part or metal deposition is required in our sensor, making the present sensor extremely accurate, very compact and cost effective. We demonstrated that our detection mechanism is capable of reliable detection of *E. coli* concentrations as low as 10^3 cfu/ml with an experimental accuracy greater than 99%.

1. Introduction

Pathogenic *Escherichia coli* (*E. coli*) is one of the most dangerous agents of food-borne disease. Consumption of contaminated food or water can be deadly, especially for children and the elderly. Although *E. coli* infection is most common in developing countries, many recent outbreaks in Europe and Northern America have been attributed to a strain of *E. coli* which has been identified among the most common causes of diseases related to food safety (WHO, 2008, http://www.cdc.gov/ecoli/2011/ecolio104/). Accurate routine testing is crucial for outbreak prevention. Nevertheless, currently available tests require time-consuming amplification of samples. The standard detection process of *E. coli* bacteria takes about 24 h to obtain results from culturing methods. Although more recent detection techniques such as PCR, ELISA and IMS offer a more rapid detection, analysis time of several hours is still required.

For the specific and rapid detection of such pathogens, bio-recognition elements such as antibodies (Rijal et al., 2005; Arora et al., 2011), nucleic acids (DNA/RNA) (Baeumner et al., 2003; Dorst et al., 2010) and bacteriophages (Balasubramanian et al., 2007; Shabani et al., 2008) have widely been used for the specific capturing of the target bacteria. Their binding can be detected by fluorescence labeling methods (Pan et al., 2005; Tombelli et al., 2005) or by label-free methods (Zourob et al., 2005; Nanduri et al., 2007; Smietana et al., 2011). Each of these recognition elements has its own advantages and disadvantages. For example, recognition based on nucleic acid, though offering high specificity, suffers from the inability to discriminate between viable and non-viable cells (Dorst et al., 2010). For antibody-based recognition elements, the drawbacks are high price, stability and cross-binding to other bacteria which may result in false positives (Balasubramanian et al., 2007). Bacteriophage, on the other hand, offers a superior alternative to other recognition elements in terms of their high specificity, fast binding, easy/low-cost production and stability. Furthermore, the bacteriophage has a unique feature to discriminate between the viable and non-viable cells. In principle the bacteriophage binds to both viable and non-viable bacteria as long as the surface receptor (lipopolysaccharides, teichoic acids, proteins) is intact and recognized by the bacteria. To see whether the bacteria are viable, the experiment can be extended past the latent period (the time from infection to bacterial lysis by the bacteriophage progeny). The signal should change since the intact bacteria are now broken (lysed).

In the fluorescent-label method, the recognition elements are labeled with specific dyes, making this procedure complex, time-consuming and the potential alteration of the properties of the analyte (Balasubramanian et al., 2007). Label-free methods, on the other hand, are based on changes occurring at the sensor surface, on changes in the analyte refractive index (ARI) or on changes in the thickness of the bio-film – all changes that could modify the optical properties of a sensor. This method is not only very fast but also allows real-time monitoring of the biomolecular interactions occurring while the process of detection takes place.

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Several schemes based on the label-free methods, like evanescent wave coupling in grating couplers (Horváth et al., 2003), leaky optical waveguides (Zourob et al., 2005) and SPR (Bae et al., 2004; Oh et al., 2005; Taylora et al., 2005; Homola, 2006) for the detection of *E. coli* have been proposed in recent past. Out of these, the SPR based sensors, both in the angular interrogation regime (Bae et al., 2004; Oh et al., 2005; Taylora et al., 2005) and spectral interrogation regime (Homola, 2006), have received much attention due to their extremely high sensitivity which is necessary for highly accurate bio-measurements. However, drawbacks associated with the SPR based sensors are numerous, such as the incorporation of bulky moving part (the high index prism used in angular interrogation regime) (Bae et al., 2004; Oh et al., 2005), the expensive and elaborated metal deposition requirements to support the surface Plasmon wave, the limitation of operation to the highly lossy visible spectral regime (~400–800 nm) (Homola, 2006), and the possibility of false positives due to input power fluctuations and/or connector losses associated with the intensity based measurements. To overcome these issues, our group has recently proposed a low-cost long-period fiber grating (LPFG) sensor for detection of *E. coli* bacteria based on physically adsorbed bacteriophages on the optical fiber surface (Smietana et al., 2011). Due to the physical adsorption mechanism and low sensitivity ~700 nm/RIU (as compared to ~1000 nm/RIU achievable using SPR based sensors), the sensor was able to detect only high concentrations of *E. coli*, with no link between bacterial concentration and spectral shift detected.

Here, we present for the first time a compact, ultra high sensitive LPFG (~2321 nm/RIU) based cost-effective, highly accurate, ultra stable, label-free, specific detection of *E. coli* using bacteriophages covalently attached on the optical fiber surface as the recognition moiety. We demonstrated a reliable detection of *E. coli* concentrations as low as 10^3 cfu/ml with an exceptional experimental accuracy (>99%). Using scanning electron microscopy (SEM), we also confirm that these measurements are the result of real bacterial binding to the optical fiber surface and not on the changes in the sample refractive index.

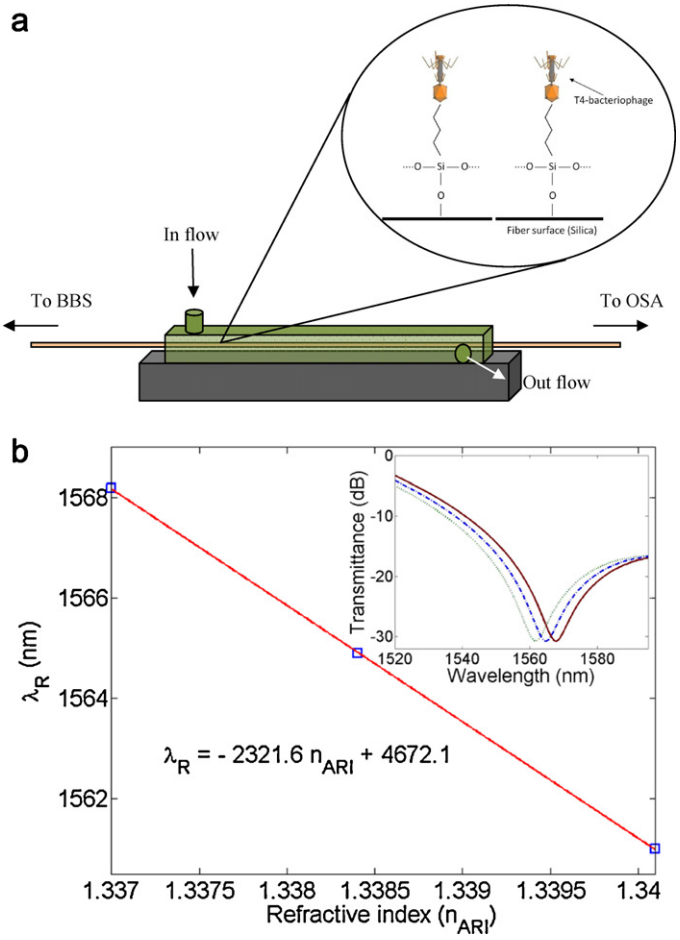

Fig. 1. (a) Schematic of the experimental arrangement; inset shows Si—O—Si covalent binding of the bacteriophages with the optical fiber surface (SiO_2) (b) Variation of the resonance wavelength with ARI; the transmission spectrum is shown in the inset.

2. Materials and methods

The LPFGs are periodic refractive index variations created within the core region of optical fiber (diameter ~9 μm) to redirect part of the optical field from the fiber core region (known as the core mode) to the cladding region (diameter ~125 μm) known as the cladding mode of the optical fiber. The specific wavelength at which the field is redirected is known as the resonance wavelength (λ_R) and is expressed as (Kashyap, 2009),

$$\lambda_R = \left(n_{eff}^{c} - n_{eff}^{cl}\right)\Lambda + (\kappa_{c\text{-}c} - \kappa_{cl-cl}) \quad (1)$$

where n_{eff}^{c} and n_{eff}^{cl} are the effective refractive indices of the core and the cladding mode; Λ is the grating period; and κ_{c-c} and κ_{cl-cl} are the self-coupling coefficients of the core mode and the cladding mode, respectively. A small fraction of the cladding mode field, evanescent field, travels outside the optical fiber, interacting with the outer region, changing the n_{eff}^{cl} and thus changing the λ_R. The LPFG sensing principle relies on measuring the changes in λ_R, the smaller the period of refractive index variation (Λ) the larger would be cladding mode evanescent field leading to high sensitivity.

2.1. LPFG fabrication

To carry out the experiments we fabricated several highly sensitive LPFGs at our lab in single mode optical fiber SMF-28™ (Corning: NY, 14831 USA). For ease of fabrication the photosensitivity of the fibers was increased by hydrogen loading of the fibers at 150 bar in hydrogen chamber for 15 days. Photosensitive LPFGs were then inscribed into the fiber core using a chromium amplitude mask (Λ = 226.8 μm) and a high-power KrF excimer laser (Lumonics™ Lasers: Pulse Master®-840) emitting at 248 nm at a pulse repetition rate of 100 Hz, pulse duration of 12 ns and peak pulse energy of 10 mJ. The LPFGs were then annealed at 150 °C for 3 h to release the excess hydrogen to stabilize their optical properties. The LPFGs so fabricated have λ_R in the low loss window of telecommunication wavelengths (~1.56 μm) for air as surrounding medium. Since the measurements were to be performed in aqueous solutions the λ_R would shift by ±350 nm shifting away from the low loss regime. To overcome it we slowly etched the cladding of the fiber in 4% HF for ~3 h. This shifted the λ_R close to its turning point ~1.58 μm, enhancing the sensitivity of the LPFGs (Shu et al., 2002).

2.2. E. coli culturing

Frozen stock of *E. coli* B was used to seed Luria-Bertani (LB) media overnight. Bacteria were harvested by centrifugation at 3000 × *g* for 10 min, followed by washing in phosphate-buffered saline (PBS) buffer. Dilutions of the overnight culture were plated on LB agar to determine the titer, expressed in colony forming units (cfu). Appropriate dilutions of bacteria stock were made in the PBS buffer from the overnight culture stock.