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Review Article

Recent Developments in Biosensor Technologies for Pathogen Detection in Water

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Abstract

Contamination of drinking water with pathogenic agents is a serious threat to the population. The potential presence of bacteria, viruses and protozoa requires efficient and effective monitoring methods that allow the detection and quantification of these pathogens. Conventional methods of pathogen detection in water primarily rely on culturing, a pre-concentration procedure and some biochemical identification which require a significant period of time ranging from 24 hours to up to a week. Recently significant efforts have been made to develop biosensors capable of rapid and real-time detection of pathogenic agents. This report summarizes the recent developments of biosensor systems used for this purpose.

INTRODUCTION

Environmental waters such as marine and estuarine waters contain different micro-organisms, including viruses and bacteria, many of which play an essential role in the nature. However, certain harmful micro-organisms can pose serious human health risks [1]. Cholera, giardiasis, cryptosporidiosis and Hepatitis A and E are only some of the diseases contracted through consumption of contaminated water [2]. The importance of efficient water quality monitoring methods has come into the focus due to increasing incidences of outbreaks of waterborne illnesses [3]. In one of the largest outbreaks, 400,000 people suffered cryptosporidiosis in Milwaukee in 1993 [4]. Theodore Escherich suggested using *Escherichia coli* (*E. coli*) as an indicator for fecal contamination due to its high density in feces and its association with typhoid bacillus [5]. Multiple tube fermentation (MTF) method for *E. coli* detection was introduced in early 1990s which was based on observing gas production in glucose broths incubated with samples at high temperatures. This method is widely recognized as the standard method for fecal contamination of water [5]. Membrane filtration (MF), a faster and more cost-efficient method, is also widely used for water quality monitoring [6]. In addition to the drawbacks such as being time-consuming, culture-based methods are further complicated by the fact that non-culturable bacteria form a certain percentage of the total bacterial population, making identification a challenge [7]. DNA amplification methods or immuno-fluorescence methods [8-9] remain too expensive to daily use and are highly complex [2]. The development of new user-friendly, portable and low-cost bioanalytical methods is in the focus of research and biosensors are in the forefront of these research works. Biosensors consist of a bioreceptor compound such as an antibody, protein or nucleic acid immobilized on a transducer surface which is capable of providing a signal (in some cases real-time) for the interaction between the bioreceptor and the analyte. Biosensors are able to detect a wide range of analytes in complex matrices and have proven a great potential in environmental monitoring, clinical diagnostics and food analysis [10,11] In this review, the reported biosensors are categorized in two groups of electrochemical and optical on the basis of their transduction mechanism. Therefore we limit the scope of this review to the recently reported developments in these two mechanisms and technologies.

ELECTROCHEMICAL BIOSENSORS

High sensitivity, easy miniaturization and usability in turbid matrices are some of the advantages of electrochemical biosensors [12]. In electrochemical biosensors, the current or the potential changes arisen from the interaction at the interface between the sensor surface and the sample matrix is measured. The techniques are classified into impedimetric, amperometric, voltammetric and potentiometric on the basis of the parameter measured [13]. Screen printing of electrodes using inks of different conductivities has lead to high through-put sensors [14].

IMPEDANCE-BASED BIOSENSORS

Impedance-based biosensors are designed on the basis of a widely used electrochemical technique; Electrochemical Impedance Spectroscopy (EIS). In EIS, a low voltage sinusoidal potential is applied at different frequencies to the electrochemical system and the impedance is measured as a

function of frequencies using the resulting current [13]. A bio-recognition element is immobilized on one of the electrodes which interacts with and binds to the analyte, causing changes in the impedance. The results of the impedance measurements are interpreted in terms of equivalent circuits [15]. The major advantage of impedance-based sensors over amperometric and potentiometric sensors is being a label-free technique. Efforts are being focused on development of novel technologies for more efficient employment of EIS concept for detection of pathogenic agents in water. Chowdhury *et al.* have reported development of a label-free polyaniline-based impedimetric biosensor for simple, rapid and inexpensive detection of *E. coli* O157:H7 [16] (Figure 1). Illustrates the schematic diagram for preparation of the sensor and its working mechanism.

In this biosensor, Anti- *E. coli* antibody as the bio-recognition element has been covalently immobilized on an electrochemically synthesized conducting polyaniline (PANI) film surface using glutaraldehyde as the cross-linker. The biosensor has been reported to detect *E. coli* O157:H7 at concentrations as low as 10^2 CFU mL^{-1} with an upper detection limit of 10^7 CFU mL^{-1}. The specificity of the developed sensor also has been indicated to be satisfactory after testing it for two other strains of similar bacteria. Another impedance-based biosensor utilizing a ferrocene-antimicrobial peptide as the bio-recognition element for detection of pathogenic *E. coli* O157:H7 has been introduced by Li *et al.* [17]. In this work the approach developed by Mannoor *et al.* [18] for the detection of *E. coli* O157:H7 using magainin I antimicrobial peptide (GIGKFLHSAGKFGKAFVGEIMKS) has been refined by introducing a ferrocene label to the peptide. To evaluate the selectivity of the biosensor, it has been exposed to non-pathogenic *E. coli* K12, *Staphylococcus epidermidis* and *Bacillus subtilis.* The preferential selectivity of the biosensor has been shown to be *E. coli* O157:H7 > non-pathogenic *E. coli* > gram positive species. The detection limit obtained by ferrocene-labeled magainin I has been reported to be 10^3 CFU mL^{-1} which is one order of magnitude better than the non-labeled magainin I-modified biosensor (10^4 CFU mL^{-1}). Amini *et al.* have recently demonstrated the applicability of impedance-based Toll-Like Receptor (TLR) 3 immunoprotein-modified Au sensors for detection of viral pathogens [19]. TLRs are the receptor proteins in the innate immune system of higher organisms which recognize pathogen-associated molecular patterns [20]. Different components of the bacterial cell wall, including lipopolysaccharide, lipoteichoic acid, and peptidoglycan are recognized by the pattern recognition receptors [21]. Several pattern recognition receptors including TLR3 recognize the viral molecular pattern; double-stranded RNA (dsRNA) [22]. In this report the applicability of TLR3-modified sensors for detection of polyinosinic–polycytidylic acid; a dsRNA mimicing molecule has been discussed.

AMPEROMETRIC BIOSENSORS

Detection of micro-organisms by amperometric biosensors includes measurement of the changes in the current due to their involvement in bioaffinity interactions at the surface of the working electrode or measurement of the current generated as a result of enzyme catalyzed redox reactions. Platinum (Pt), gold (Au), graphite, modified forms of carbon or conducting polymers are typical materials used as working electrodes. Antibodies capable of binding to specific ligands are immobilized on the surface of the working electrode. Binding of the ligand to the antibodies will give rise to a current signal which indicates the detection response. A second enzyme-antibody complex which can bind to the target ligand on the electrode surface can be employed for signal amplification. Tang *et al.* have developed an amperometric method using a bienzyme biosensor for the detection of *E. coli* density based on determination of phenol produced by enzymatic reactions in the *E. coli* solution [23]. The biosensor has been constructed by covalent immobilization of laccase and horseradish peroxidase (HRP) on indium tin oxide (ITO) electrodes through a self-assembled monolayer of (3-aminopropyl) triethoxysilane. A high sensitivity by the bienzyme biosensor has been observed for the determination of the polyphenolic compounds, which is microbially generated from the salicylic acid (SA), added into the culture medium during the *E. coli* metabolism. As the amount of the polyphenolic compounds is dependent on the *E. coli* density, the developed bienzyme biosensor has been employed to detect the *E. coli* density in a rapid and highly sensitive manner after the incubation of *E. coli* with salicylic acid in culture medium for 2.5 h at 37 ˚C. Chronoamperometry has been used as the detection method and the amplified response current has been obtained for the substrate recycling of the polyphenolic compounds driven by bienzyme-catalyzed oxidation and

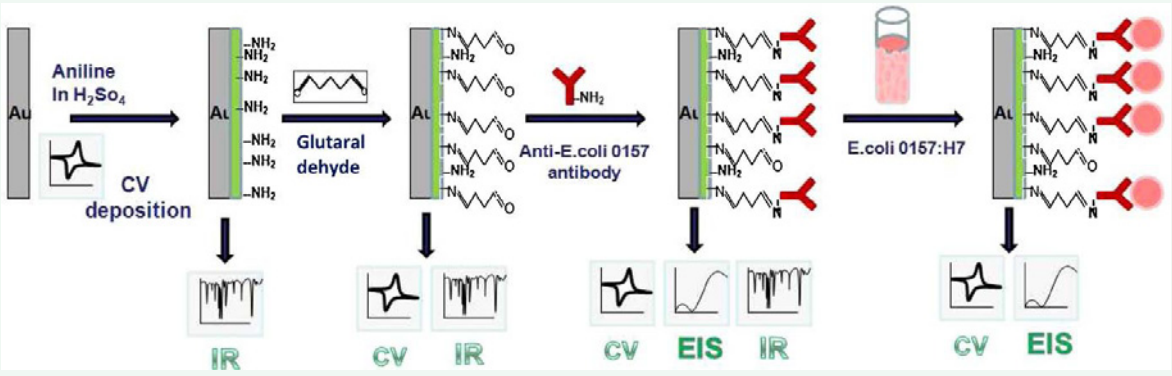

Figure 1 Schematic illustration of the preparation of the polyaniline based impedimetric biosensor and its working mechanism. Reprinted from Chowdhury et al. [16] (Copyright 2012) with permission from Elsevier.

electrochemical reduction. The developed biosensor has been capable of detecting polyphenolic compounds in the nanomolar concentration range. The amplified response current obtained by the bienzyme biosensor and chronoamperometry has been linear with the variation of *E. coli* density between 1.6×10^{3} and 1.0×10^{7} cells mL^{-1}, and the detection limit has been 9.7×10^{2} cells mL^{-1}. The method developed by Tang *et al.* has been reported to have the advantages of being simple, fast and highly sensitive in comparison to conventional microbiological techniques. Cheng *et al.* also have developed an atyrosinase (Tyr) biosensor based on Fe_3O_4 magnetic nanoparticles (MNPs)-coated carbon nanotubes (CNTs) nanocomposite and have employed it for the coliform concentration detection in a flow injection assay system [24]. MNPs being negatively charged have been absorbed onto the surface of CNTs which in turn have been wrapped with cationic polyelectrolyte poly (dimethyldiallylammonium chloride) (PDDA). A glassy carbon electrode (GCE) surface has been modified by the Fe_3O_4 MNPs-coated CNT nanocomposites and then Tyr has been loaded on the modified electrode through glutaraldehyde.

A good microenvironment has been provided by the immobilization matrix for retaining the bioactivity of Tyr and the CNTs incorporated into the nanocomposite have resulted in improved electrochemical detection of phenol. The dynamic linear range for the developed Tyr biosensor for phenol has been reported to be broad (1.0×10^{-8}–3.9×10^{-5} mol L^{-1}) and the detection limit has been as low as 5.0×10^{-9} mol L^{-1}. This biosensor has been integrated in a flow injection analysis system to monitor *E. coli* concentrations as a representative of coliforms. The current responses obtained by this biosensor in the flow injection analysis system have been proportional to the concentration of bacteria ranging from 20 to 1×10^{5} CFU mL^{-1} with detection limit of 10 CFU mL^{-1} and the assay time of ~ 4 h. This biosensor has been shown to be a versatile tool for rapid and automatic clinical diagnostics and water quality monitoring.

VOLTAMMETRIC BIOSENSORS

Different voltammetric methods such as cyclic voltammetry, square wave voltammetry and differential pulse voltammetry have shown important advantages in the analysis of environmental samples [25-27]. These methods have been also employed for pathogen detection in water samples. Fernandes *et al.* have introduced a highly sensitive electrochemical genosensor based on multiwalled carbon nanotubes-chitosan-bismuth and lead sulfide nanoparticles for the detection of pathogenic *Aeromonas* [28]. Lead sulfide nanoparticles coated with 5′-(NH2) oligonucleotide through 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) cross-linkers have been used as signalizing probe DNA (szDNA) and another complementary sequence of DNA with thiol modification which can strongly get adsorbed on the gold surface has been employed as the fixing probe DNA (fDNA). The hybridization of these two probes with the DNA sequence of the target *Aeromonas* (tDNA) (fDNA-tDNA-szDNA) has been detected by differential pulse voltammetry (DPV) after the electro-deposition of the lead nanoparticles (PbS) released from sz-DNA on the glass carbon electrode surface modified with MWCNT–Chi–Bi which improves the deposition and signal transduction. The developed biosensor has been reported to have the highest sensibility for target gene detection in comparison to related biosensors and even polymerase chain reaction (PCR). The detection limit for this biosensor has been 1.0×10^{-14} mol L^{-1} and it has been capable of detecting *Aeromonas* in spiked tap water samples at concentrations lower than 10^{2} CFU mL^{-1}. Schematic presentations of the biosensor development and its working principle have been illustrated in (Figure 2).

Li *et al.* also have recently developed another biosensor for detection of *E. coli* O157:H7 which is based on HRP-mimicking hemin/G-quadruplex wrapped GOx nanocomposites [29]. In this work the graphene oxide (GOx) has been used as the nanocarrier to immobilize thionine (Thi) and the Au nanoparticle-coated SiO_2 nanocomposites (Au–SiO_2) through electrostatic adsorption and the adsorption among nanomaterials. Then on the GOx–Thi–Au@SiO_2 nanocomposites a large amount of signal DNA (S2) and G-quadruplex has been immobilized. Afterwards, interpolating hemin into the G-quadruplex has lead to the hemin/G-quadruplex structure as HRP-mimicking DNAzyme. *E. coli* attaching and effacing (eaeA) gene (S) (5′-GTCACAGTTGCAGGCCTGGTTACAACATTATG-3′) [30] has been selected as the target ligand due to being an excellent genomic marker to *E. coli* O157:H7 and differential pulse voltammetry (DPV) has been employed as the detection method. The developed biosensor has been shown to detect *E. coli* O157:H7 using a calibration curve with a dynamic linear range of 0.02 to 50.0 nmol L^{-1} and a detection limit of 0.01 nmol L^{-1} (S/N=3). The schematic illustration of the stages for GOx–Thi–Au@SiO_2 nanocomposite preparation and the biosensor fabrication and detection mechanism has been shown in (Figure 3).

POTENTIOMETRIC BIOSENSORS

The most investigated potentiometric biosensors include ion-selective field effect transistor (ISFET)-based sensors and light-addressable potentiometric sensors (LAPS). The potentiometric measurements are based on the development of electrochemical potential in proportion to the activity of analyte (a_1) which can be explained by Nernst equation:

$$E = E^{0} \pm \left(\frac{RT}{nF}\right) \ln a_1$$

where E_0 is the standard potential for a_1 = 1 mol L^{-1}, R is the gas constant, F is the Faraday constant, T is the temperature in K, n is the total number of charges of ion i, and the sign (+ or −) is for cations and anions, respectively [13]. The outer layer of the potentiometric biosensors is selectively permeable. A bioactive element in the biosensor; usually an enzyme catalyzes the consumption or generation of a chemical species which is measured by conventional electrochemical techniques. Potentiometric methods have a large dynamic range and are in particular sensitive at low concentrations due to their logarithmic concentration response. ISFET-based biosensors have shown poor detection limit due to the incompatibility of the immobilization procedures with the ISFET-based biosensor development technology [12]. In these biosensors an electric field is employed to create regions of excess charge in a semiconductor substrate to enhance or reduce local electrical conductivity [31]. Light-addressable potentiometric sensors (LAPS) which have

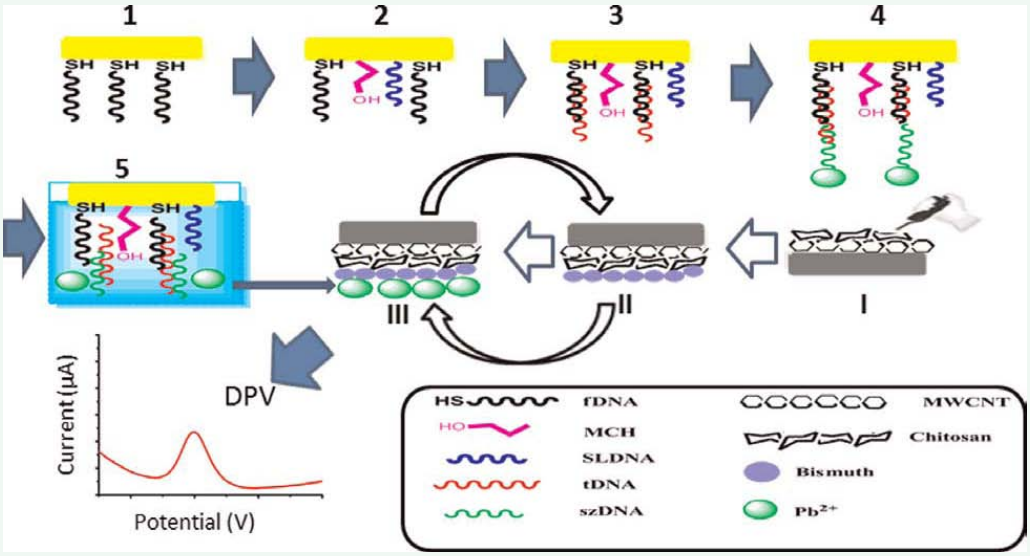

Figure 2 Schematic illustration of the preparation of the biosensor based on multiwalled carbon nanotubes–chitosan–bismuth and lead sulfide nanoparticles and its working mechanism. Reprinted from Fernandes et al. [28] (Copyright 2015) with permission from Elsevier.

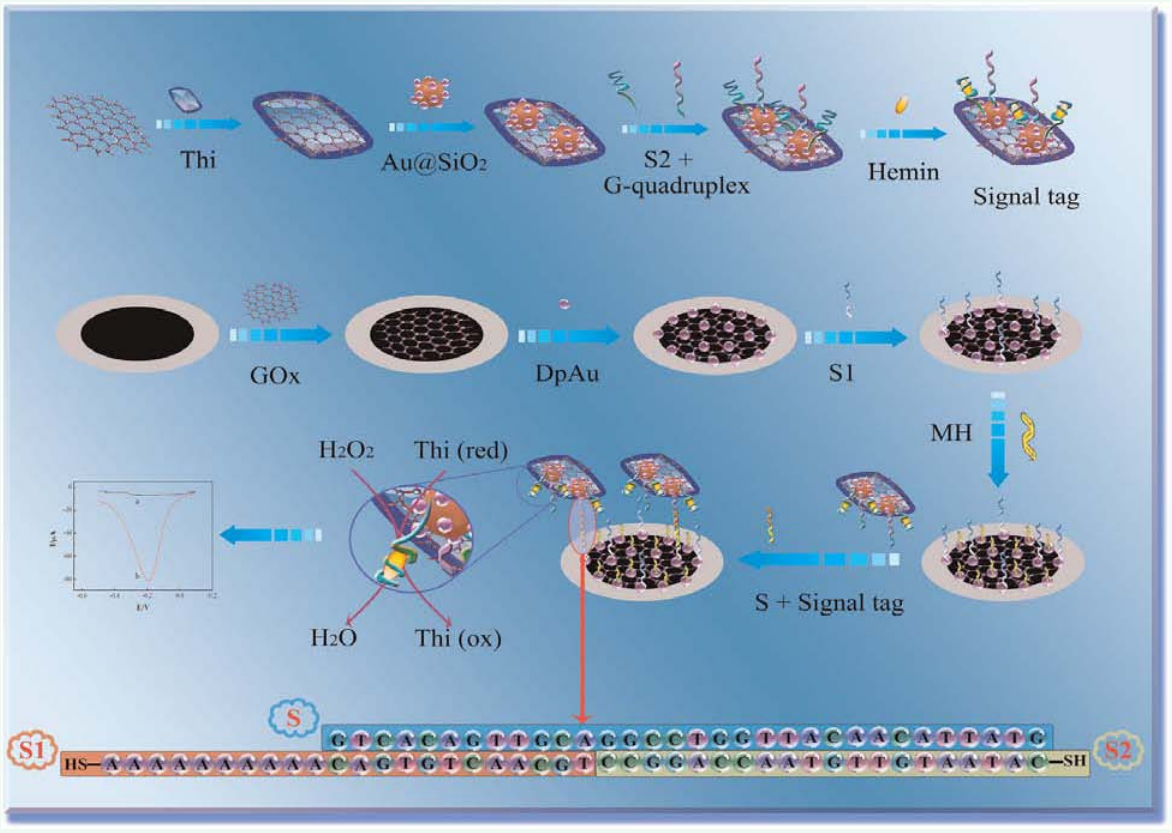

Figure 3 Schematic illustration of the preparation of the biosensor based on HRP-mimicking hemin/G-quadruplex wrapped GOx nanocomposites and its working mechanism. Reprinted from Li et al. [29] (Copyright 2015) with permission from Elsevier.

been developed on the basis of field effect transistors (FET) have been shown to have a good potential for pathogen detection [10]. In LAPS a transient photocurrent is coupled to a thin layer of insulated n-doped or p-doped silicon which is in contact with the electrolyte in which the immunoreaction of interest occurs. The potential changes at the silicon interface can be detected through the difference in charge distribution between the FET and the surface of the insulator. The alternating photocurrent generated by a light source is measured by LAPS and the changes in potential are transduced into voltage per time differentials.

This technique has been employed by Ercole *et al.* for detection of *E. coli* in vegetables which can be easily expanded to water analysis as well. In this work the detection of *E. coli* in lettuces and carrots at concentrations as low as 10 cells mL^{-1} have been achieved after washing them in peptone water and blending them to recover the bacterial content in the liquid medium in 1.5 hr [32]. A potemtiometric aptamer-biosensor for real-time detection of non-pathogenic *E. coli* CECT 675 as a model organism for pathogenic *E. coli* O157:H7 has been developed more recently by Zelada-Guillén *et al.* [33]. In this biosensor, covalently immobilized aptamers have been used as the bio-recognition element and single-walled carbon nanotubes have been employed as an excellent ion-to-electron transducer. The electrical potential has been shown to change dramatically by the selective interaction between the aptamer and the target making the direct detection of the target possible and allowing for both interspecies and interstrain selectivity. The developed biosensor has not given a detectable potentiometric signal when using *Salmonella enterica*, *Lactobacillus casei*, and a different strain of *E. coli* (CECT 4558) indicating its selectivity. The experimental set-up for sample pre-treatment and detection using the biosensor has been illustrated in (Figure 4).

OPTICAL BIOSENSORS

As a powerful detection tool, optical biosenros are employed in biomedical and pharmaceutical research, homeland security as well as environmental monitoring [34]. In this review we focus only on two common optical sensor categories; surface plasmon resonance (SPR)-based biosensors and evanescent field-based optical fiber biosensors.

SURFACE PLASMON RESONANCE-BASED BIOSENSORS

Liedberg *et al.* reported biosensing on the basis of SPR in 1983 for the first time [35] and since then SPR-based biosensors

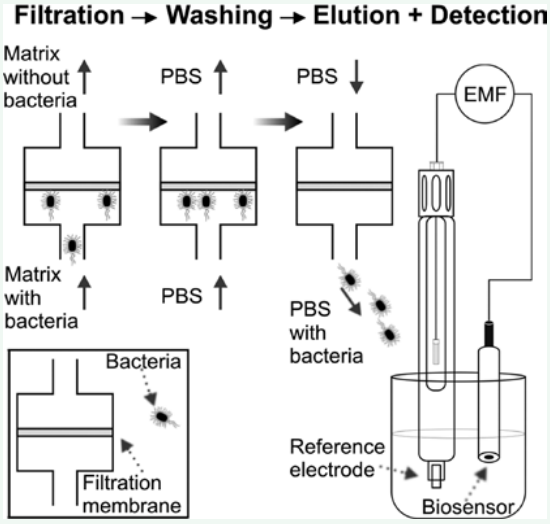

Figure 4 The experimental set-up for sample pre-treatment and *E. coli* detection using the potentiometric aptamer-based biosensor. Reprinted from Zelada-Guillén et al. [33] Copyright (2010) American Chemical Society.

have been studied extensively. In SPR as an optical phenomenon, the resonance condition is fulfilled by excitation of electrons and generation of an electron density wave; surface plasmon wave. The SPR sensors are usually made from a noble metal such as gold coated on a dielectric material such as quartz. At the interface between metal and dielectric material, an evanescent electromagnetic field is generated and propagates into the ambient medium. As the evanescent field diminishes from the surface exponentially, only interactions in the close vicinity of the surface are detected by SPR [36]. The intensity of the reflected light is dramatically reduced due to the occurrence of SPR. The resonance wavelength or angle is dependent on the refractive index of the layer adjacent to the metal surface. Changes in the reflected angle, wavelength, or reflection intensity can be followed to monitor the shifts in the resonance. The refractive index (RI) of the interface can be obtained from the resonance angle or wavelength [37]. Krestchmann configuration which consists of a metallic layer deposited on a quartz prism is the most common geometry for SPR. Tapered fiber geometry has been recently introduced and employed for SPR-based sensing as well [38]. Some of the advantages of the fiber-based SPR sensors include low cost and potential multichannel and remote sensing [13]. Recently an M13 bacteriophage-based SPR detection method for *Salmonella* has been developed by Karoonuthaisiri *et al.* [39]. This method has been shown to be specific and has had detection limits of 8.0×10^{7} and 1.3×10^{7} CFU mL^{-1} for one-time and five-time immobilized sensors, respectively. This study has indicated the applicability of a rapid and label-free SPR assay for pathogen detection (detection of *Salmonella*) using M13 bacteriophages expressing target-specific peptides as a binder. In another study Huang *et al.* have focused on the importance of fluidic conditions and probing depth in SPR-based biosensors for pathogen detection [40]. Importance of these parameters is due to their effect on the diffusion-driven transfer of the analyte from the liquid sample to the sensor which results from the hindrance caused by the large size of the analyte and the subsequent specific capture by the immobilized bio-recognition elements. In this report Huang *et al.* have indicated that only in a narrow window of flow rates, the balance between the mass transfer rate of the analyte and the stability of binding between the analyte and the bio-recognition element of the surface is achieved. Also it has been shown that the enhancement of the sensor response can be achieved by probing of the sensor surface by surface plasmon waves with the probing depth matching to the size of the target analyte. Especially, using long range surface plasmons has lead to the improvement of the sensitivity for detection of model *E. coli* by a factor of three in comparison to other surface plasmons. Schematic illustration of the optical setup and the architecture of the sensor developed by them have been illustrated in (Figure 5).

EVANESCENT FIELD-BASED OPTICAL FIBER SENSORS

The changes in the refractive index due to analyte binding alter the evanescent field which in turn can be detected by fiber optic sensors. If the sensing surface of the fiber is modified with specific bio-recognition elements, target analyte will bind specifically to the sensor surface. This binding modulates the refractive index at the sensor surface which leads to changes in the evanescent field which causes changes in optical throughput. The total internal

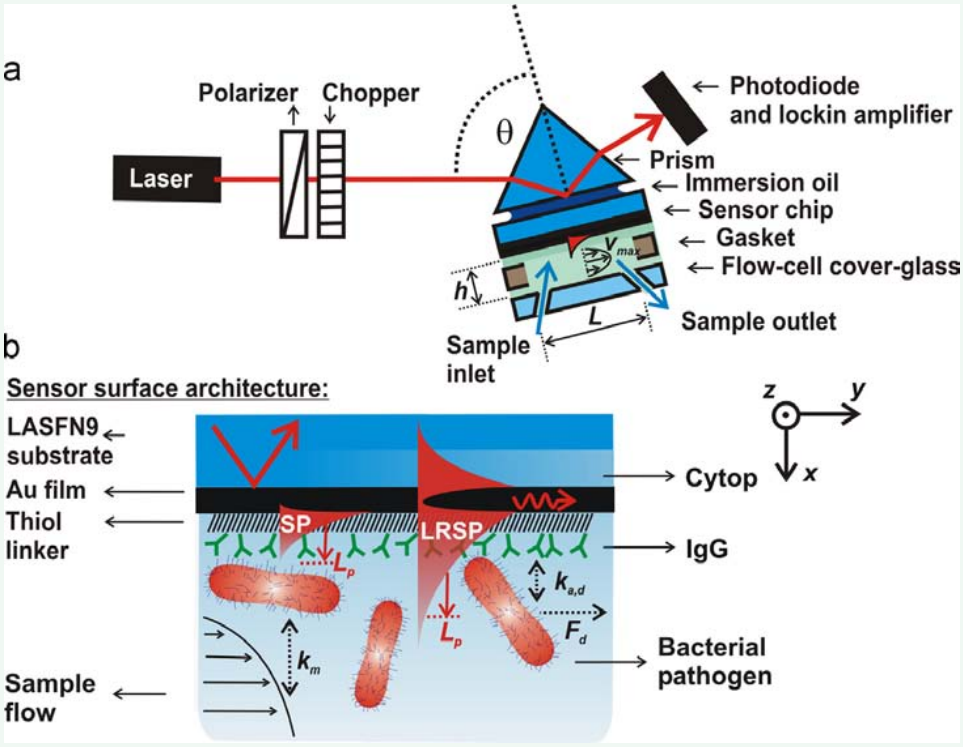

Figure 5 Schematic illustration of (a) the optical setup and (b) the architecture of the sensor chip layer based on long range surface plasmon with antibody bio-recognition elements for sensing the target analyte. Reprinted from Huang et al. [40] (Copyright 2014) with permission from Elsevier.

reflection of the transmitted light happens in the core of the fiber and as a result minimal loss of light is experienced in optical fibers. The propagating light has two components including the guided field in the core and evanescent field in the coating which diminishes exponentially. The interaction of this evanescent field with the surroundings is of great importance in evanescent field-based optical fiber sensors. Very small refractive index disruptions at the sensor surface as a result of analyte binding can lead to significant changes in the optical transmission due to the very small dimensions of the evanescent field (a few hundred nanometers). An evanescent wave DNA-aptamer biosensor based on long period gratings has been developed by Queirós *et al.* for specific detection of the outer membrane proteins of *E. coli* [41]. In this work sensing probes have been obtained by functionalization of long period gratings incised in single mode fiber. The aptamer raised against outer membranes proteins of *E. coli* [42] containing 36 nucleotides has been employed as the bio-recognition element. Two immobilization methods namely electrostatic assembly and covalent binding also have been investigated. The biosensor developed has enabled the specific detection of the proteins of the outer membranes of *E. coli* for the determination of *E. coli* in water through following the resonance wavelength shift which occurs as a result of the binding events and the subsequent changes in the refractive index. The sensors have been reported to have linear responses between 0.1 nmol L^{-1} to 10 nmol L^{-1} of outer membranes proteins of *E. coli* and the sensitivities have been shown to be -0.1563 ± 0.005 nm $decade^{-1}$ [Outer membranes proteins of *E. coli*, mol L^{-1}] for electrostatic immobilization method and -0.1597 ± 0.004 nm $decade^{-1}$ [Outer membranes proteins of *E. coli*, mol L^{-1}] for covalent immobilization method. The sensors have been regenerated under low pH conditions and have been reused for at least three subsequent detections with a deviation less than 0.1%. Being simple in terms of design and analysis, the developed biosensor has provided a versatile platform for detection of *E. coli* proteins and therefore alarming the presence of *E. coli* in water samples. In another recent report, Xiao *et al.* have introduced a portable evanescent wave fiber biosensor for sensitive detection of *Shigella* [43]. In this biosensor, a DNA probe capable of hybridization with a fluorescently labeled complementary DNA is covalently immobilized onto the fiber-optic biosensors. The detection sensitivity has been reported to be as low as 10^{-10} mol L^{-1} for synthesized oligonucleotides. For the regeneration of the sensor surface 0.5% sodium dodecyl sulfate solution (pH 1.9) has been used and the sensor has been shown to be reusable for over 30 times. The comparison of the results obtained by the real time polymerase chain reaction (PCR) and the fiber optic biosensor has shown that these two methods yield comparable results and they also have similar limits of detection of 0.1 nmol L^{-1} (or 10^2 CFU mL^{-1} *Shigella*). The advantages of the fiber optic biosensor over the existing detection methods however has been reported as speed, simplicity, and suitability for on-site detection as well as reusability for over 30 times. (Figure 6) illustrates the schematic of the evanescent wave fiber biosensor system.

DISCUSSION AND CONCLUSION

For a biosensor to be applicable in real pathogen detection, desired characteristics such as accuracy, near real-time assay, sensitivity, specificity, reproducibility, stability and ease of use should be taken into account. Very low (ideally zero) number

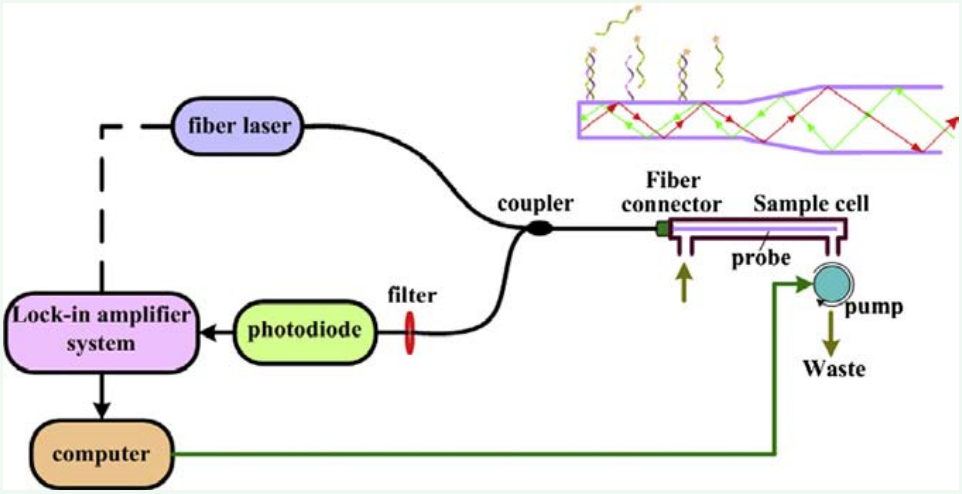

Figure 6 Schematic illustration of the evanescent wave fiber biosensor system. Reprinted from Xiao et al. [43] (Copyright 2014) with permission from Elsevier.

of false-positive and false-negative results is one of the crucial requirements for a biosensor assay to be acceptable. One of the major advantages of biosensors over conventional methods is being rapid and less time-consuming so the desirable assay time for a biosensor is considered less than 1 hr. The biosensor assay should be quantitative and reproducible. High mechanical and biochemical stability of the biosensor is also another requirement. Being easy to use and simple also is an important factor which will remove the need for training and skilled personnel [1, 13]. In biosensor design, complicated methods and procedures should be avoided. However, integration of some steps in biosensor structure is inevitable. For instance, although in some cases biosensors can be applied for analysis in complex target samples with no enrichment or pre-treatment, as mostly the analyte in environmental water samples is dilute, it is important that the innovations in biosensor technologies include integration of a purification or concentration step. Miniaturisation is also another significant factor which makes biosensor devices suitable for on-site analysis. Most of the biosensors for pathogen detection rely on the interaction between the antibodies as the bio-recognition elements and their specific antigens. These antigens are prone to change or deterioration by time. Targeting the DNA signature of any pathogen however, would be a better and more stable approach for detection and quantification of that pathogen as DNA is a stable molecule. Development and synthesis of new bio-recognition elements such as peptides, nucleonic acids, etc with high affinity towards specific analytes is another feature of the advancement of biosensor technologies which should be focused on. Nonspecific adsorption when analyzing complex environmental samples can limit the application of biosensors due to its effect on the selectivity of the biosensor. Dilution of the sample and blocking the unreacted surface sites can help reduce the nonspecific adsorption significantly. In SPR-based immunosensors to correct the contribution from the nonspecific adsorption, a reference channel has been used where a closely similar bio-recognition element to that of the measurement channel is immobilized however the ligand of this bio-recognition element is absent from the sample of interest. Subtracting the reference channel signal from the measurement channel signal will eliminate the effect of the nonspecific absorption. Although optical sensors provide an exciting opportunity for pathogen detection in water, their complexity and high cost puts them on the back foot especially for on-site applications. Electrochemical sensors on the other hand are sensitive and easy-to-use however they do not have the selectivity required for most in-field uses. The need for more efficient biosensors for on-site analysis of real environmental samples is still high. The newly developed technologies should of course be validated and standardized by comparison to already existing and commonly accepted and used methods in terms of results, sensitivity and selectivity.

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