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Short communication

Evanescent wave absorbance based fiber optic biosensor for label-free detection of *E. coli* at 280 nm wavelength

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ABSTRACT

A novel label-free technique for the detection of pathogens based on evanescent wave absorbance (EWA) changes at 280 nm from a U-bent optical fiber sensor is demonstrated. Bending a decladded fiber into a U-shaped structure enhances the penetration depth of evanescent waves and hence sensitivity of the probe. We show that the enhanced EWA response from such U-bent probes, caused by the inherent optical absorbance properties of bacterial cells or biomolecules specifically bound to the sensor surface, can be exploited for the detection of pathogens. A portable optical set-up with a UV light emitting diode, a spectrometer and U-bent fiber optic probe of 200 μm core diameter, 0.75 mm bend radius and effective probe length of 1 cm demonstrated an ability to detect less than 1000 cfu/ml.

1. Introduction

Real-time and sensitive detection of common food or water borne pathogens like *Escherichia coli*, *Salmonella* is of critical importance to counter the spread of diseases caused by them. Several label-free optical sensing techniques such as, surface plasmon resonance (SPR) (Taylor et al., 2006), grating coupled waveguides (Hórváth et al., 2003), metal clad waveguides (Zourob et al., 2005), ring resonator (Ramachandran et al., 2008), photonic crystals (Ouyang et al., 2007), and fiber Bragg's gratings (DeLisa et al., 2000) have been investigated for pathogen detection. However, many of these devices could be expensive due to the complexity in instrumentation and sensor fabrication procedures.

The cost and complexity in fabrication and operation may be reduced by utilizing the inherent optical scattering or absorbance properties of pathogens in UV and visible wavelength range for sensing purposes (Acharya et al., 2006, 2007). Evanescent wave based sensors using optical fibers are useful tools for making sensitive absorbance and fluorescence measurements. Mutharasan and coworkers (Haddock et al., 2003; Rijal et al., 2005) have demonstrated an evanescent wave based fiber optic biosensor for detection of *E. coli* using a biconical tapered single mode fiber operating at 470 nm wavelength.

Recently, we have demonstrated the possibility of immunodetection of biomolecules such as IgG using evanescent wave absorbance from straight fiber optic probes at 280 nm wavelength (Sai et al., 2010). However, straight fiber optic probes are unsuitable for detecting micron sized analytes such as bacteria due to low penetration depth of the evanescent field, particularly for the UV region. The evanescent field intensity and/or its penetration depth can be improved by modifying the fiber probe geometry (Leung et al., 2007). U-bent optical fiber probes are an attractive choice because of their enhanced absorbance sensitivity, simpler probe design and fabrication (Gupta et al., 1996; Khijwania and Gupta, 2000). Recently, we have shown the advantages of U-bent fiber probes over straight fiber probes by developing a sensitive EWA based localized surface plasmon resonance (LSPR) biosensor (Sai et al., 2009).

This communication demonstrates a novel, label-free detection technique for bacteria by combining the advantages of optical absorbance properties of proteins associated with bacteria at 280 nm and enhanced EWA in U-bent probes, as illustrated in

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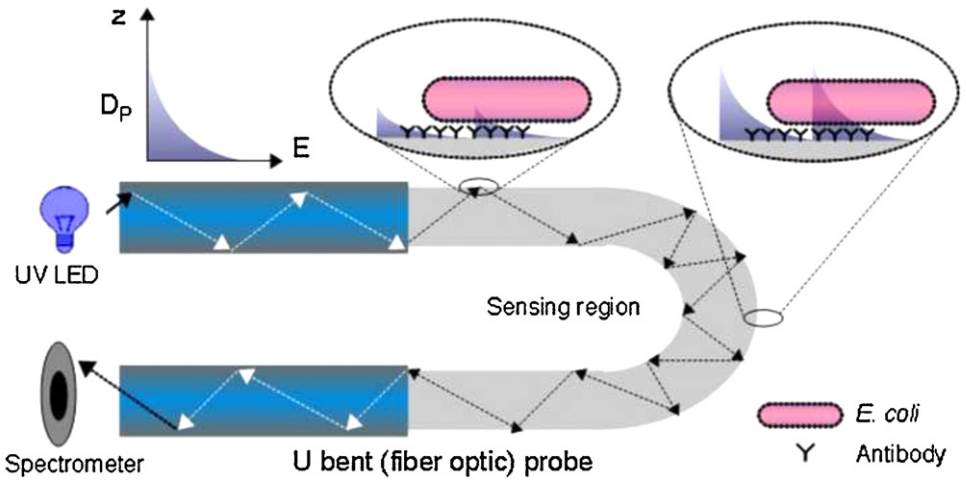

Scheme 1. Enhancement of penetration depth D_P in bend fiber probe, enabling evanescent field overlap for detection of *E. coli* (D_P and biomolecules not drawn to scale).

Scheme 1. *E. coli* cells were captured on the probe surface immobilized with antibodies against *E. coli*. The potential of U-bent probes for the detection of larger size analytes such as pathogens is demonstrated with the help of FITC (as a chromophore) stained *E. coli* cells by measuring EWA at visible wavelengths. Subsequently, proof-of-concept for label-free detection of pathogens at 280 nm is established using a simple optical set-up consisting of only three components, *i.e.*, a UV LED, a U-bent multimode fiber optic probe, and a fiber optic spectrometer.

2. Materials and methods

2.1. Fiber optic probe preparation

Fiber optic probes were fabricated as described in our earlier work (Sai et al., 2009). Briefly, 20 cm and 40 cm long, 200 μm core diameter fibers (Ceramoptec®, Germany) were decladded at the center using surgical blades to obtain probe lengths of 5 cm or 2 cm for straight and U-bent probes respectively. U-bent probes were made by exposing the center of the 2 cm of decladded region to butane flame and bent into a U-shape (Figure S-1). The bend radii of the probes were measured using an optical microscope and only probes having bend radius of ~0.75 mm were used for the experiments.

Solarization resistance of the high-OH fiber probes and their suitability for applications at 280 nm wavelength were discussed in our earlier publication (Sai et al., 2010). Surface functionalization and immobilization of antibodies on fiber optic probes were carried out as per Sai et al. (2010). Briefly, fiber probes were amine functionalized using 3-((2-aminoethyl amino)-propyl) trimethoxy silane (Sigma–Aldrich) and immobilized with monoclonal antibody against *E. coli* (Fitzgerald Antibodies, USA) with the help of glutaraldehyde as a cross linker. Probes were dipped in 5 mg/ml of bovine serum albumin (Bangalore Genie, India) for 30 min to reduce non-specific binding.

2.2. Optical setup

The optical set-up (Figure S-2 of Supplementary material) used for the experiments is similar to our previous studies (Sai et al., 2009). Light from the UV LED (T9H28C, λ_{max} = 280 nm, FWHM = 15 nm, 441–520 μW, Roithner Lasertechnik GmbH, Austria) was coupled into one end of the U-bent probe. The other end of the probe was coupled with a fiber optic spectrometer (USB 4000, Ocean Optics®, USA) to record absorbance measurements. Spectra were acquired every 100 ms and the signal was averaged over 100 samples.

The U-bent fiber portion was held within a custom-made flow cell made from a glass capillary channel (ϕ = 2.5 mm) with inlet and outlet (Figure S-2). The flow cell was filled with 250 μl of 0.85% saline as a reference for absorbance measurements. Each time 500 μl volume of a given analyte was introduced into the flow cell and real-time EWA changes were monitored. Needless to say, the first 250 μl acted as a flushing volume and the remaining as incubating volume.

3. Results and discussion

3.1. Experiments with FITC stained E. coli cells

The superiority of U-bent probes over straight probes for EWA based pathogen sensing is demonstrated by designing experiments in the visible wavelength range with FITC stained *E. coli* cells (ATCC-35218) as target analyte. The *E. coli* cells were stained with FITC (FITC on celite, Sigma–Aldrich) by incubating the cells in 4 μM (0.2 ml of 20 μM in 1 ml of cell culture) solution of FITC prepared in borate buffer (pH 8.3) for 15 min. The cells were centrifuged at 2000 rpm for 10 min to remove the excess dye (supernatant) and cell dilutions were prepared subsequently. The optical setup used for these experiments is as described above, except a broad spectrum white LED is used as the light source.

The EWA changes at 500 nm due to binding of FITC stained *E. coli* cells were monitored real time as shown in Fig. 1A. The presence of *E. coli* cells on the probe surface was confirmed through fluorescence microscopy. The average surface density of *E. coli* bound to the probes was *ca.* 4900 cells/mm^2 after incubation with 10^6 cfu/ml. Interestingly, the maximum change in EWA due to the binding of FITC stained *E. coli* to U-bent probe surface was ~0.12, which is more than the value obtained after treating amine functionalized U-bent probes with 4 μM FITC (Figs. S-8a and S-7b respectively). Given the significantly small number of FITC molecules labeled to *E. coli* bound to the U-bent probe surface, such a high EWA response can be attributed to the enhanced penetration depth (D_P) of evanescent waves.

The enhanced D_P in the U-bent region can be due to the presence of incident rays closer to the critical angle in the sensing region, which is not possible to achieve in straight fiber probes (Gupta et al., 1996). The estimated values of ($D_{P\,Min}$, $D_{P\,Max}$) in aqueous medium of U-bent probe (bend radius = 0.75 mm) for 500 nm wavelength are at least (134.78 nm and 534.16 nm) compared to (134.78 nm and

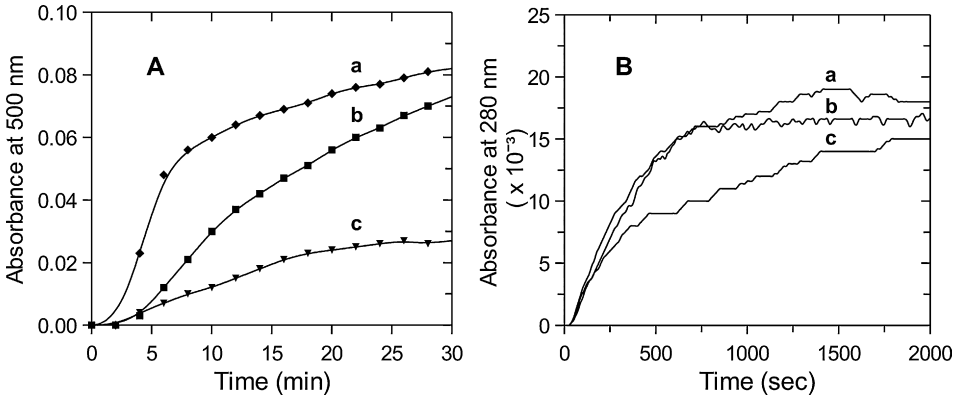

Fig. 1. (A) Time resolved EWA values at 500 nm obtained due to binding of FITC stained *E. coli* at (a) 10^7, (b) 10^6, and (c) 10^5 cfu/ml to biofunctionalized U-bent probes. (B) Time resolved absorbance changes at 280 nm obtained from three different probes (a, b and c) due to binding of monoclonal antibody to glutaraldehyde treated U-bent probe.

175.58 nm) for straight probes as per Khijwania and Gupta (1998) (Figure S-6). Further, the refractive index changes (from about 1.33 to 1.37) in the immediate vicinity of the fiber due to bacteria binding on it leads to higher losses, resulting in ~25% transmission loss from the fiber in the bent region with respect to the transmission before bacteria binds. The net effect is an increase in apparent absorbance as measured at the output of the fiber.

This result illustrates the potential of U-bent probes for EWA based detection of analytes of larger size such as bacteria. With the proof of concept of enhanced EWA in U-bent probes, all the subsequent experiments were performed using UV LED at around 280 nm wavelength.

3.2. *E. coli* detection based on EWA changes at 280 nm

Before investigating pathogen detection using U-bent probes at 280 nm, absorbance changes at 280 nm due to antibody (specific to *E. coli*) immobilization on glutaraldehyde treated U-bent probe surface were monitored in real-time. Absorbance response due to immobilization was found to be very consistent with an average value of 0.017 ± 0.0015 ($n = 3$) (Fig. 1B). The absorbance per unit probe length (0.008 abs units/cm) for U-bent probes is almost equal to that for straight probes (Sai et al., 2010), which indicates that bending the fiber has negligible effect on absorbance response caused by smaller biomolecules (size « penetration depth) bound to the probe surface. D_P values for the straight probes for 280 nm (in aqueous medium) vary from 69.71 nm to 87.56 nm, whereas U-bent probes (bend radius = 0.75 mm) are estimated to have a maximum of at least 449.7 nm in the bend region (Figure S-6).

The antibody immobilized U-bent probes (freshly prepared) were subjected to a wide range of *E. coli* cell concentrations (10^1–10^8 cfu/ml) to determine the dynamic range of the probes. Each probe was incubated with a cell concentration of 10 cfu/ml for 20 min followed by subsequent higher concentrations of *E. coli*. EWA response for 10 and 100 cfu/ml was insignificant due to diffusion limited binding of *E. coli* cells to the U-bent probes. A significant rise in absorbance was observed for cell concentrations above 10^2 cfu/ml and was more prominent above 10^5 cfu/ml (Figure S-10). EWA values as high as 0.26 ± 0.04 were observed using these probes. Interestingly, the response from U-bent probes was an order more than 5 cm long straight probes indicating the superiority of U-bent probes over straight probes for pathogen detection.

Dose response was obtained by subjecting biofunctionalized U-bent probes to a specific *E. coli* concentration between 10^3 and 10^6 cfu/ml. Real time absorbance changes are shown in Fig. 2A. No significant drop in absorbance was observed after flushing the flow cell with 500 μl of the reference solution, indicating no desorption of bound cells. Saturated response from U-bent probes for different *E. coli* concentrations was plotted to obtain a dose response curve as shown in Fig. 2B. The U-bent probes gave rise to a linear relationship between absorbance change and $\log_{10}$ (analyte concentration). These probes were able to detect 250 *E. coli* cells in 250 μl consistently.

Non-specific binding (NSB) studies performed with polyclonal antibody (against *E. coli* O157 strain, non-specific to *E. coli* strain used in this study) bound U-bent probes show a negligible rise in absorbance. The maximum change in absorbance due to NSB in the presence of 10^7 cfu/ml was only ~0.009, however, dropped more

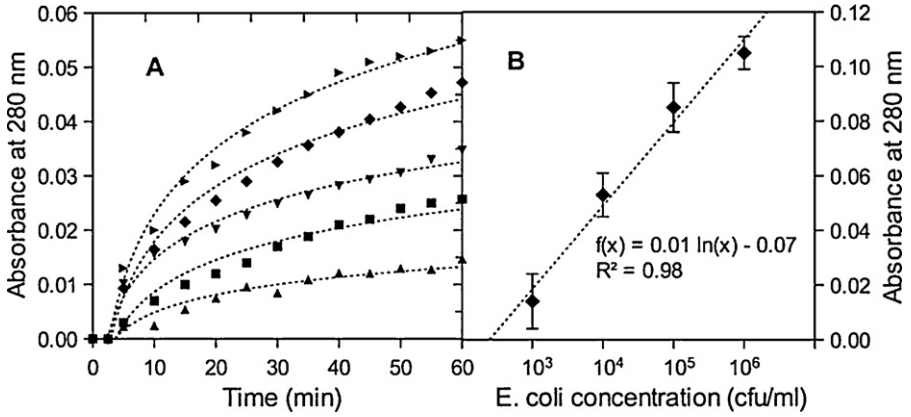

Fig. 2. (A) Time resolved absorbance response at 280 nm obtained for 10^6 (►), 10^5 (♦), 10^4 (▼), 10^3 (■) cfu/ml of *E. coli* on U-bent probes of length 1 cm and bend diameter 1.5 mm and 10^8 (▲) cfu/ml of *E. coli* on a 5 cm long straight probe, (B) Dose response obtained from U-bent probes after saturation for different *E. coli* concentrations ($n > 3$). The effective analyte volume used for pathogen detection was 250 μL.

than 50% after loosely bound cells were flushed with reference solution. Thus, EWA based technique using U-bent probe resulted in minimum detection limit of 10^3 cfu/ml, where its average response is more than thrice that for NSB (Figure S-11). The EWA response from U-bent probes for 10^3 cfu/ml is higher than straight probes incubated with 10^8 cfu/ml as shown in Fig. 2A. Thus, U-bent fiber probes are several orders more sensitive than straight probes (10^3 vs. 10^8 cfu/ml) due to the higher penetration depth.

The demonstrated detection limit of these probes is better than or equal to most label-free optical sensors reported hitherto (10^4 cfu/ml as in Fan et al., 2008; Homola, 2008 to 10^2 cfu/ml as in Waswa et al., 2006; Oh et al., 2003). In addition, these probes are at least an order less sensitive to bulk refractive index changes than SPR based biosensors (Sai et al., 2009) due to higher numerical aperture of the probes UV region (Sai et al., 2010). The performance of U-bent fiber optic biosensor can be further improved by using (i) smaller sample volumes under continuous or re-circulating flow to overcome the diffusion limited binding of cells at lower concentrations (Leung et al., 2008) and (ii) fully optimized probe design. Further studies are in progress to improve the EWA sensitivity of U-bent probes by increasing evanescent field strength and depth of penetration with the help of smaller fiber core diameter and optimized probe geometry.

4. Conclusion

In summary, we have demonstrated real-time label-free detection of ~250 *E. coli* cells present in 0.25 ml of sample with the help of U-bent fiber optic probes by exploiting their optical absorption properties at 280 nm. Modest detection limits (1000 cfu/ml) are obtained as a result of trade-off between sensitivity and simplified sensor design. Nevertheless, this technique is of immense use as the infectious dose for certain pathogenic strains of bacteria widely varies between 10^6 cfu and 10 cfu (Lim, 2003). Owing to the cost-effectiveness and ease of fabrication, the proposed probe and sensor design could be the best choice for the development of a portable device for pathogen detection in resource-poor conditions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.12.014.

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