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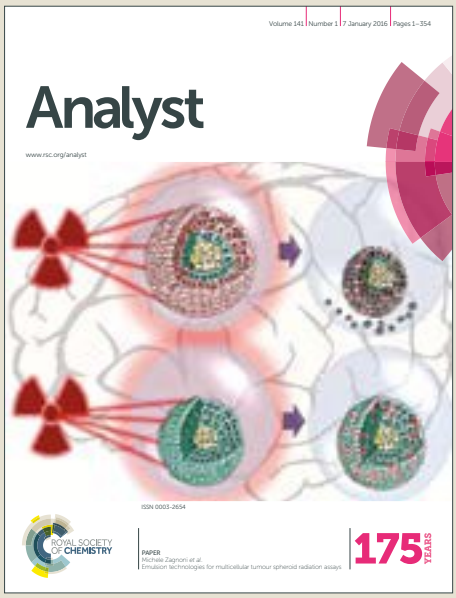

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ARTICLE

Emerging Biosensor Platforms for the Assessment of Water-Borne Pathogens

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Nishant Kumar,[†‡b] Yuan Hu,[†a] Suman Singh[*‡b] and Boris Mizaikoff[*a]

Pathogens are key contaminants in water responsible for the generation of various water-borne diseases, and includes viruses, fungi, bacteria, and protozoan parasites. The pathogenic effects of these species in water depend on their shape, size, composition, and structure. The resulting water-borne diseases are a serious threat to environment including humans and animals, and are directly responsible for environmental deterioration and pollution. The potential presence of these pathogens requires sensitive, powerful, efficient, and ideally real-time monitoring methods for their reproducible quantification. Conventional methods for pathogen detection mainly rely on time-consuming enrichment steps followed by biochemical identification strategies, which require assay times ranging from 24 h to up to a week. In recent years, significant efforts have been made towards the development of biosensing technologies enabling rapid and close-to-real-time detection of water-borne pathogens. This review summarizes recent developments on biosensors and sensing systems based on a variety of transducer technologies for water quality monitoring with specific focus on rapid pathogen detection.

1 Introduction

Waterborne diseases are worldwide problem, which is assessed to bring about more than 2.2 million deaths per year along with higher instances of ailments including cholera, diarrhea, giardiasis, cryptosporidiosis, and Hepatitis A and E.[1, 2] Globally, a monetary loss of about 12 billion US dollars per year is the associated economic effect.[3] For example, estuarine and marine water contains certain microorganisms, which have toxic effects on humans.[4] As of now, it is evaluated that there are >1,400 types of pathogens contaminating people, which includes bacteria (538 species), viruses (208 types), parasitic protozoa (57 species), and a few fungi and helminths species.[1, 5] The pathogenic effects of these species in water depend on their shape, size, composition, and structure. For example, in a seminal review Young has detailed the role of bacteria morphology.[6] Likewise, the shape of biological species/pathogens has biological relevance, as it assist in fighting against primary and secondary pressures, which include nutrient acquisition, cell division, predators, attachment to surfaces, passive dispersal, active motility, and internal or external differentiation.

The presence of pathogens in water is creating a threat to human survival. Table 1 provides general data on pathogens that are associated with different diseases as well as are persistent in drinking-water supply.[7] Despite the fact that water-borne outbreaks have been deteriorating drastically since the 1900s, the worldwide impact of infectious water-borne diseases remains considerable. At least 1,870 outbreaks were concerned with drinking water in the period of 1920 to 2002. From 1991 to 2002, 433,947 illness were reported in the United States of America (USA) resulting from the protozoan agents *Giardia, Naegleria fowleri, Crytosporidium* and the bacteria *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Legionella*, and *Vibrio cholera*.[8] There were 134 water-associated outbreaks reported by 38 States in USA and Puerto Rico between 2007 and 2009. This led to the occurrence of at least 13,966 cases comprising 81 outbreaks of acute gastrointestinal illness, 24 of dermatologic illnesses, and 17 reporting acute respiratory illness. The leading pathogens, which were involved in these cases were *Crytosporidium, Escherichia coli* O157:H7, *Pseudomonas* spp., *Shigella sonnei*, and *Legionella* spp. In 2010, approximately 25 outbreaks were reported in USA, which were concerned with drinking water.[9] In Germany 2011, *E.coli* (strain O104:H4) was responsible for severe cases of diarrhea, which resulted from the consumption of uncooked sprouts. Contaminated water was used for irrigation purpose which caused the pathogenic infection.[10] In 2012, 18 cases of cholera were reported from the United Kingdom, Austria, and Sweden. In the same year, United Kingdom, Ireland, Belgium, and Germany faced 9,591 cases of *Cryptosporidium* largely due to *C. parvum* gp60 subtype IIaA15GR1. In the same year, 10 waterborne outbreaks were reported in EU caused by *E. coli* O157:H7.[11] In 2013, an

[a] Ulm University, Institute of Analytical and Bioanalytical Chemistry, Ulm, Germany. E-mail: boris.mizaikoff@uni-ulm.de
[b] CSIR-Central Scientific Instruments Organisation, Chandigarh, India E-mail: ssingh@csio.res.in
[‡] Academy of Scientific and Innovative Research (AcSIR-CSIO), Chandigarh 160030, India
† Equal contribution for first authorship.

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outbreak of acute gastroenteritis (AGE) was notified at the Gipuzkoa Epidemiology Unit at a domestic appliance factory, though first signs of outbreak were observed in June 2013.[12] Analysis of water confirmed the presence of norovirus and rotavirus. This outbreak affected 238 people. In 2014, according to the World Health Organisation (WHO) approx. 94,000 deaths occurred due to diarrhoea in the Western Pacific Region via contaminated water.[13] In 2015, the United Nations International Children's Emergency Fund (UNICEF) reported that around 884 million people across the world use some form of contaminated drinking water ressource.[14]

The WHO has reported that enhancing the water quality would decrease the worldwide effects of sickness by around 4%.[15] Hence, there is a significant demand for the development of efficient, rapid, and sensitive analytical techniques for the detection of water-borne pathogens. Effective testing on the presence of waterborne pathogens requires methods of analysis that meet a number of challenging criteria, which aid in reducing outbreaks in future. Pathogenic detection techniques are required to respond rapidly and sensitively, as the presence of even single pathogenic organisms in the water may cause severe infections. To meet this increasing demand, biosensors offer user-friendly, rapid, selective, portable, and sensitive analytical platforms for the detection of water-borne pathogens.

Biosensors are devices or assays that consist of a biorecognition element, which is coupled to a signal transducer capable of providing an-ideally quantitative-analytical signal,

Table 1 Waterborne pathogens and their significance in water supplies. Adapted from the WHO Guidelines for Drinking Water Quality.[7]

Pathogen	Associated disease	Health significance	Persistence in water supplies	Resistance to chlorine	Relative infectivity
Bacteria					
Burkholderia pseudomallei	Melioidosis	High	May multiply	Low	Low
Campylobacter jejuni, C. coli	Gastroenteritis	High	Moderate	Low	Moderate
Escherichia coli - Pathogenic	Gastroenteritis	High	Moderate	Low	Low
E. coli – Enterohaemorrhagic	Gastroenteritis, Hemolytic-uremia	High	Moderate	Low	High
Legionella spp.	Legionnaires' disease	High	May multiply	Low	Moderate
Non-tuberculous mycobacteria	Pulmonary disease, skin infection	Low	May multiply	High	Low
Pseudomonas aeruginosa	Pulmonary disease, skin infection	Moderate	May multiply	Moderate	Low
Salmonella typhi	Typhoid Fever	High	Moderate	Low	Low
Salmonellae enterica	Salmonellosis	High	May multiply	Low	Low
Shigella spp.	Shigellosis	High	Short	Low	High
Vibrio cholerae	Cholera	High	Short to long	Low	Low
Yersinia enterocolitica	Gastroenteritis	Moderate	Long	Low	Low
Viruses					
Adenoviruses	Gastroenteritis, respiratory infection	Moderate	Long	Moderate	High
Enteroviruses	Gastroenteritis	High	Long	Moderate	High
Astroviruses	Gastroenteritis	Moderate	Long	Moderate	High
Hepatitis virus A, E	Hepatitis	High	Long	Moderate	High
Noroviruses	Gastroenteritis	High	Long	Moderate	High
Sapoviruses	Gastroenteritis	High	Long	Moderate	High
Rotavirus	Gastroenteritis	High	Long	Moderate	High
Protozoa					
Acanthamoeba spp.	Keratitis, encephalitis	High	May multiply	Low	High
Cryptosporidium parvum	Cryptosporidiosis	High	Long	High	High
Cyclospora cayetanensis	Gastroenteritis	High	Long	High	High

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Entamoeba histolytica	Amoebic dysentery	High	Moderate	High	High
Giardia intestinalis	Giardiasis (Beaver fever)	High	Moderate	High	High
Naegleria fowleri	Primary amoebic meningoencephalitis	High	May multiply	Low	Moderate
Toxoplasma gondii	Toxoplasmosis	High	Long	High	High
Helminths					
Dracunculus medinensis	Dracunculiasis (Guinea worm disease)	High	Moderate	Moderate	High
Schistosoma spp.	Schistosomiasis	High	Short	Moderate	High

resulting from the interaction between biorecognition element and target analyte/species.[16] Among the more commonly applied biorecognition elements are enzymes, antibodies, oligonucleotide probes, aptamers, and cell-surface molecules,[17] as well as phages.[18] The recognition elements are immobilized such that specific interactions with the target species – here, water-borne pathogens - is ensured, while the signal transduction process ideally remains free from interferences. The biochemical signal generating from chemical or enzyme catalysed reactions is converted into an electrical signal by the transducer. Some of the commonly used transducers have been discussed in coming sections.

This review focusses especially on biosensing methods for the detection of water-borne pathogens using various types of transducer platforms complemented by selected state-of-the-art application examples highlighting the particular benefits of each technology.

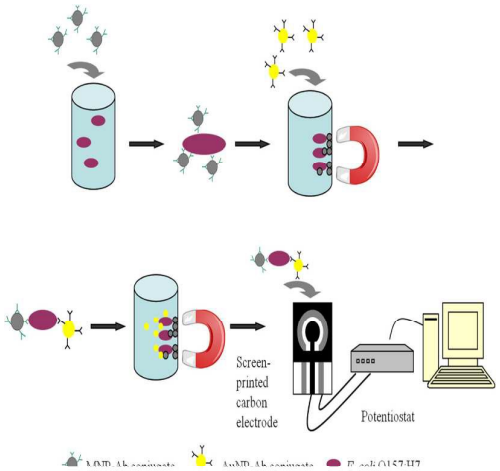

Fig. 1 Schematic of a gold nanoparticle (AuNP) based biosensor. Target cells in a sample were captured by magnetic nanoparticle (MNP)-antibody (Ab) conjugates and separated by a magnet. Then, the cells were labeled with AuNPs. The MNP-Ab-cell-Ab-AuNP complexes were transferred onto a screen-printed carbon electrode chip connected to a potentiostat for electrochemical analysis. Adapted from Wang et al. [26] with permission from BioMed Central.

2 Classification of biosensors

Biosensors are usually classified based on the mode of physiochemical signal transduction. The transducer plays a vital role within a biosensor, as the (bio)chemical interaction/signal is reproducibly translated into a physical quantity. Transduction methods include predominantly electrochemical, optical, and piezoelectric principles, however, are continuously expanding into utilizing other physical effects also.

2.1. Electrochemical biosensors

An electrochemical biosensor, as defined by IUPAC in 1999 is a self-contained integrated device, which can perform quantitative or semi-quantitative analysis of biorecognition catalysed reactions using an electrochemical transduction element.[19] Electrochemical biosensors measure the current produced from oxidation and reduction reactions and current produced is directly related to the concentration of the electroactive species present/produced.

Despite the fact that biosensing devices may combine with an assortment of (bio)recognition elements, electrochemical detection technique prevalently take advantage of enzymes. This may be attributed not only to their specific binding capabilities, but also to their (bio)catalytic activity giving rise to inherent signal enhancements.[20-22] For electrochemical biosensors, the transducer needs to a conductor, and conducive to appropriate surface modification steps for attachment of the biorecognition element. Electrochemical biosensors are further classified into voltammetric, potentiometric, amperometric, and impedance-based biosensors.

2.1.1. Voltammetric biosensors

Voltammetric biosensors detect an analyte by determining the change in current as a function of applied potential. For the analysis of environmental samples, different voltammetric techniques have been used including cyclic voltammetry, differential pulse voltammetry, and square wave

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voltammetry.[23-25] Recently, Wang et al. developed a state-of-the-art electrochemical biosensor using antibody-modified nanoparticles for the detection of *E. coli* O157:H7.[26] For detection purposes, *E. coli* O157:H7 colonies from frozen culture stored at -70 °C were grown on trypticase soy agar plates. Afterwards, a single colony was isolated and grown overnight at 37 °C after inoculating in tryptic soy broth. Now, in another tube of tryptic soy broth, 1 mL of the liquid culture was transferred and overnight incubated at 37 °C. Before performing each experiment, 1 mL of this liquid culture was transferred again to a new broth tube and maintained at 37 °C for 6 h. Figure 1 shows a schematic of such gold nanoparticle labeled biosensors. In this study, two innovative nanoparticle approaches were applied for designing the biosensor: (i) polymer-coated magnetic nanoparticles (MNPs), which help to separate the bacteria from the sample matrix, and (ii) carbohydrate-capped AuNPs, which label the separated bacteria via formation of a sandwich structure during signal generation. The signals were generated due to interaction of separated bacteria with carbohydrate-capped AuNPs. Thus generated signals were determined by differential pulse voltammetry (DPV) using a screen-printed carbon electrode chip. The lower limit of detection was found to be 10^1 CFU (colony forming unit)/ml with a dynamic range of 10^1 to 10^6 CFU/ml. The time required for the bacterial extraction and detection was less than 45 min.

Das et al. have developed an electrochemical genosensor for the water-borne pathogen *Salmonella typhi* (*S.Typhi*) using gold nanoparticle-mercaptosilane modified screen-printed electrodes.[27] In this study, a system of integrated self-assembled layer of organosilane 3-mercaptopropyltrimethoxy silane (MPTS) was fabricated at the surface of the screen-printed electrode (SPE), and electrochemically deposited gold nanoparticles employing Vi gene as a molecular marker for the detection of *Salmonella typhi* were immobilized (Figure 2). Onto the gold nanoparticle modified SPE, thiolated DNA probe was immobilized for a DNA hybridization assay using methylene blue (MB) as redox (i.e. electroactive) hybridization indicator. The signal was evaluated using differential pulse voltammetry (DPV). The linearity of this biosensor was found to be 1.0×10^{-11} to 0.5×10^{-8} M, and the detection limit was 50 (±2.1) pM. The as-developed electrochemical biosensor could be regenerated and reused for up to four times.

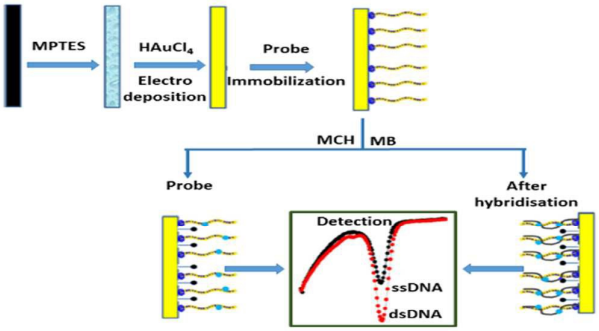

Fig. 2 Schematic representation of a MPTS-AuNPs modified SPE platform serving as genosensor (With minor changes in representation). Adapted from Das et al. [27] with permission from Elsevier.

2.1.2. Potentiometric biosensors

Potentiometric biosensors determine the electrical potential difference between a working and reference electrode in an electrochemical cell, ideally without any significant current flowing in between. The potential of the reference electrode remains invariant during the measurement, whereas the working electrode undergoes significant changes in potential upon changes in analyte concentration. Such biosensors are usually based on so-called ion-selective electrodes (ISEs), and ion-sensitive field effect transistors (ISFETs).[28] The output signal generates due to accumulation of ions at ion-selective membrane interface. In other words, we it can be said that these sensors/biosensors help in the determination of ion activity in an electrochemical reaction. Potentiometric sensors are good for measuring low concentrations in tiny sample volumes.[29] Many reviews have been dedicated on potentiometric sensors/biosensors.[30-32] Several studies have been reported on the detection of water-borne pathogens using potentiometric

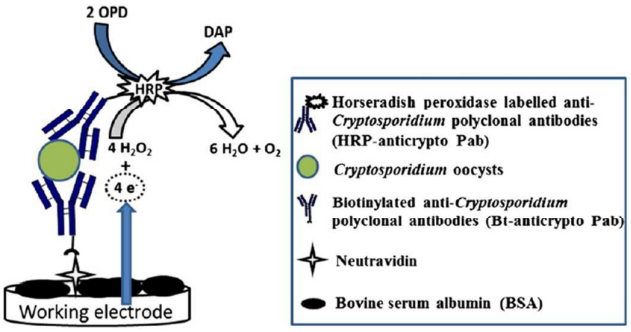

Fig. 3 Principle of a potentiometric immunosensor based on direct electron transfer resulting from the conversion of hydrogen peroxide in oxygen and water in the presence of the mediator and proton donor o-phenylenediamine (OPD). The oxidation of OPD by HRP results in the production of 2,3-diaminophenazine (DAP). Adapted from Laczka et al. [36] with permission from Elsevier.

biosensors.[33-35]

Laczka et al. reported a novel electrochemical method for rapidly sensing the parasitic protozoan *Cryptosporidium parvum*.[36] The detection of this water-borne protozoa is of specific interest for drinking water safety due to its resistance against standard methods of water disinfection.[37-39] Using a solid phase extraction (SPE) and horseradish peroxidase (HRP) labelled antibodies binding to the *Cryptosporidium parvum*, the species were detected potentiometrically. O-phenylenediamine (OPD) acted as a proton donor, while HRP catalyzed the conversion of hydrogen peroxide to oxygen and water in its presence. The reduction of hydrogen peroxide involved a direct electron transfer,

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which generates an electrical potential difference between working and reference electrodes. This potential difference is directly proportional to the rate of the enzymatic reaction, which in turn related to the concentration of *Cryptosporidium parvum oocysts* (Fig. 3). This method allowed the detection of 5 × 10^2 *Cryptosporidium oocysts* per mL in 60 min.

Karapetis et al. developed a miniaturized potentiometric cholera toxin sensor using graphene nanosheets with incorporated lipid films.[40] Cholera toxin (CT) is produced by bacterium *Vibrio cholera*, which is responsible for epidemic diseases leading to rapid dehydration and cause death within a few hours of exposure. In this study, the natural cholera toxin receptor known as Ganglioside GM1 was immobilized onto the lipid film providing excellent selectivity. The proposed sensor was reusable, reproducible, and selective across a wide range of concentrations (10 × 10^{-9} M to 10 × 10^{-6} M). The sensor revealed adequate response even during the analysis of real water samples. A schematic of the experimental setup is shown in Fig. 4.

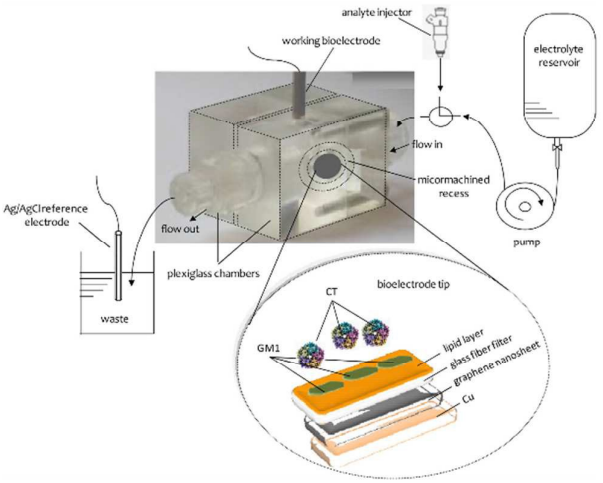

Fig. 4 Schematic of the experimental setup and detail of the bioelectrode surface. Adapted from Karapetis et al. [40] with permission from Wiley.

2.1.3. Amperometric biosensors

Amperometric biosensor detect the analyte by determining the changes in current after bioaffinity interactions occur at the surface of the working electrode. Escherichia coli (E.Coli) O157:H7 is the most common Shiga toxin producing strain of E.coli, which cause severe bloody diarrhea, stomach cramps, vomiting or even life threatening hemolytic uremic syndrome.[41] Xu et al. developed an electrochemical biosensor to detect E.coli O157:H7.[42] This biosensor was fabricated by integration of bifunctional glucose oxidase (GOx)-polydopamine (PDA) based polymeric nanocomposites (PMNCs) and Prussian blue (PB) modified screen-printed interdigitated microelectrodes (SP-IDMEs). Firstly, by the self-polymerization of dopamine (DA) core-shell magnetic beads (MBs)-GOx@PDA PMNCs were synthesized. Gold nanoparticles (AuNPs) were dispersed at the surface of PMNCs via biochemical synthesis to achieve more efficient adsorption of antibodies (ABs) and GOx. Thereafter, using filtration, the unbound PMNCs were separated, and transferred into glucose solution to allow the enzymatic reaction to occur. The change in current response was measured using both cyclic voltammetry (CV) and amperometric techniques via PB-modified SP-IDMEs. The detection limit of this biosensor was found to be 10^2 CFU/ml. A schematic of this biosensor principle is shown in Figure 5.

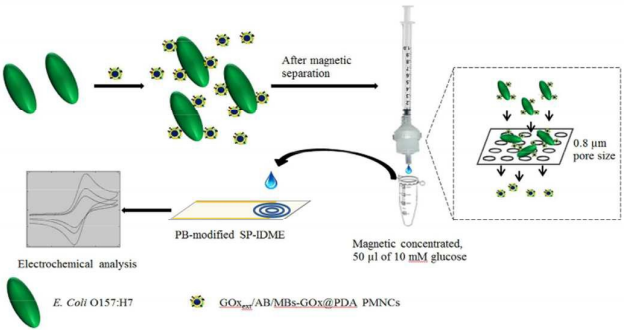

Fig. 5 Electrochemical biosensor for the detection of E. coli O157:H7. Adapted from Xu et al. [42] with permission from Royal Society of Chemistry.

Davis et al. have developed an enzyme-linked immunosorbent assay (ELISA) based amperometric biosensing strip for rapid and cost-effective detection of *L. monocytogenes* using AuNP-modified screen printed carbon electrodes (SPCE).[43] The purpose of the modification of an electrode surface using AuNPs was to create a large electrode surface, which will assist fast electron transfer and will contribute to the conductivity. The detection limit of the amperometric biosensing strip was determined at 10^2 CFU/g of food. The developed biosensor requires one hour for an accurate determination, and revealed an excellent analytical response in real-world samples.

2.1.4. Impedance-based biosensors

Electrical impedance spectroscopy (EIS) based biosensors are of increasing popularity. At different frequencies, low voltage sinusoidal potentials are applied to the electrochemical system. Via the resulting current, impedance changes are determined as a function of frequency.[44] The results obtained from such impedance measurements are evaluated in terms of equivalent circuits.[45] Due to its sensitivity and simplicity of the measurement, significant interest has emerged for the detection of water-borne pathogens using impedance-based techniques. Recently, Chen et al. have reported an impedance-based biosensor for the sensitive detection of *L. Monocytogenes*, which is a water-borne pathogen.[46] Monoclonal antibodies (MAbs) were immobilized at the surface of magnetic nanoparticles (MNPs) using the biotin-streptavidin system, which assists in separating Listeria cells efficiently from the sample matrix. Moreover, at the surface of

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AuNPs polyclonal antibodies (PAbs) and urease were used to establish MNP-MAb-Listeria-PAb-AuNP-urease sandwich complexes. Subsequently, using 200 µL of PBST (0.5% Tween-20 in PBS) and deionized water the complexes were washed sequentially three times to remove unbounded PAb, urease-modified AuNPs, and conductive ions, respectively. Afterwards, these complexes were resuspended in 200 µL of 1 mM urea. The magnetic separation of complexes was done for 2 min after incubating for 30 min, and 20 µL of supernatant was transferred onto the microelectrode for analysis. The lower detection limit of this biosensor for *L. monocytogenes* was found to be 30 CFU/mL, with the fundamental principle shown in Figure 6.

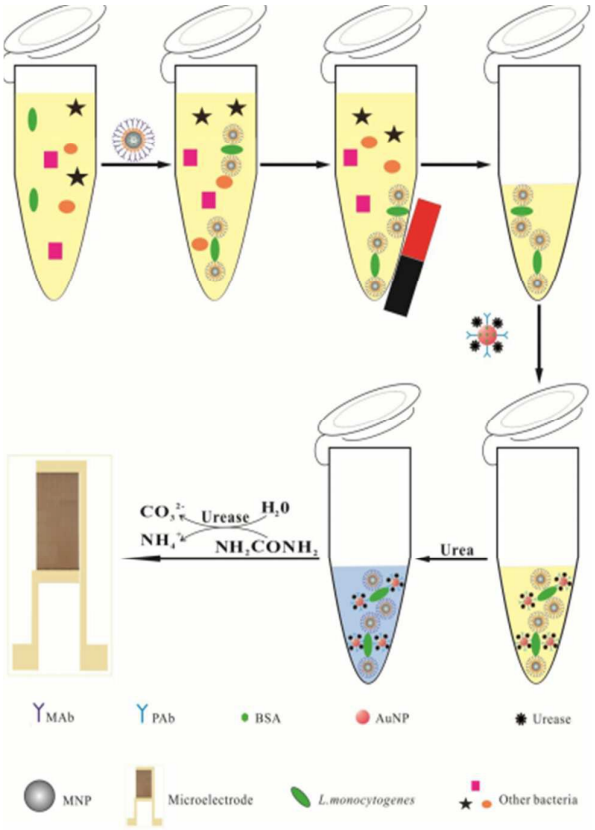

Fig. 6 Impedance-based biosensor using immunomagnetic separation and urease catalysis. Adapted from Chen et al, [46] with permission from Elsevier.

Dong et al. have shown a label-free electrochemical impedance immunosensor by immobilizing anti-Salmonella antibodies onto gold nanoparticles and poly(amidoamine)-multiwalled carbon nanotubes-chitosan nanocomposite films modifying glassy carbon electrodes (AuNPs/PAMAM-MWCNT-Chi/GCE) for the detection of *Salmonella typhimurium.*[47] A linear response function of the biosensor was found in the range of 1.0×10^3 to 1.0×10^7 CFU/mL with a detection limit of 5.0×10^2 CFU/mL. A schematic is shown in Figure 7.

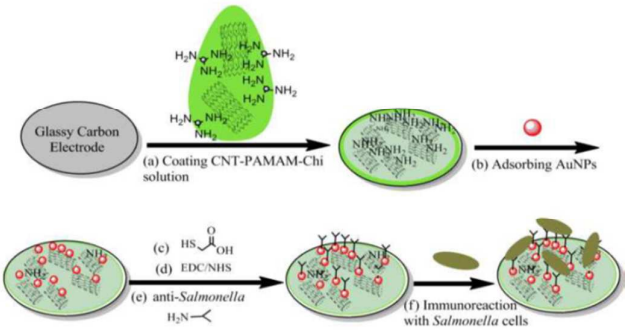

Fig. 7 Schematic of an immunosensor fabrication process. (a) dropping of PAMAM-MWCNT-Chit membrane; (b) assembly of AuNPs; (c) assembly of mercaptoacetic acid; (d) activation of carboxyl groups with EDC/NHS; (e) capture of anti-*Salmonella* antibodies; (f) immunoreaction of anti-*Salmonella* and *Salmonella* cells. Adapted from Dong et al. [47] with permission from Elsevier.

2.2. Optical biosensors

Optical biosensors have been promoted as promising alternative transducer platform for pathogen analysis using a variety of optical sensing modalities. These types of biosensor are capable of detecting even minor changes of analytes, thus allowing sensitive and specific measurements. Herein, the most prominent types of optical biosensors are discussed based on surface plasmon resonance (SPR), evanescent field sensing via optical fibers/waveguides, infrared and Raman spectroscopy, fluorescence, chemiluminescence, and colorimetry.

2.2.1. Surface plasmon resonance (SPR) biosensors

Surface plasmon resonance (SPR) is an optical technique that uses the occurrence of surface wave propagating along noble metals under resonance conditions in dependence of the refractive index adjacent to the noble metal surface, i.e., sensor surface.[48, 49] In SPR biosensors, the bioreceptor is immobilized at the sensor surface, and the analyte solution usually passes via a microfluidic channel across the biomolecular recognition interface. The direct interaction between analyte and ligand results in a change in refractive index close to the sensor surface, which is determined as a change of the resonance angle of the surface plasmon. Thus, SPR is considered a label-free technique even enabling dynamic/kinetic measurements. The major benefits associated with this technique are that it simply responds to changes in refractive index induced by molecular binding events, and does not require additional reagents, assays, and laborious sample preparation steps. At the same time, the entire specificity of SPR-based sensors results from the biorecognition step. There are various reports on SPR-based biosensors for the detection of water-borne pathogens.[50-52] Recently, Singh et al. have developed a SPR-based biosensor

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for the detection of *Salmonella*.[53] This biosensor has been fabricated by generating self-assembled monolayers of 5'-thiolated single-stranded DNA (ssDNA) probe attached to the gold surface of a SPR chip. This DNA biosensor has been investigated for label-free real-time monitoring of *Salmonella* with a detection limit of 2 fM. A schematic of this SPR-based biosensor scheme is shown Fig. 8.

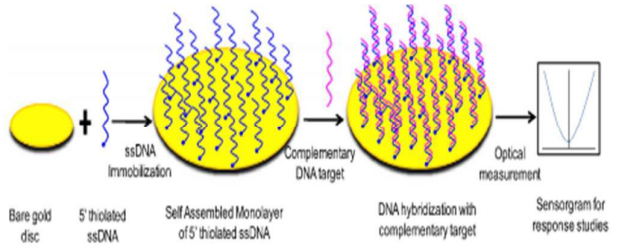

Fig. 8 SPR-based optical DNA biosensor. Adapted from Singh et al. [53] with permission from Springer.

Nanduri et al. have detected *L. Monocytogenes* pathogen with SPR sensors using phage-displayed scFv antibody to the virulence factor actin polymerization protein (ActA) as biorecognition element.[54] Via physical adsorption, phage Lm P4:A8 expressing the scFv antibody fused to the pIII surface protein, and was immobilized at the sensor surface. The detection limit of this biosensor was estimated at 2×10^6 CFU/ml. The dissociation constant (K_d) for the interaction of phage-displayed scFv and soluble ActA was found to be 4.5 nM.

2.2.2. Evanescent field based fiberoptic biosensors

When light propagates via a core-only high-index (i.e., $n_{fiber}>n_{sample}$) optical fiber on the basis of total internal reflection (TIR), an electromagnetic field (a.k.a., evanescent field or evanescent wave) is generated at the waveguide/sample interface. This field decays exponentially with distance from the interface. An evanescent wave can thus be used to excite fluorescence in proximity of the sensing surface, e.g., in fluorescently labeled biomolecules bound to the optical sensor surface.[55]

In recent years optical fibers have been successfully used as biosensor platform in various spectral regimes, as they are rapidly responding, specific, sensitive, and cost effective. Furthermore, they are suitable for close to real-time monitoring and on-site detection. Consequently, fiberoptic biosensors have been used in a wide range of applications.[56, 57] In the simplest form, evanescent field based optical fiber or waveguide biosensors observe changes in refractive index due to analyte binding events via appropriate biorecognition elements, thus directly affecting the optical conditions for generating an evanescent field.[58, 59] Consequently, a variety of optical transduction schemes can be used taking advantage pf evanescent field sensing concepts including like fluorescence detection, monitoring of refractive index changes, absorbance spectroscopy via the evanescent field or detecting spectroscopic shifts.

Evanescent field fiberoptic biosensors are increasingly being applied for the detection of pathogens in food and water supplies, food processing facilities, and homeland security operations.[60] These biosensors can be used for multiplexed pathogen detection or to complement other analytical techniques. Bharadwaj et al. developed a U-shaped evanescent wave absorbance (EWA) fiber optic biosensor for the label-free detection of *E.coli* at a wavelength of 280 nm.[61] The penetration depth of the evanescent wave was enhanced by bending an unclad fiber segment into a U-shaped structure. The enhanced EWA response caused by the inherent optical absorbance properties of the biomolecules specifically bound to the surface of the sensor were used for the detection of pathogens. The detection limit of the proposed sensor was found to be 1000 CFU/ml, and is schematically shown in Figure 9.

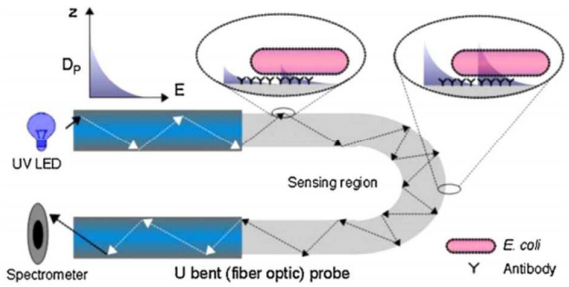

Fig. 9 Enhancement of the penetration depth DP via a U-shaped fiber probe enabling the evanescent field detection of E.coli. Adapted from Bhardwaj et al. [61] with permission from Elsevier.

This biosensor has enabled the specific detection of the proteins of the outer membranes of *E. coli* for the determination of *E. coli* in water. A portable evanescent wave fiber biosensor has been developed for Shigella detection using DNA as a biorecognition element.[62] The DNA probe is capable of hybridization with a fluorescently labeled complementary DNA strand that is covalently immobilized onto the fiberoptic biosensors. In a recent report, a fluorescent immunosensor has been developed using an optical waveguide for fast and sensitive detection of microcystin-LR in lakes.[63] Microcystin-LR (MC-LR) is one of the most toxic cyclic heptapeptide cyanotoxins released by cyanobacterial blooms in surface waters. The 3-mercaptopropyl trimethoxysilane/N-(4-maleimidobutyryloxy) succinimide (MTS/GMBS) was used to activate the surface of waveguide chip to facilitate the immobilization of bovine serum albumin (BSA)-MC-LR conjugate.

2.2.3. Long period grating (LPG) based biosensors

Among the (bio)sensors using optical fibers, long-period gratings (LPG) are a particularly interesting platform.[64] In an LPG, the refractive index of the fiber core is periodically modulated with a period (Λ) in the range of hundreds of micrometers.[65] As a result of these modulations, coupling of

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the core mode into a series of cladding modes occurs, which results in the appearance of resonance peaks.[66] The evanescent field of each cladding mode interacts with the environment surrounding the fiber. Hence, LPGs assists mode coupling at resonance wavelengths that are sensitive to variations in refractive index of the surrounding medium, i.e., sample. The advantages of LPG sensors include simple fabrication and easiness in adjusting the resonance wavelength within the spectrum emitted by the light source via the grating period. While LPG-based biosensors have demonstrated their generic utility, they appear less exploited to date for the detection of water-borne pathogens.

Tripathi et al. reported the label-free, bacteriophage-based detection of *E. coli* using particularly sensitive long-period fiber gratings (LPFGs).[67] For this study, the bacteriophage T4 was covalently immobilized at the fiber surface. Binding of *E.coli* was subsequently monitored. An LPG-based biosensor has been fabricated for the detection of *Escherichia coli* (*E.coli*) outer membrane proteins (EcOMPs) via the generated evanescent field. For that purpose, DNA-aptamer-based optical biosensor were applied where long period gratings were serving as a refractometric platform.[68] Via the functionalization of LPGs within a single-mode fiber, sensing areas were obtained using two types of immobilization: (i) electrostatic assembly, and (ii) covalent binding. Monitoring changes of the resonance wavelength enabled the specific recognition of EcOMPs in water. The sensor revealed linear response in the range of 0.1 nM to 10 nM.

2.2.4. Infrared and Raman spectroscopy based biosensor

Infrared (IR) and Raman spectroscopies provide highly discriminatory vibrational fingerprints on a diversity of samples based on their chemical composition, enabling long-term monitoring, high reproducibility, accurate determination and characterization of pathogens.[69]

Both vibrational spectroscopic techniques are effective and non-destructive, and enable analyzing the phenotype of pathogen samples including dynamic changes in their metabolism and structure occurring due to environmental stress.[70] For example, Al-Qadiri et al. used Fourier transform infrared (FT-IR) absorbance spectroscopy to study the effect of chlorine-induced bacterial injury in a mixed bacterial culture of *E.coli* and Pseudomonas aeruginosa.[71] Changes were found in the spectral features of injured bacterial cells, which revealed apparent denaturation reflected between 1800 and 1300 cm^{-1} concerning ester functional groups of lipids, structural proteins, and nucleic acids. Their study suggests that infrared spectroscopy may be applicable for assessing the presence of injured and viable, but not culturable water-borne pathogens, which were underestimated or not discernible using traditional microbiological procedures. Lee-Montiel et al. developed a strategy for the detection and quantification of water-borne poliovirus using a combination of FT-IR spectroscopy and cell culture.[72] Buffalo green monkey kidney (BGMK) cells infected with different virus titers were investigated at 1–12 hours post-infection utilizing FTIR spectroscopy to monitor changes in the absorbance patterns of cell components (e.g. lipids, proteins, nucleic acids, and sugars) subsequent to poliovirus infection. An overview of this method is shown in Figure 10.

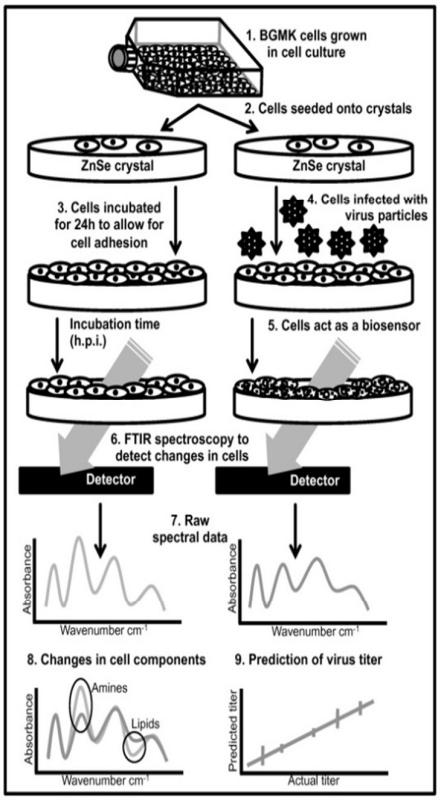

Fig 10 Schematic representation of a viral detection method using cell culture and FTIR spectroscopy (not to scale). Adapted from Lee-Montiel et al. [72] with permission from BioMed Central.

Raman scattering provides complementary information to infrared spectroscopy offering a wide range of potential analytical wavelengths, as well as laser excitation sources (i.e. UV, near infrared or visible). However, Raman signals are inherently much weaker, which renders surface enhanced Raman spectroscopy (SERS) relevant for ultrasensitive pathogen sensing. Therefore, considerable progress has been made in developing SERS biosensor platforms for pathogens.73 Currently, there are two types of SERS approaches commonly used to detect pathogens. The first one is a label-based strategy using an immunosorbent assay format similar to ELISA, and a SERS tag as a quantitative reporter to produce sensitive and distinct signals. Biorecognition molecules such as antibodies and aptamers are necessary for specific binding of pathogens. Recently, Wang et al. proposed a surface-enhanced Raman scattering (SERS) immunosensor for staphylococcal enterotoxin B (SEB) detection on a microplate by utilizing the 4-nitrothiophenol (4-NTP)-modified Au@Ag core–shell nanoparticles (NP) as a signal reporter/SERS tag coupled to an SEB monoclonal antibody (mAb).[74] The

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encoded 4-NTP in the Au@AgNP leads to substantial electromagnetic enhancement between AuNPs and the Ag-shell, and differentiates SERS signals between the sample and the microplate. A limit of detection (LOD) of 1.3 pg/mL SEB was obtained with this method. The schematic of the working principle

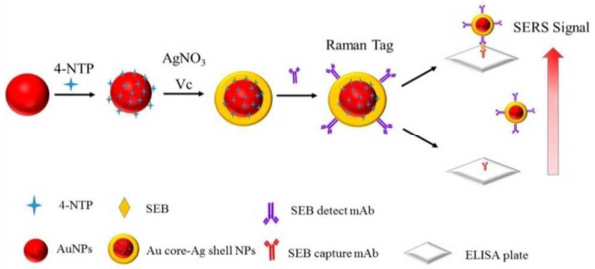

Fig 11 Scheme of an Au@Ag core–shell structure-based SERS immunosensor for SEB. Adapted from Wang et al. [74] with permission from American Chemical

of this SERS-based immunoassay is given in Figure 11.

The second type of SERS biosensor, which directly detects the intrinsic vibrational fingerprint of pathogens in the vicinity of nanostructured noble metal surfaces, operates label-free and is able to simultaneously analyze various pathogens. Wang et al. synthesized the positively charged PEI-modified Au-coated magnetic microspheres (Fe_3O_4@Au@PEI) to capture, enrich, and separate negatively charged bacteria, which were then magnetically immobilized onto a Si substrate for establishing SERS substrates with a high density of hot spots.[75] Au@Ag NPs in ethanol were further added and dried at the substrate to cover the blank surface. The bacteria SERS signal was then synergistically enhanced by the combination of Fe_3O_4@Au@PEI and Au@Ag NPs. Hence, a label-free SERS pathogens detection termed capture–enrichment–enhancement (CEE) three-step method was established, and was verified by the spiked tests in tap water and milk and its detection of Gram-positive bacterium *E. coli* and Gram-positive bacterium *S. aureus* at a detection limit as low as 10^3 cells per mL. Figure 12 illustrates the general scheme of this concept.

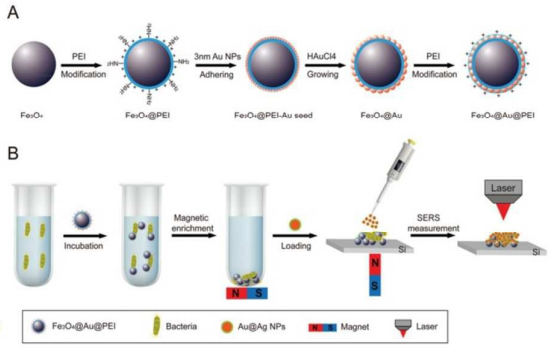

Fig. 12 Schematic of (A) the synthesis of high-performance Fe_3O_4@Au@PEI microspheres, and (B) the CEE three-step procedure for rapid SERS detection of bacterial pathogens. Adapted from Wang *et. al.*[9] with permission from Royal Society of Chemistry.

2.2.5. Fluorescence and chemiluminescence biosensors

Optical biosensors for pathogens involving detection via luminescence (i.e., fluorescence and chemiluminescence) represent a powerful analytical technique offering high sensitivity, flexibility, easy readout, and ease of operation. Furthermore, quantitative determination is enabled across a sizeable dynamic range. The vast majority of luminescent assays rely on appropriate labels, and the labelled components are predominantly antibodies, aptamers, proteins, amino acids, and peptides, which are then used as specific probes for the detection of a particular target. In recent decades, fluorophores (e.g., organic dyes), semiconductor quantum dots (QDs), and other fluorescent nanoparticles have been considered as effective labels in fluorescence-based biosensors. Most commonly, these reactive fluorescent labels selectively bind to a specific region or functional group on the target analyte. A sandwich assay was developed by Dogan et al. combining immuno-magnetic separation (IMS) based on iron oxide core gold shell (Fe_3O_4@Au) magnetic nanoparticles and chitosan modified CdTe QDs as a fluorescence label for the quantitative detection of *E.coli.*[76] Figure 13 gives a scheme of the proposed method.

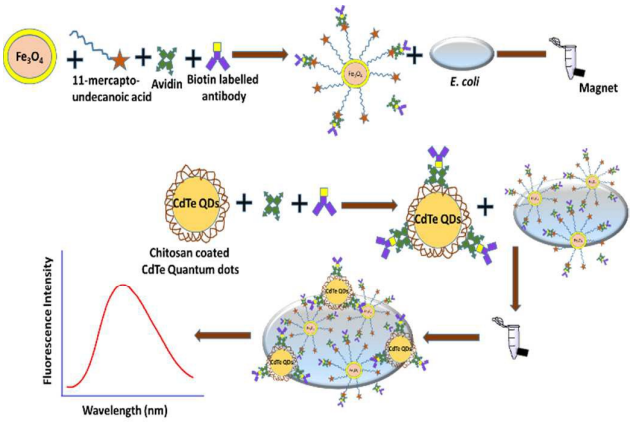

Fig 13 Schematic illustration of the overall strategy using a fluorescence-based sandwich immunoassay for the enumeration of *E.coli.* Adapted from Dogan et al [76] with permission from Elsevier.

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Apart from conventional fluorescence assays, Förster resonance energy transfer (FRET) based techniques have been widely applied in ultrasensitive detection schemes, which are usually avoiding sample separation or washing steps. FRET describes the energy transfer process between fluorescence particles serving as energy donor, and quencher particles serving as acceptors whereby the fluorescence of the donor may be quenched by the acceptor, if they are within a certain distance (i.e., Förster distance) of each other (usually < 10 nm). The changes of fluorescence intensity during the FRET process may be used to monitor concentrations of target analytes. Jin et al. designed a FRET-based platform for bacteria detection, in which gold nanoparticles (AuNPs, acceptor) were functionalized with bacteria targeting aptamers, while upconversion nanoparticles (UCNPs, donor) were modified with corresponding complementary DNA (cDNA).[77] *Via* complementary pairing of the aptamers and cDNA, FRET occurred owing to the spectral overlap between UCNPs fluorescence emission and AuNPs absorption, thereby resulting in quenching of the UCNPs fluorescence. If the target bacteria are present, the aptamers would preferentially bind to the bacteria to form a three-dimensional structure, and induce aptamers dissociation from cDNA leading to the liberation of UCNPs, and to subsequent recovery of upconverted fluorescence. This aptasensor was tested by detecting *E. coli* in real-world food and water samples (e.g., tap/pond water, milk). Schematic illustration of the developed FRET biosensor is shown in Figure 14.

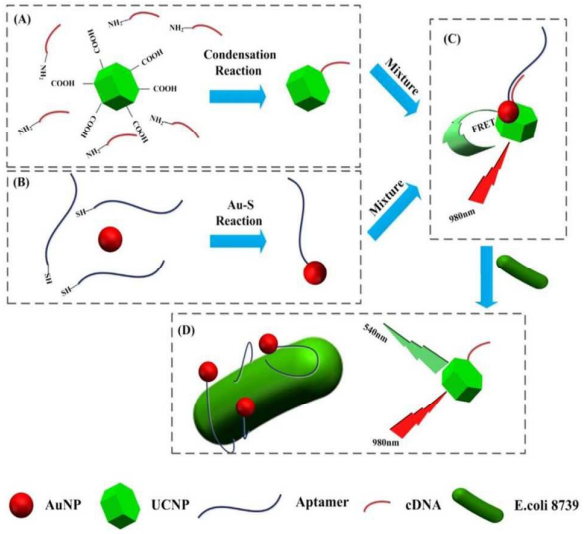

Fig 14 Schematic illustration of upconversion nanoparticles based FRET aptasensor for rapid and ultrasenstive bacteria detection. (A) The amino-modified complementary DNA of the aptamer is attached to the carboxyl-functionalized UCNPs by condensation reaction. (B) Conjugating thiol-modified aptamers to the AuNPs via Au-S chemistry. (C) The FRET system is established between a donor-acceptor pair: UCNPs-cDNA hybridized with AuNPs-aptamers. (D) By introducing target bacteria into the FRET system, aptamers preferentially bind to target bacteria resulting in the dissociation of cDNA, thereby aptamers-DNA pairs are destroyed and the green fluorescence recovers.

Instead of labeling fluorophores to the target analyte, Zhang et al. reported a novel fluorescence system for sensing viable *S.enteritidis*, on the basis of specific aptamer recognition and the target-induced assembly of G-quadruplex DNA.[78] In the present of target *S.enteritidis*, the specific aptamer sequence in the designed capture probe complex would bind, meanwhile the hybridized signal target sequence would be released, and finally generate G-quadruplex structures after a series of reactions. The fluorescent dye N-Methyl mesoporphyrin IX (NMM) could bind to the produced G-quadruplex structures, and then emit intensive fluorescent signals to achieve *S.enteritidis* detection. This biosensor was capable to discriminate viable *S.enteritidis* from dead species. The principle of this sensor is illustrated in Figure 15.

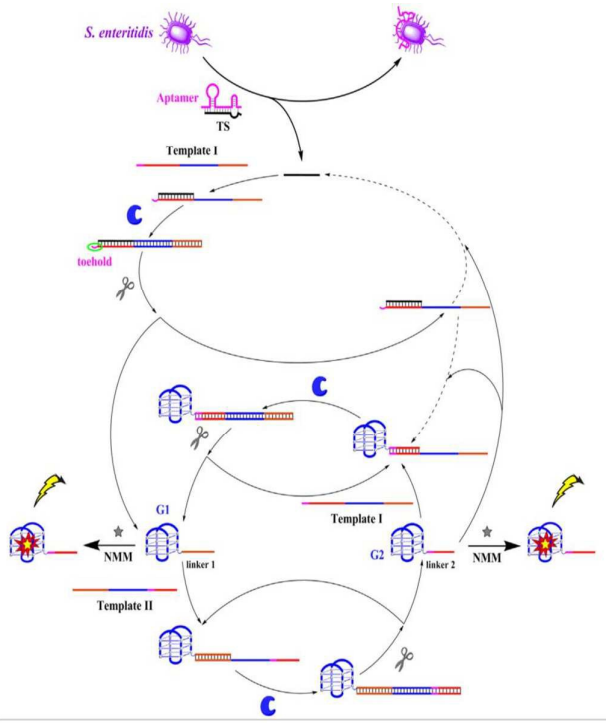

Fig 15 Schematic illustration of a label-free, modification-free, and DNA extraction-free fluorescent detection strategy for S. enteritidis based on the cascaded two-stage toehold strand-displacement-driven assembly of G-quadruplex structures. The dashed arrows represent the two different products including the target sequences (TS) in the same reaction. Adapted from Zhang et al [78] with permission from Elsevier.

In contrast to fluorescence, chemiluminescence (CL) biosensors promise an ultralow background, superior sensitivity, extended calibration range, and simple instrumentation since no external light source or sophisticated optics are involved. Chemiluminescence sandwich ELISA has been extensively exploited in clinical analysis. In a typical CL biosensor for pathogens - as presented for example by Wolter et al. - species-specific antibodies were immobilized onto a substrate.[79] Target pathogens were then captured at the prepared substrate surface by the antibodies, and subsequently bonded to specific secondary antibodies labeled with biotin. Chemiluminescence detection could be accomplished after addition of a horseradish peroxidase (HRP)-labeled streptavidin and CL substrates due to CL emitted

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during the HRP-catalyzed reaction of luminol and hydrogen peroxide. CL was recorded via a sensitive charge-coupled device (CCD) camera. Figure 16 displays the principle of a typical CL assay.

2.2.6. Colorimetric biosensors

Colorimetry is an ideal method for developing low-cost biosensors applicable in sensing scenarios where mostly expected pathogens are addressed, and read-out may be attained by simply monitoring visual color changes with the naked eye. Such methods do not require expert users and promise a straightforward, cost-efficient, and convenient strategy. For example, the pronounced localized surface plasmon resonance (LSPRs) of gold colloids occurs within the visible spectrum, and color changes depending on the inter-particle distance of nanoparticles have been exploited for designing colorimetric sensors.[80, 81] Similarly, Thakur et al. developed a polyaniline nanoparticle (PANI NP) based colorimetric sensor for monitoring bacterial growth relying on estimates of its metabolic product.[82] Herein, the conducting polymer, i.e., polyaniline is highly sensitive to the presence of protons in its microenvironment, and a visible color change could be observed, if proton doping occurs, which could be triggered by the interaction of polyaniline with metabolic products released by the pathogens. Figure 17 gives an overview on the fundamental mechanism of this colorimetric biosensor.

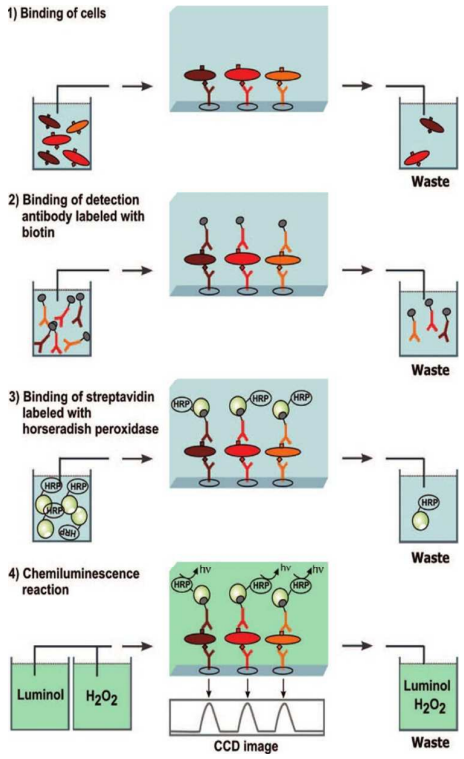

Fig 16 Scheme of the assay principle illustrated for the detection of three different bacteria at the antibody microarray surface. Adapted from Wolter et al [79] with permission from Elsevier.

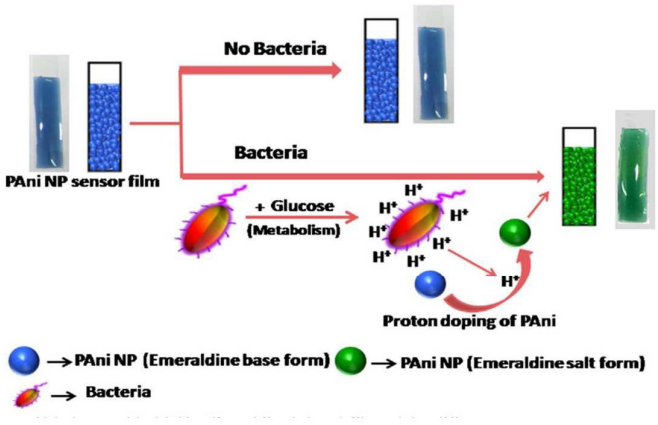

Fig 17 Schematic representation of the mechanisms for monitoring bacterial growth using PAni-Pec NPs as colorimetric sensor. PAni-Pec NPs = Polyaniline–pectin nanoparticles. Adapted from Thakur et al [82] with permission from Elsevier.

Frequently, colorimetric bioassays are based on ELISA schemes. Conventional ELISA uses an enzyme as label that catalyzes a chromogenic substrate, while in plasmonic ELISA tests an immunocomplex is conjugated to a substrate that affects the growth/agglomeration/accumulation of gold nanoparticles. Bui et al. introduced a sensitive enzyme-free immunoassay based on plasmonic colorimetry via gold nanoparticles in conjunction with signal amplification via cysteine-loaded liposomes.[83] In the presence of a pathogen, cysteine acted as a cross-linker, and aided the rapid aggregation of AuNPs. Thus assembled nanoparticles showed revealed light absorbance at higher wavelengths. Thus, a typical

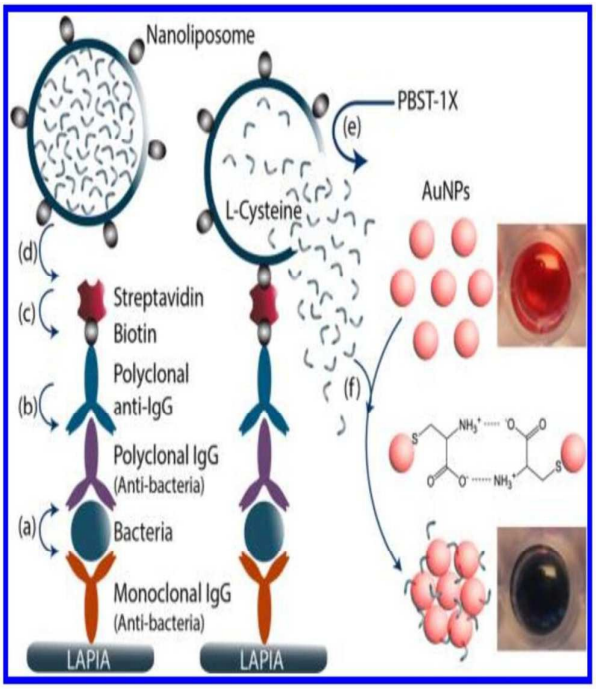

Fig 18 Schematic of a liposome-amplified plasmonic immunoassay (LAPIA). One bacterium, molecule, or antigen may rapidly trigger a chemical cascade leading to a chromogenic aggregation of AuNPs. The reaction proceeds in different steps: (a) Capture of the target (biomarker, pathogen, toxin) using sandwich immunoassay. (b) After washing steps, biotinylated secondary antibody (polyclonal anti-IgG) is interacted with the immunocomplex. (c) After incubation and washing, streptavidin is added to interact and bind to biotinylated IgG. (d) After washing steps, biotin-conjugated liposomes containing cysteine are added to the medium followed by AuNPs solution. (e) Addition of PBS-Tween-20 1X to the medium causes the breakdown of the liposomes and the release of cysteine, leading to immediate aggregation of gold nanoparticles and a color shift from red to dark blue (f). Adapted from Bui et al [83] with permission from American Chemical Society.

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color shift of the solution from red to dark blue could be observed when aggregation was generated, and the detection of pathogens was accomplished even at 6.7 attomolar (600 molecules in 150 µL) concentration levels. The obtained results have been verified by visually detecting a single-digit live pathogen of *Salmonella*, *Listeria*, and *E. coli* O157 in food samples. The entire analytical process is shown in Figure 18.

Furthermore, colorimetric dyes (e.g., hydroxy naphthol blue (HNB), calcein, etc.) has also been used to indicate positive signals generated by genetic-based detection of pathogens. A portable point of care (POC) device was thus assembled by Safavieh et al. for the identification of bacterial pathogens using isothermal DNA amplification and colorimetric read-out.[84] *E.coli* (as Gram-negative bacteria) was detected as well as *S.aureus* (as Gram-positive bacteria) via the unambiguous color change of HNB and calcein dyes. The present POC device was fabricated using a flexible ribbon polyethylene substrate ensuring low manufacturing costs and a simple method. The detection limit of the resulting POC device for *E. coli* was 30 CFU mL^{-1}, and 200 CFU mL^{-1} for *S. aureus*.

2.3. Piezoelectric biosensors

Piezoelectric sensors are responsive to binding of an analyte by the induced increase in mass, which in turn changes the oscillation frequency of a piezoelectric material/crystal along with an associated electric read-out.[85] The transducer in piezoelectric biosensors is made from piezoelectric materials (e.g., quartz), and the biosensing interface is again coated or immobilized onto the piezoelectric surface that oscillates at its natural frequency.[19] If a target analyte binds to the sensing surface, a frequency shift will be caused, which will produce changes in piezoelectric current directly proportional to the induced mass change.

Regarding the application of piezoelectric biosensors for detecting water-borne pathogens, only few reports are published to date. Wang et al. developed a quartz crystal microbalance (QCM), which is based on the piezoelectric effect for the detection of *E.coli* 0157:H7 DNA based on nanogold particles amplification.[86] Gold nanoparticles were immobilized onto the thiolated surface of the Au electrode. Then, thiolated single-stranded DNA (ssDNA) was fixed via Au-SH bonding. This ssDNA probe was exposed to the complementary target DNA of *E.coli* 0157:H7 gene eaeA. This caused a mass change, and a corresponding frequency shift of the QCM resonance. The outer avidin-coated Au nanoparticles could combine with the target DNA to increase the mass. The detection limit of this biosensor was found to be 2.0×10^3 CFU/mL. The fabrication scheme of such QCM-based biosensors is shown in Figure 19.

Babacan et al. have reported a piezoelectric based biosensor for the detection of *Salmonella typhimuium.*[87] In this report, for the activation of the piezoelectric biosensor protein A antibody was immobilized to detect *S.typhimurium*. The detection system consisted of an advanced design for the flow cell combined with a flow injection analysis system. The sensor had a response in the range of 5 to 65 Hz within 30 min with R^2 = 0.95 for *S.typhimurium* concentrations of 10^7 to 10^9 CFU/mL at continuous flow conditions, and 3 to 75 Hz within 40 min with R^2 = 0.96 for *S.typhimurium* concentrations of 10^6 to 10^{10} CFU/mL at stop-flow conditions.

The detection of Escherichia coli O157:H7 was reported using a piezoelectric biosensor-quartz crystal microbalance (QCM).[88] The antibody-functionalized gold nanoparticles (AuNPs) were used as detection verifiers and amplifiers. The

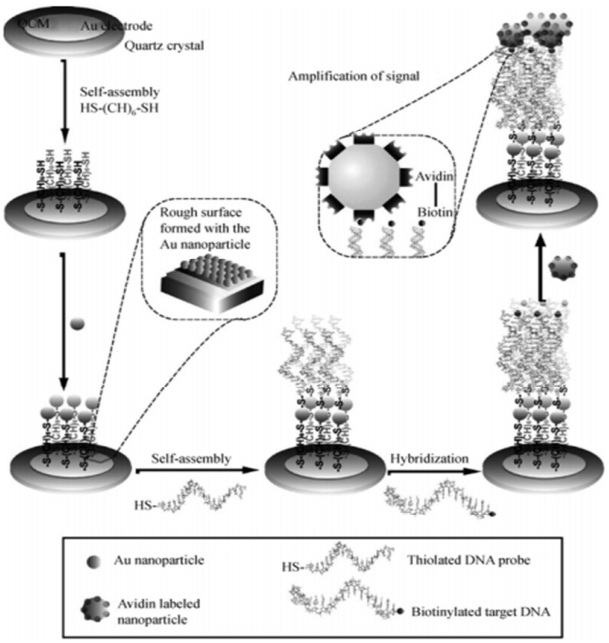

Fig 19 Illustration of QCM DNA biosensor fabrication and detection procedure. Adapted from Wang et al. [86] with permission from Springer.

capture antibodies for E. coli O157:H7 were first immobilized onto the QCM chip. The sample containing E. coli O157:H7 was circulated through the system in the presence of 10 ml of brain heart infusion (BHI) broth for 18 h. The cells of E. coli O157:H7 specifically captured and enriched on the chip surface of the QCM were identified by QCM frequency changes. Authors claim that the real-time monitoring method for viable E. coli O157:H7 developed in this study can be used to enrich and detect viable cells simultaneously within 24h.

3 Current challenges and future prospects

The main requirements for reliable pathogen analysis include specificity, sensitivity, reproducibility, and reliability of the obtained results next to detection speed, automation, and ultimately, low cost. To date, even though a plethora of reports are available on biosensors for pathogen detection in aqueous environment, but still they face challenges during application in real-world environmental samples.

A major challenge is posed by the numerous and potentially interfering microbial species present along with

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particulate matter, organic/inorganic constituents and contaminants, and molecular agglomerates and associates, e.g., from humic substances. Consequently, nonspecific interactions and adsorption with and at the biosensing interface limits the life time, specificity, and reliability of biosensors applied in complex environmental samples. In addition, frequently biosensors used in pathogen detection rely on the interaction of a natural receptor, i.e., usually antibodies serving as the biorecognition element with their corresponding counterparts. Natural receptors themselves usually operate at comparatively stringent environmental conditions (i.e., pH, ionic strength, etc.), and if immobilized at a transducer surface may be prone to accelerated degeneration resulting in a loss of selectivity, binding activity, and/or binding capacity. Hence, not only will the sensor performance in general degenerate with extended application time, but the robustness of the calibration will be detrimentally affected.

These challenges may be address by taking advantage of more robust molecular detection schemes such as addressing the DNA signature of pathogens given that DNA is an inherently robust molecule. Alternatively, natural receptors such as enzymes and antibodies may increasingly be replaced by advanced biomimetic recognition elements such as peptides, aptamers, and molecularly imprinted/templated polymers providing similar recognition abilities, yet, increasingly robust sensing interfaces.

Evaluating the transducers, it is evident that in particular electrochemical sensors/electrodes have evolved into the biosensor 'workhorse' given their maturity, robustness, ease of preparation, and low cost. Electrochemical sensors are generally characterized by high sensitivity, low cost, and ease of use. However, most electrochemical detection schemes are of limited inherent molecular selectivity, and thus rely on the (bio)molecular recognition scheme. In addition, electroactive interferants may convolute the obtained signal by, e.g., unwanted redox reactions.

In contrast, optical biosensors – probably the main alternative transducer category – are still frequently challenged by the required limits of detection in pathogen analysis. Given the progress in photonics technologies including waveguides, light sources, detectors, and integrated optics it appears that optical sensing platforms are increasingly competitive with electrochemical transduction schemes. This generically holds true independent of the utilized spectral window (i.e., in particular visible, near-infrared, and mid-infrared wavelength regimes) and interaction principles (i.e., from absorption to fluorescence, SPR, Raman scattering, etc.). Noteworthy, some optical sensing schemes (e.g., in the mid-infrared) have to appropriately deal with interferences by the aqueous matrix itself potentially mitigated via appropriate surface functionalization strategies.

With the increasing emergence of optical sensing schemes taking advantage of evanescent fields/waves emanating at the interface of high-refractive-index waveguides and lower index samples (e.g., aqueous media), and ideal optical sensing platform is provided with tailorable surface chemistries for immobilizing chemo or bioreceptors.

Last but not least, optical amplification schemes including surface enhanced Raman, infrared, and fluorescence enable lowering the achievable limits of detection into the required regime. Notwithstanding, more reliable and reproducible surfaces and interfaces – usually involving nanostructured noble metals - remain an issue of future research for improving the quantification capabilities of surface enhanced optical techniques during pathogen detection in complex environments.

For the detection of water borne pathogens, culture-dependent methods are still extensively used despite limitations of low sensitivity and excessive time required to obtain genuine results. In order to mitigate such problems in pathogen detection, advanced sample filtration and pre-concentration techniques including usage of charged membrane filters to process large volumes of water, have been implemented. Here, microfluidic devices play an increasingly important role for pathogen detection because of their large surface to volume ratio, multiplexing possibility and minute sample requirement. Easy, cost-effective, ultrasensitive and selective detection of water-borne pathogen is being done nowadays using nano-dielectrophoretic microfluidic device integrated with, e.g., SERS.[89, 90] Microfluidic devices have the potential to shorten the assay time in comnbination with almost any transduction scheme.[57,91] Liposomes are also being used in sensing applications as liposomes tagged probes is capable of detecting the analyte selectively without enzymatic amplification.[92] Up-converting phosphor technology (UPT) is another method which provide specific signals in assay without amplification of enzymes.[93] Moreover, lack of auto-fluorescence in UPT produces the signals with very low noise. Beside, 'green nanotechnology' is among the most promising emerging research areas contributing to advanced pathogen sensing.[94, 95] This technology is capable of minimizing human health risks caused by water-borne pathogens. The use of biologically synthesized nanomaterials offer selective, sensitive and cost-effective detection of pathogens without usage of toxic chemicals which in turn deteriorate the environment.[96] In addition to this, molecular imprinted polymer (MIP) also have the potential for specific detection of pathogens. Actually, these are artificial receptor ligands which can bind specifically to the target analyte. The target specific MIP nanoparticles are more resistant to biological and chemical damage in comparison to antibodies which make them a potential candidate for detection of water-borne pathogens.[97]

Despite the remaining challenges, a wide variety of transduction technologies and molecular recognition schemes increasingly combined with integrated sampling/microfluidic concepts are evidently advancing biosensor platforms and their capabilities for detecting and monitoring water-borne pathogens in real-world aqueous environments, and will lead to their increasing usage.

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