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# Environmental protection based on the nanobiosensing of bacterial lipopolysaccharides (LPSs): material and method overview

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Lipopolysaccharide (LPS) or endotoxin control is critical for environmental and healthcare issues. LPSs are responsible for several infections, including septic and shock sepsis, and are found in water samples. Accurate and specific diagnosis of endotoxin is one of the most challenging issues in medical bacteriology. Enzyme-linked immunosorbent assay (ELISA), plating and culture-based methods, and Limulus amoebocyte lysate (LAL) assay are the conventional techniques in quantifying LPS in research and medical laboratories. However, these methods have been restricted due to their disadvantages, such as low sensitivity and time-consuming and complicated procedures. Therefore, the development of new and advanced methods is demanding, particularly in the biological and medical fields. Biosensor technology is an innovative method that developed extensively in the past decade. Biosensors are classified based on the type of transducer and bioreceptor. So in this review, various types of biosensors, such as optical (fluorescence, SERS, FRET, and SPR), electrochemical, photoelectrochemical, and electrochemiluminescence, on the biosensing of LPs were investigated. Also, the critical role of advanced nanomaterials on the performance of the above-mentioned biosensors is discussed. In addition, the application of different labels on the efficient usage of biosensors for LPS is surveyed comprehensively. Also, various bio-elements (aptamer, DNA, miRNA, peptide, enzyme, antibody, etc.) on the structure of the LPS biosensor are investigated. Finally, bio-analytical parameters that affect the performance of LPS biosensors are surveyed.

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## 1. Introduction

Gram-negative bacteria are described by an envelope that contains two membranes: an outer membrane (OM) that separates the cell from its environment and an inner membrane (IM) that surrounds cytoplasmic components.<sup>1,2</sup> Gram-negative bacteria envelope surrounds periplasm that is an aqueous cellular compartment, which encloses the peptidoglycan cell wall. Therefore, OM encompasses a crucial role in the protection of Gram-negative bacteria against environmental treatments. Outstandingly, in distinction to many biological membranes, the OM of most Gram-negative microorganisms is not a lipid bilayer.<sup>1</sup> Instead, it is an especially unbalanced bilayer that contains phospholipids within the inner leaflet and lipopolysaccharide (LPS) molecules in the outer leaflet.<sup>3</sup> Consistent with previous studies, LPS is the endotoxin portion

of the Gram-negative bacterial cell wall. LPS is found in high levels in clinical necrotizing enterocolitis (NEC) that acts as a pro-inflammatory stimulus in the gastrointestinal (GI) tract. LPS performs numerous functions in Gram-negative bacteria. The essential role of LPS is to help as a main structural part of the OM. Also, LPS is a crucial section of the cell wall in some Gram-negative bacteria.<sup>4</sup> The results show that the LPS molecules transform OM into a permeable membrane that is effective against small molecules and bacteria that can overcome the phospholipid bilayer, rendering Gram-negative bacteria resistant to natural antibacterial agents of some compounds.<sup>5,6</sup> LPS may also play an essential role in bacterial-host interactions by regulating the host immune system.<sup>7</sup> LPS is the cause of a wide range of infections in humans, including sepsis, severe fever, and shock sepsis, and is found in different types of water, including groundwater, freshwater, and saline.<sup>8</sup>

## 2. Biological effect of LPS

LPS is a molecule that forms a part of the bacterial cell wall of Gram-negative bacteria and helps to stabilize the bacterial cell wall. The LPS is made up of three parts.<sup>9</sup> (A) The O-side (O antigen) is the main site of immunospecificity, (B) a central polysaccharide component close to the lipid A matrix, which is

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exposed to the extracellular milieu; and (C) lipid A is the real fever component of LPS. Lipid A is surrounded by the cell barrier of Gram-negative bacterium and is the most conserved part of the LPS structure.<sup>10–12</sup> The other two components are outside, away from the bacterial cell wall. Lipid A aids as an anchor for the LPS component. LPS acts as an inner and outer portion of the external cell wall and internal membrane of Gram-negative bacterial cells.<sup>13</sup> A representative design of the Gram-negative bacteria cell wall structure is presented in Fig. 1.

LPS provides a protecting and stabilizing mechanical maintenance to the cell and aids in protecting the integrity of the cell wall, and therefore, survival of the cell mainly in high level stress, such as dehydration. The pathogenicity of the LPS is almost entirely due to the region III component, lipid A (endotoxin), which in several texts is mentioned as catechin.<sup>14,15</sup> The main pathogenicity of lipid A may primarily be obvious itself by the increase of alteration of metabolism in small doses and fever.<sup>16,17</sup> Therefore, LPS is one of the target antigens for diagnosis and potential candidates for vaccine and drug development. Even though the name “endotoxin” is infrequently used to refer to any cell-associated bacterial toxin, in bacteriology, it is accurately reserved to refer to the LPS complex related to the outer membrane of Gram-negative microorganisms such as *Pseudomonas*, *Bordetella pertussis*, *Escherichia coli*, *Salmonella*, *Haemophilus influenza*, *Shigella*, *Neisseria*, and *Vibrio cholera*. Some important endotoxin (LPS) producing bacteria and related diseases are summarized in Table 1.

Based on Table 1, there are a variety of diseases associated with bacterial endotoxin. Therefore, rapid and specific diagnosis of endotoxin plays a critical role in controlling and treating the disease. In the following, some of the most important and widely used methods of endotoxin diagnosis are discussed. These severe effects instantly demand approaches for sensitive quantification such as endotoxin from pharmaceutical and biological products. Limulus amoebocyte lysate assay (LAL), rabbit pyrogen test, enzyme-linked immunosorbent assay (ELISA), and fluorescence-based methods are the most important and widely used techniques for the determination of LPS.<sup>31,32</sup> To strengthen and increase the sensitivity and specificity of routine methods in recent years, various methods have been developed, of which nanotechnology-based methods are one of the most important. The present article aims to investigate the diagnostic methods of lipopolysaccharide, emphasizing biosensors developed in this

field. This study also addresses the challenges of routine testing and the importance of developing biosensors. As mentioned in the introduction, endotoxin is one of the most important pathogens associated with Gram-negative bacteria, so that its accurate, specific, and rapid identification can play a crucial role in controlling and treating the disease. In addition, due to the exceptional properties of endotoxin, this biomolecule has been considered by researchers in pharmacy, so the expansion of advanced diagnostic methods will be desirable. In the review, routine diagnostic methods on endotoxin monitoring are introduced, and their strengths/weaknesses are discussed. In addition to the comprehensive introduction of biosensors as a specific and sensitive method for endotoxin detection, the most up-to-date developed biosensors in this field have been reviewed. For the complete nobility of the readers of this article, a table containing general information of the introduced LPS biosensors such as analytical features of used techniques and platforms have been considered appropriately. So in this review, various types of biosensors such as optical (fluorescence, SERS, FRET, and SPR), electrochemical, photoelectrochemical, and electrochemiluminescence were investigated. Also, the critical role of advanced nanomaterials on the performance of the above-mentioned biosensors is discussed. In addition, the application of different labels on the efficient usage of biosensors for LPS is surveyed comprehensively. Also, various bioelements (aptamer, DNA, miRNA, peptide, enzyme, antibody, etc.) on the structure of the LPS biosensor were investigated. Finally, bioanalytical parameters, which affect the performance of LPS biosensors are surveyed.

### 3. Conventional methods in the detection of endotoxin (LPS)

Culturing and plating techniques are the out-of-date and ancient identified conventional incidental methods that aided as the gold standard method for bacterial endotoxin detection.<sup>33–35</sup> The critical limitation is that each microorganism needs its own supplements and also its culture media for optimum growth.<sup>36,37</sup> Similarly, new fresh media should be considered for each microbe, and each microbe will develop positive or negative colonies over time.<sup>38,39</sup> The limulus amoeba cell lysate (LAL) enzyme assay is usually the preferred gold standard for endotoxin detection.<sup>40,41</sup> LAL is obtained from

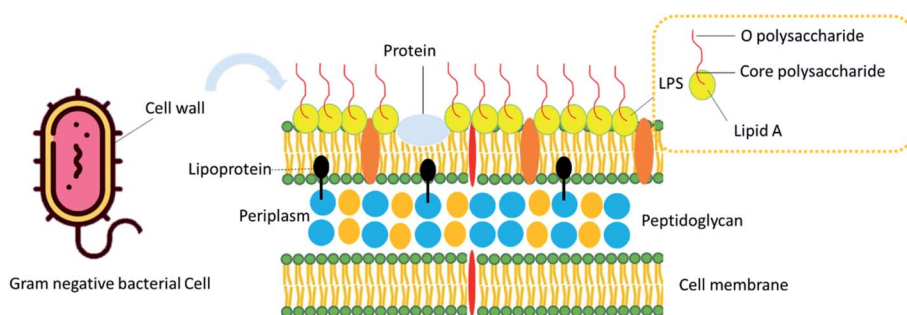


Fig. 1 Schematic illustration of the Gram-negative bacteria cell wall structure.



**Table 1** Endotoxin (LPS) producing bacteria and related disease

| Bacterium  | Role and diseases  | Ref.      |
|--|--|-----------|
| <i>Pseudomonas aeruginosa</i>                        | Persistent infections, mainly in the genetic ailment cystic fibrosis, facilitating biofilm formation, antibiotic resistance                                      | 18        |
| <i>Escherichia coli</i> and <i>Bacteroides dorei</i> | Neurodegeneration diseases, hemolytic uremic syndrome (HUS), diarrhea that may range from watery to bloody, stomach pains and cramps, loss of appetite or nausea | 19 and 20 |
| <i>Haemophilus influenzae</i>                        | Vomiting, cholecystitis, cholangitis, neonatal meningitis  | 21–23     |
| <i>Salmonella</i>                                    | Meningitis, septicemia and pneumonia, Texas Children's Hospital (TCH) disease, meningitis and sepsis, necrotizing myositis and septic shock                      | 24 and 25 |
| <i>Neisseria gonorrhoeae</i>                         | Bacteremia and meningitis, enterocolitis, tachycardia, fever, hepatomegaly, splenomegaly, diarrhea, vomiting   | 25 and 26 |
| <i>Vibrio cholera</i>                                | Ophthalmia neonatorum endocervicitis urethritis, systemic neonatal infection   | 27        |
| <i>Staphylococcus aureus</i>                         | Wound infection, watery diarrhea leading to serious dehydration. Gastroenteritis, septicemia   | 28 and 29 |
| <i>Bordetella pertussis</i>                          | Toxic shock syndrome, septic arthritis, pneumonia, endocarditis, pneumonia, boils, folliculitis, impetigo, cellulitis  | 30        |
|  | Respiratory tract infection, pertussis, diphtheria   |           |

amoebic cells (similar to human leukocytes) of the horseshoe crab family (horseshoe crab family), including the horseshoe crab family (East Asia) and the horseshoe crab family (Atlantic Ocean).<sup>37</sup> The LAL test is used both in the industry and in the laboratory to measure bacterial endotoxin in various samples.<sup>42</sup> The principle of this technique is based on the coagulation process that occurs in the blood lymph of the horseshoe crab (*Limulus polyphemus*) in the existence of LPS.<sup>43,44</sup> Various types of LAL based on fluorescent and colorimetric methods have been established for the sensitive recognition of LPS. Even though these tests have presented hopeful detection limits and sensitivity, many of them need tedious and complex processes.<sup>45,46</sup> LAL assays' sensitivity depends on the processing method, the sample type, and the dilution factor.<sup>47</sup> The enzyme-

linked immunosorbent assay (ELISA) is an immunological assay frequently used to measure proteins, antibodies, antigens, and glycoproteins in biological samples. Some examples include pregnancy tests, diagnosis of HIV infection, and measurement of cytokines or soluble receptors in serum or cell supernatant. ELISA is another widely used test in bacteriology and endotoxin diagnosis. ELISA method is sensitive, robust, and reliable. Since LPS is a very heterogeneous class of substances, it is still not possible to develop antibodies with sufficient affinity and broad specificity, so this method is not in the market.<sup>48</sup>

Since large-scale commercial production and detection of endotoxin has become an absolute requirement, controlling endotoxin levels is important, primarily because it can cause some severe physiological responses.<sup>49</sup> Therefore, it is very

**Table 2** Analytical parameters of conventional methods in detection of LPS

| Method       | Sample           | Technique                            | LOD/sensitivity  | Ref. |
|--------------|------------------|--------------------------------------|--|------|
| Cultivation  | —                | Culture & plating                    | 1.5 CFU mL <sup>-1</sup>                               | 50   |
| LAL          | Milk             | Chromogenic assay                    | ~103 CFU mL <sup>-1</sup>                              | 45   |
| LAL          | Beef             | Violet red bile agar overlay         | 5067.6 ng g <sup>-1</sup>                              | 51   |
| LAL          | Physiological    | Violet red bile agar overlay         | 7.00–7.49/g <sup>-1</sup>                              | 52   |
| LAL          | Milk             | —                                    | 1 : 10 <sup>4</sup> to 1 : 10 <sup>9</sup>             | 48   |
| LAL          | Plasma           | —                                    | 0.005 to 0.001 µg mL <sup>-1</sup>                     | 53   |
| ELISA        | Complex mixtures | ELISA using poly-L-lysine            | 1 µg mL <sup>-1</sup>                                  | 54   |
| ELISA        | Murine           | Sandwich capture ELISA               | 1 ng mL <sup>-1</sup>                                  | 55   |
| ELISA        | Clinical         | ELISA-bacteriophage receptor protein | 0.05 EU mL <sup>-1</sup> up to 500 EU mL <sup>-1</sup> | 56   |
| ELISA        | Human biofluid   | ELISA-high coating efficiency        | ~0.2 µg  | 57   |
| Kit          | Biological       | Endpoint chromogenic assay           | 0.1 EU mL <sup>-1</sup>                                | 58   |
| Kinetic-QCL  | Clinical         | Endpoint chromogenic assay           | 0.005 EU mL <sup>-1</sup>                              | 59   |
| Pyrosate kit | Clinical         | Gel clot assay                       | 1.0 EU mL <sup>-1</sup>                                | 60   |
| EndoLISA     | Biological       | Fluorescence                         | 0.005 EU mL <sup>-1</sup>                              | 56   |



Table 3 Comparison advantages and limitations of traditional methods in the detection of LPS

| Method      | Advantages  | Limitation  | Ref.      |
|-------------|---|---|-----------|
| Cultivation | It evaluates living (cultureable) microorganisms, recognizes live cells in the sample, easily quantifies the cells in the sample, and achieves high sensitivity in the appropriate medium | Advanced skills are required for optimal results, unique dietary supplements are required, and unique nutritional media are required for optimal growth. Time and resource intensive, relies on phenotypic biochemical characterization | 61 and 62 |
| LAL         | Acceptable sensitivity & specificity, cost effective  | Time consuming, complicated procedures, serious ecological problems   | 63 and 64 |
| ELISA       | Rapid, cost saving, acceptable sensitivity, and specificity, flexibility  | Difficult antibody pretreatment, cross reactive between the captured antibody and target, need for standard ELISA kit   | 65 and 66 |

important in pharmaceutical manufacturing, life sciences and medical research.<sup>43</sup> The commercial excess of various endotoxin detection kits on the market underscores the demand and need for early detection, regulation, and monitoring of endotoxin levels in different sectors. Compared to the official United States Pharmacopeia (USP) rabbit pyrogen test, the LAL test has been shown to be not only more sensitive to endotoxin, but also easier, faster, and cheaper to perform. Commercially available kits are based on various principles. Each strategy has its own advantages over other strategies. Everything is sensitive, reliable, efficient, and usually recommended over the old and slow rabbit pyrogen test. Each method has its own lower bound for endotoxin detection and its own incubation time, depending on the kit used.

Some practical and frequently used methods and also commercial products in the quantification of endotoxin are summarized in Table 2.

All the above techniques have certain drawbacks, such as attachment of additional agent (BSA), the use of high amounts of the coating antigen, cross-reactive reactions, false-positive results, and the possibility of variation of antigenic sites by chemical treatment. Some critical advantages and limitations of conventional methods are summarized in Table 3.

Due to the high importance of endotoxin (LPS) in various diseases, the development of advanced methods for its accurate and rapid diagnosis is an essential aim of researchers. Due to its exceptional properties, nanotechnology-based biosensing is more attractive to scientists. Biosensors are among the most important types of advanced technologies that have been developed extensively in recent years. The most important and latest diagnostic method (biosensing) for the detection of endotoxin will be discussed in the following subsections.

## 4. Biosensors; modern methods for the recognition of LPS

A biosensor is an analytical instrument that contains a biological sensor element (bioreceptor) that is closely connected or integrated with a transducer. The first biosensor was developed

for the recognition of glucose in 1962.<sup>62</sup> Meanwhile, in 1956 Prof. Clark developed the first “real” biosensor (Clark electrode) towards detection of oxygen in biological samples. Professor Clark is known as the “father of biosensors”, but the term “biosensors” was coined by Karl in 1972.<sup>67,68</sup> The technology converts biologically induced recognition events into a measurable signal (transduction), and analyses are acquired on screen after a series of processing. Biosensors are classified based on various subjects. As previously mentioned, the transducer is one of the crucial parts of the biosensor. Accordingly, biosensors are classified into three main subclasses, which include electrochemical, optical, and mechanical biosensors. Meanwhile, electrochemical and optical biosensors are very popular in medical diagnoses.

Electrochemical (EC) biosensors proposition advantages such as low background noise, high specificity of their biological recognition process, and better signal to noise ratios. Furthermore, measurement with electrochemical method measurement needs small sample volumes. Electrochemical recognition is extensively chosen for the transduction of biosensors due to its simplicity of construction, low cost, portability, and user-friendliness.<sup>69</sup> The biosensor has a simple, single overall structure that features direct conversion, high bioselectivity, high sensitivity, miniaturization, electrical/optoelectronic readout, continuous monitoring, ease of use, and cost effectiveness. Electrochemical biosensors are categorized into voltammetric, potentiometric, conductometric, or impedimetric based on transducing methods. Voltammetry sensors apply an electric potential to the working electrode and measure the current by electrochemical reduction or oxidation of the analyte.<sup>70</sup> The current response is typically acquired in a peak comparative to the analyte concentration.<sup>70</sup> Moreover, voltammetry contributes significantly to the boom in the concept of chemically modified electrodes.<sup>71</sup> Findings show numerous kinds of voltammetric biosensors effectively planned over wide surfaces of modifying elements and transducers.<sup>71</sup> Potentiometric technique, one of the earliest and most influential methods, occupies a permanent position in sensory analysis. This analytical method is an intelligent instrument for



many practical applications because it can detect different ions of different concentrations and can be used in inexpensive measuring instruments.<sup>72</sup> A potentiometric biosensor is well-defined as a tool combining a biological sensing component attached to a potential electrochemical transducer. Potentiometric biosensors generally rely on simpler species and biochemical reactions that result in electrochemical detection. The analytical signal produced by a potentiometric biosensor is an electrical potential.<sup>73</sup> However, impedance is generated in a complete alternating current (AC) circuit with a voltage-current phase angle gap due to inductive and capacitive effects. In the case of impedimetric biosensors, the impedance consists of capacitive and partial ohm resistance due to complex interactions with small amplitude voltage signals as a function of frequency.<sup>74,75</sup> A piezoelectric sensor is one of the most important sensors that can be used in home applications, medical applications, and energy self-sufficiency sensors.<sup>76,77</sup> So far, researchers have been able to produce a next-generation piezoelectric sensor that is highly efficient, robust, lightweight, and can be used as an energy self-sufficient sensor.<sup>78</sup> Piezoelectric sensors convert mechanical energies such as pressure, force, pressure, and vibration into electrical signals.<sup>79</sup> Some of the advantages and disadvantages of different electrochemical transducers are summarized in Table 4.

To date, various types of electrochemical biosensors have been developed for the recognition of LPS. In this section, some EC-based biosensors were surveyed. Also, the role of different nanomaterials on their analytical performance was discussed.

#### 4.1 Optical biosensors for detection of LPS

Another essential type of biosensor is the opto-sensor. Optical biosensors have shown valuable performance in discovering biological systems and promoting primary progress in drug discovery, clinical diagnostics, environmental monitoring, and food process control.<sup>88,89</sup> Simplicity in their pretreatment and possible influence on the nature of target molecules and

robustness, high sensitivity, reliability, and potential to be incorporated on a single chip are the critical advantages of optical biosensors.<sup>88,89</sup> Optical biosensors are categorized into two main groups; labeling and label-free method. Biosensing based on surface plasmon resonance (SPR) and fluorescence is the most popular and extensively used method in research and medical fields, such as pathogen recognition.

Examination of optical biosensors shows that despite the many advantages over routine methods, they have limitations and disadvantages that researchers in future studies should consider. Here are some examples of these problems. Optical biosensors have a medium or low selectivity, the main reason for which can be found in the method used. Low reproducibility as well as poor stability are other challenges related to the development of optical biosensors that should be addressed. The development of portable and cellophane-based biosensors can improve their attractiveness and popularity. So, developing such devices should be the goal of future studies.

Interestingly, a smartphone-based biosensor was developed to detect LPS on a grating-coupled surface plasmon resonance. Fixed biodevices provide POC detection for environmental and biomedical targets with attractive simplicity and portability benefits over distance and satisfactory analytical results (linear range; 570–730 ng mL<sup>-1</sup> and LOD = 32.5 ng mL<sup>-1</sup>).<sup>90</sup> Streptavidin-Horseradish peroxidase-modified hybridization chain reaction (HCRHRP) nanocomposites have been used as signal amplifiers or signal reporting elements for sensitive quantification of LPS. The planned genosensor quantitatively shows a handheld spectrophotometer with wide linear range and good LOD (1–150 ng mL<sup>-1</sup> and 50 pg mL<sup>-1</sup>, respectively) for future clinical diagnosis, food industry, and environment. It opened up a new perspective on LPS detection in monitoring.<sup>91</sup> An interesting immunosensor based on the electrochemiluminescence (ECL) method was fabricated to determine LPS. Planned ECL peptide biosensor offered a novel path in evaluating LPS expression on the bacteria surface and showed

**Table 4** Some of the advantages and disadvantages of different electrochemical transducers

| Type of detection method | Advantages  | Disadvantages  | Ref.      |
|--------------------------|---|--|-----------|
| Potentiometric           | Simple assembly technique, mass production, cost-effective, simple monitoring instrument/in field detection, fast operation, high sensitivity towards targeted ions, easy management system for POC measurement | Low selectivity, temperature-dependent system, impact solution changes, enormous mistake caused by logarithmic response, need additional components such as B. For signal amplification as the potential depends on the concentration of the analyte | 80–82     |
| Voltammetric             | Real-time detection, simultaneous analysis of several samples   | Environmentally sensitive, time consuming, and temperature sensitive   | 83 and 84 |
| Impedometric             | Small amplitude perturbation from steady state  | The sensitivity of measurement depends on the technical accuracy of the equipment and on the operating techniques  | 85 and 86 |
| Piezoelectric            | High frequency response, high sensitivity, high accuracy, high dynamic range  | Poor spatial resolution, dynamic sensing only, charge leakages   | 87        |





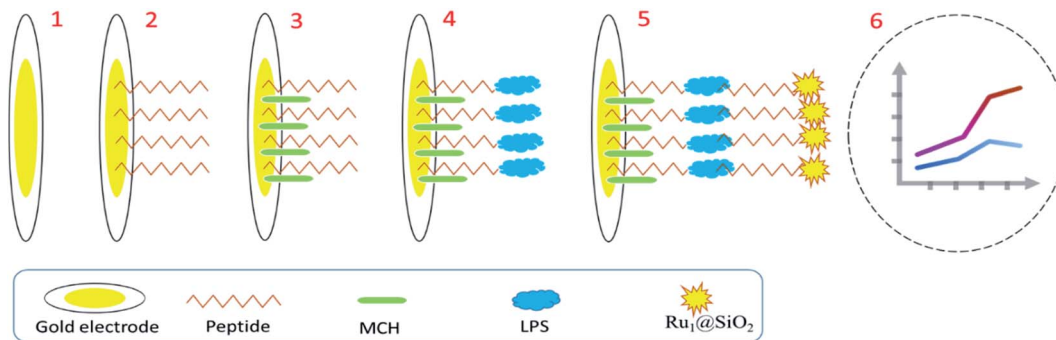


Fig. 2 Biosensing of LPS using ECL-based method.<sup>87</sup>

huge potential in the clinical analysis of LPS in related diseases. This study (Fig. 2) shows a strong gold–sulfur bond between the gold electrode surface and the thiol group of the peptide. In the presence of LPS, the peptide on the ECL biosensor can effectively capture LPS and produce LPS/peptide/AuE. The proposed system showed a wide linear detection range of 1.0–500 ng mL<sup>−1</sup> and a LOD of 0.3 ng mL<sup>−1</sup>.<sup>87</sup>

Magnetic nanoparticles (MNPs) are useable in optical sensing. The MNPs may improve the refractive index alteration for refractive index sensing, or over with suitable coatings, be turned into fluorescent or plasmonic sensors.<sup>92,93</sup> Iron oxide MNPs were employed as prospective therapy platforms, and due to their magnetic property, bacteria detection has drawn excessive consideration. These MNPs have generally been used as drug delivery, magnetic hyperthermia agents, and bioimaging contrast agents to identify and treat bacterial infections.<sup>90</sup> Carbon nanotubes (CNTs) have appeared as one of the most intensively explored nanostructured materials.<sup>91–93</sup> CNTs are hollow carbon buildings with a nanometer-scale diameter and a relatively more important length, and one or more walls.<sup>91,94</sup> The advantage of CNTs over other nanomaterials lies in the extraordinary combination of optical, electrical, magnetic, mechanical, and chemical properties that offers great potential for a wide range of applications, including biosensors.<sup>95</sup> Accordingly, several biosensors based on CNTs were developed for identifying pathogenic bacteria. For example, a single-walled carbon nanotube (SWCNT) based biosensor was fabricated to detect *Staphylococcus aureus*. The fixed tool detected *S. aureus* with a limit of detection (LOD) of 10<sup>4</sup> CFU mL<sup>−1</sup>.<sup>96</sup> Also, for rapid and sensitive detection of *Yersinia enterocolitica*, SWCNT was used appropriately. The concentration range for the SWCNT-based biosensor was 10<sup>6</sup> to 10<sup>4</sup> CFU mL<sup>−1</sup>, and the optimal limit of detection (LOD) of the SWCNT-based biosensor was determined to be 10<sup>4</sup> CFU mL<sup>−1</sup> of *Y. enterocolitica* in both clinical and phosphate buffer solution (PBS).<sup>97</sup> Screen-printed carbon electrodes (SPCE) modified with polymers molecularly imprinted (PMI) were applied to detect *P. aeruginosa*'s LPS. The developed bio-system was able to selectively identify *P. aeruginosa*'s LPS from the similar *E. coli* endotoxin with good LOD (16.7 μg mL<sup>−1</sup>).<sup>98</sup> The human Toll-Like Receptor-4 (TLR-4) is a protein responsible for sensing the LPS of Gram-negative bacteria. The TLR-4 was immobilized on the micro gold

electrode by binding a modified self-assembled monolayer (mSAM). The formation of TLR-4 protein dimers increased resistance to charge transfer from a solution-based redox probe in response to varying concentrations of its target. In this work, linear range and LOD were (10<sup>4</sup> and 10<sup>5</sup> cells per mL, 100 cells per mL), respectively.<sup>99</sup>

A different type of optical biosensor was fabricated to detect Gram-negative LPS based on T4 bacteriophage. This work presented recombinant adhesive phage protein as a receptor molecule in biosensing technology for the first time. Obtained analytical properties were acceptable (linear range: 0–0.07 mg mL<sup>−1</sup>, LOD: 50 mg L<sup>−1</sup>).<sup>100</sup> Through presenting an LPS specific binding peptide to a usual aggregation-induced emission (AIE) fluorophore, 1-(4-carboxylbenzene)-1,2,2-triphenyl (CTPY), a robust, specific, and sensitive optical biosensor based on the fluorescent method was settled. Such biodevices with rapid clearance and highly specific detection based on LPS specific binding peptides hold significant potential in diverse biological and clinical applications. The developed platform can achieve LPS sensing in a wide linear range of 0.1 to 1 μM and good LOD (6.97 nM).<sup>101</sup> Ultra-sensitive detection of LPS based on combined amplification of dual enzymes was designated recently. With the effective signal amplification, the bio-system revealed high sensitivity with wide range linearity (2.5–1000 pg mL<sup>−1</sup>) and an acceptable LOD1 (pg mL<sup>−1</sup>). In addition, advanced biosensors can clearly distinguish LPS from interference and have high specificity for LPS detection. This biosensor is also effectively used to measure LPS in actual food samples and has the potential to be applied to detect food safety.<sup>102</sup>

In early 2001, an original theory termed “aggregation-induced emission (AIE)” was first recommended by Tang *et al.* AIE luminogens (AIEgens) display negligible or weak emission in dilute solution but are released intensely in the aggregate or solid state.<sup>103,104</sup> AIEgens are categorized into two basic biomedical platforms, AIE NP probes and AIE molecular probes, which have been advanced for numerous biomedical applications.<sup>105,106</sup> Their exclusive properties in terms of stability, brightness, biocompatibility, and therapeutic functions have increased the constant development of original designs.<sup>107,108</sup> Until now, several AIE molecular probes have been established for monitoring bacterial labeling, cancer cell ablation, biological processes, and cellular organelle labelling with low



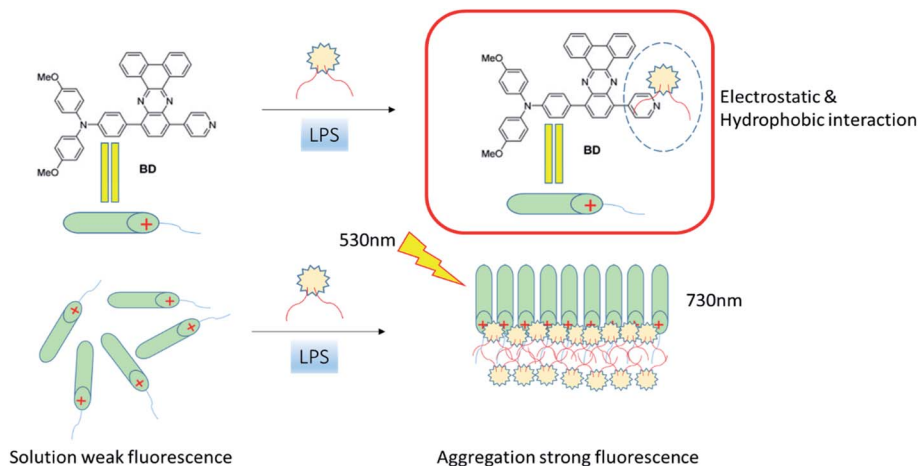


Fig. 3 AIE fluorescent platform for the detection of lipopolysaccharide, bacterial imaging, and photodynamic antibacterial therapy.<sup>107</sup>

background, high specificity, and tremendous photo stability.<sup>109,110</sup> AIE fluorescent platform was planned to detect LPS, photodynamic antibacterial therapy, and bacterial imaging. Created photoelectrochemical biosensor, Fig. 3, presented good analytical output (linear range:  $0\text{--}2.6 \times 10^8$  M and LOD:  $2.6 \times 10^8$  M), which showed great potential for detecting endotoxin in biological and food samples.<sup>107</sup>

Graphene has been used in designing different biosensors of several transduction styles because of its electrical conductivity, capacity to immobilize other molecules, large surface area, and high electron transfer rate.<sup>111,112</sup> In addition, the conjugated arrangement of graphene can facilitate electron transfer between the transducer and the bioreceptor, resulting in high signal sensitivity for electrochemical biosensors.<sup>113,114</sup> Moreover, graphene-based nanomaterial was employed as a quencher in the transducer to produce fluorescent biosensors. Findings indicated that reduced graphene oxide (rGO), graphene oxide (GO), and graphene (G) have a very high efficiency of fluorescent quenching.<sup>115</sup> Several rGO nanocomposite-based electrochemical biosensors were developed in the past decade for monitoring food-borne pathogenic bacteria.<sup>116</sup> A DNA aptamer genosensor was established for *Streptococcus pneumonia* detection based on the GO combination fluorescent method with exceptional LOD of  $15 \text{ CFU mL}^{-1}$ .<sup>117</sup>

Peptide-assembled graphene oxide was employed for endotoxin detection. Engineered fluorescent biosensor, Fig. 4, showed potential in selective detection of LPS from different bacterial strains and LPS on the membrane of living *E. coli*.<sup>115</sup>

Also, synthesized CdTe quantum dots (QDs) conjugated with concanavalin A (Con A) were proposed as an original bio device for specific and selective quantification of LPS. Wide linearity and acceptable sensitivity were reported in this research.<sup>116</sup>

Interestingly, a cell-based fluorescent optical biosensor was advanced to detect and recognize LPS, which is carried out in a 96-well microplate with high efficiency, which is user-friendly and nondestructive. This practical study combined the promoter sequence of the critical signaling pathway Enhanced Green Fluorescence Protein (EGFP) and gene ZC3H12A (encoding MCP1P1 protein) with building a recombinant plasmid, which was transferred into 293/hTLR4-MD2-CD14 cells through lipid-mediated, DNA-transfection technique. LPS could bind to TLR4 and co-receptors-induced signaling pathways could result in green fluorescent protein expression. The developed biosensor showed suitable analytical results (linear range:  $5\text{--}200 \mu\text{g mL}^{-1}$ , LOD:  $0.075 \mu\text{g mL}^{-1}$ ) applicable for endotoxin screening in food industries and medical approaches.<sup>102</sup> Also, an aptamer-based impedance genosensor was established for the detection of negative bacteria LPS. In

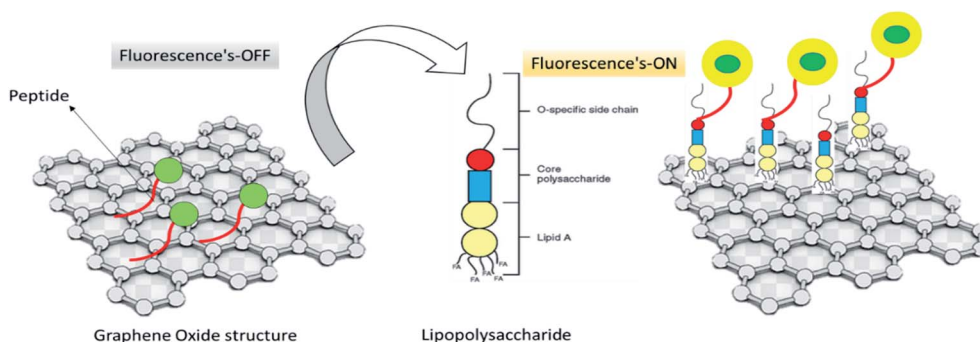


Fig. 4 Peptide-based biosensor for detection of LPS.<sup>115</sup>



this report, amine-terminated single-stranded DNA (ssDNA) was used as a probe that displayed high affinity to LPS and was immobilized on a gold electrode *via* 3-mercaptopropionic acid (MPA) as a linker. The designed system showed acceptable analytical results (linear range: 0.001–1 ng mL<sup>-1</sup>, LOD: 1 pg mL<sup>-1</sup>).<sup>118</sup> A robust living cell-based optical transcriptional biosensor to detect LPS with a red fluorescent protein reporter system was fabricated properly. In the developed system, 293/hTLR4A-MD2-CD14pGL4.26-mcherry-NF- $\kappa$ B cells were used as a recognition element. The engineered biosensor has great potential for use in bacteria sensitive identification (linear range: 0.01 to 100 ng mL<sup>-1</sup> and LOD: 1 ng mL<sup>-1</sup>), the LPS detection in food industries, biological products, and supporting the control of food-borne diseases.<sup>119</sup> Highly sensitive recognition (linearity: 1–10<sup>5</sup> ng mL<sup>-1</sup>, LOD: 1.73 ng mL<sup>-1</sup>) of LPS using a genosensor based on hybridization chain reaction (HCR) was advanced recently. In the presented technique, two complementary stable species of biotinylated DNA hairpins concurred in solution up to the introduction of a recognition probe initiated an HCR cascade. The LPS react specifically with the conjugate DNA, which was captured by the ethanolamine aptamer attached to the reaction fit surface (Fig. 5).<sup>120</sup>

Lipoteichoic acid (LTA) is a surface-binding amphipathic substance derived from Gram-positive bacteria and is a regulator of autolytic wall enzymes. It is generally released from bacterial cells after lysozyme-induced lysis from beta-lactam antibiotics, cationic peptides, and leukocytes.<sup>120,121</sup> Original research directly detects bacteremia by using host–pathogen interactions of LTA from Gram-positive bacteria and LPS from Gram-negative bacteria. The developed optical biosensor was

sensitive (LOD = 4 ng mL<sup>-1</sup>), rapid, and achieved an accurate diagnosis of bacteremia at the POC (Fig. 6).<sup>122</sup>

The optical waveguide biosensor offers the facility to recognize the kinetics of the bio-molecular interaction on an online source without the requisite for extrinsic labeling of the biomolecules.<sup>123,124</sup> Accordingly, a waveguide-based optical biosensor was settled for the high-sensitive detection of Gram-negative bacteria LPS. Waveguide-based biosystem demonstrated specific and rapid operation in the identification of pathogen biomarkers from small samples of serum or plasma, agreeing for the timely and sensitive analysis (linearity: 3.13  $\mu$ g mL<sup>-1</sup> to 200  $\mu$ g mL<sup>-1</sup>, LOD: 25  $\mu$ g mL<sup>-1</sup>) of infection at the POC.<sup>125</sup> A mechanical QCM biosensor was fabricated to detect the interaction of low molecular weight chitosan and its *N*-acylated derivatives and chitoliposomes with endotoxin. This work determined the attachment and detachment rate quantities for LPS binding to chitosan. The affinity constants were calculated based on alterations in the oscillation frequency.<sup>126</sup>

Due to its incredible chemical specificity and capacity to offer a fingerprint-like spectrum for complicated aqueous solutions, Raman spectroscopy (RS) has ended up as one of the maximum promising optical techniques. RS employs easy and reasonably-priced instrumentation, requiring minimum pattern preparation. Raman scattering now has no longer located extensive software with the organic count because of its inherently vulnerable signal. However, to date, techniques to decorate the sensitivity have been employed, namely, resonance Raman effects, which offers a 10<sup>2</sup>–10<sup>6</sup> enhancement, and surface-enhanced Raman spectroscopy (SERS), which ends up in as much as 10<sup>8</sup> enhancements.<sup>127,128</sup> It is generally accepted that the enhancement is due to the binding of the molecular

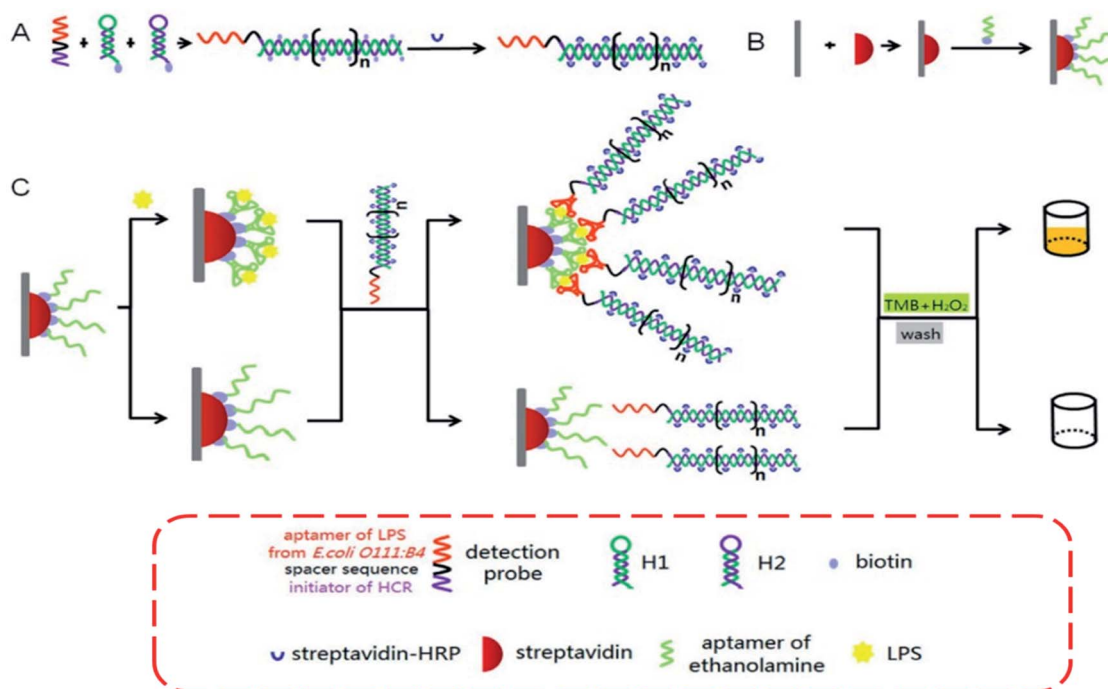


Fig. 5 Graphic depiction of HCR-based aptasensor for the sensitive detection of LPS.<sup>120</sup>





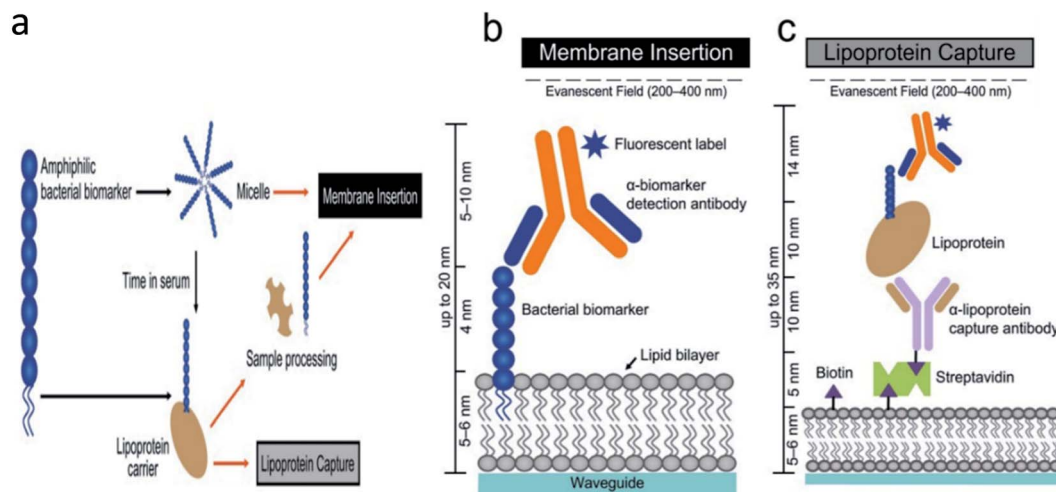


Fig. 6 Schematic of the developed optical biosensor.<sup>122</sup>

vibrations of the analyte molecule on the surface of the nanostructure to the locally enhanced electromagnetic field. These fields are created on the surface of metal nanostructures by quasiparticle vibrations called surface polarities and are formed when electrons on the metal surface interact with photons from incident light.<sup>129</sup>

The endotoxin launched within the side of the antibacterial system was monitored with the aid of the designed SERS detection strategy. The outcomes received with the aid of using SERS evaluation have been steady with the ones of the ELISA kit.<sup>130</sup> An innovative work describes the use of SERS for the label-free quantitative nanoscale identification of the LPS adsorbed on the surface of 50 nm AuNPs. This SERS method was an efficient tool to detect LPS on the AuNP surface and the basis for the development of a novel specific and sensitive LPS-detection sensor based on the use of SERS and AuNPs.<sup>131</sup> FRET or Förster resonance energy transfer is a flexible and sensitive device for qualitative and quantitative evaluation of organic interactions and processes.<sup>132</sup> The access to a wide range of fluorescent materials, in conjunction with improved, easy-to-use, spectrometers and very state-of-the-art microscopes, have made FRET a completely exceptional methodology for biosensing.<sup>132</sup> Fluorophores, which can be applied in FRET, now embody natural dyes, semiconductor quantum dots, fluorescent proteins, metallic chelates, several noble metals and different nanoparticles, and also polymers, basically fluorescent amino acids and organic cofactors.<sup>132,133</sup> Recently, a fluorescent turn-on sensor for detecting micromolar levels of bacterial LPS has been industrialized. Polydiacetylene liposome surface modified with fabricated pentyllysine peptide and histidine have effectively displayed fluorescence signal only in the presence of LPS. A significant increase in the fluorescence signal was perceived at *E. coli* concentrations as low as  $4.91 \times 10^8$  CFU mL<sup>-1</sup> with liposomes at a concentration of 40.0 μM. The sensor worked on the principle of FRET.<sup>134</sup>

Highlights of Table 5 include the following: (A) the majority of studies to date have been on ocular bacteria, and other bacteria have received less attention. Therefore, future studies

could be aimed at developing biosensors for other bacteria. (B) As shown in the table, the fluorescence technique has been more popular with researchers due to its inherent advantages: simple and reasonably predictive and easily miniaturized. (C) As revealed in Table 5, labeled biosensors have received more attention in most studies. The easy detection method is the main feature of this type of biosensor. In other words, there is no need to interpret the results due to direct diagnosis. (D) The table shows that metal nanoparticles are more prevalent in developing biosensors due to significant features such as low cytotoxicity to biomass cells, ability to bind multiple targeted compounds, and large surface-to-volume ratio. (E) Analytical results of various studies show a linear relationship between μM to nM. Compared to routine methods, these results are acceptable, but their strengthening in future studies seems necessary. According to the findings detailed in Table 5, biosensors based on metal and carbon nanoparticles are more sensitive.<sup>138</sup>

As discussed, optical biosensors are one of the most important and widely used methods of detecting bacterial endotoxin. Due to several advantages, including high specificity, sensitivity, small size, and cost-effectiveness, their development has increased extensively in recent years. In addition, optical biosensors have high environmental compatibility and good migration capability. On the other hand, as shown in the table, optical biosensors have the appropriate analytical properties in detecting real samples. Despite the advantages of optical biosensors to achieve ideal platforms, some of their weaknesses, such as sensitivity to the environment such as pH should be addressed in future studies.

#### 4.2 Developed electrochemical biosensors for detection of LPS

Recently a three-dimensional gold nanoparticles/ferrocene/liposome cluster (GFLC) was developed for electrochemical biosensing of LPS. In this cluster, gold nanoparticles, ferrocene, and liposome clusters act as signal amplification components,



Table 5 Developed optical biosensors for detection of endotoxin<sup>a</sup>

| Bacterium   | Technique                                       | Method                            | Sample           | NPs                     | Linear range  | LOD                               | Ref. |
|---|---|-----------------------------------|------------------|-------------------------|---|-----------------------------------|------|
| <i>E. coli</i>  | Grating-coupled SPR                             | Label-free                        | Clinical         | Metallic                | 570–730 nm  | 32.5 ng mL <sup>-1</sup>          | 85   |
| <i>E. coli</i>  | UV-Vis spectrophotometry                        | Colorimetric/ immunosensor        | Clinical         | Nickel                  | 0.07 mg mL <sup>-1</sup>                              | 50 mg L <sup>-1</sup>             | 100  |
| <i>S. typhosa</i> , <i>K. pneumonia</i> , <i>E. coli</i> , <i>P. aeruginosa</i> | Fluorescence                                    | Fluorescent/ immunosensor         | Clinical         | CTPY-AIE                | 0.1–1 μM  | 6.97 nM                           | 101  |
| Gram-negative bacterium   | ChA, SWV, EIS                                   | Labeled genosensor                | Food             | Gold                    | 2.5–1000 pg mL <sup>-1</sup>                          | 1 pg mL <sup>-1</sup>             | 102  |
| <i>E. coli</i>  | Fluorescence emission spectra, AIE              | Labeled – fluorescence            | Culture media    | Inorganic nanoparticles | $2.6 \times 10^8$ M                                   | $2.6 \times 10^8$ M               | 107  |
| <i>E. coli</i>  | Fluorescence emission spectra, Raman scattering | Labeled – fluorescence            | Clinical         | GO                      | 0 to 20 nM  | 130 pM                            | 115  |
| <i>Serratia marcescens</i>  | UV-visible spectra                              | Fluorescence labels- immunosensor | Culture media    | QDs                     | $1 \times 10$ to $1 \times 10^6$ CFU mL <sup>-1</sup> | 10–9 ng mL <sup>-1</sup>          | 116  |
| <i>E. coli</i>  | UV-Vis absorption spectra                       | Fluorescence labels/ immunosensor | Clinical         | Ru@SiO <sub>2</sub>     | 1.0–500 ng mL <sup>-1</sup>                           | 0.3 ng mL <sup>-1</sup>           | 24   |
| <i>E. coli</i>  | Cell-based fluorescent                          | Fluorescence labels/ immunosensor | Serum            | —                       | 5–200 μg mL <sup>-1</sup>                             | 0.075 μg mL <sup>-1</sup>         | 135  |
| <i>E. coli</i>  | EIS, CVs  | —                                 | —                | —                       | 0.001–1 ng mL   | 1 pg mL <sup>-1</sup>             | 118  |
| <i>E. coli</i>  | Fluorescent                                     | Aptamer-based impedance           | Serum            | —                       | 0.01 to 100 ng mL <sup>-1</sup>                       | 1 ng mL <sup>-1</sup>             | 119  |
| <i>E. coli</i>  | Fluorescent                                     | Genosensor/labeled                | Serum            | Streptavidin            | $1-10^5$ ng mL <sup>-1</sup>                          | 1.73 ng mL <sup>-1</sup>          | 136  |
| <i>S. typhimurium</i> and <i>Staphylococcus</i>                                 | Fluorescent                                     | Waveguide-based optical biosensor | Clinical samples | Streptavidin–biotin     | —   | 4 ng mL <sup>-1</sup>             | 137  |
| <i>S. typhimurium</i>   | Fluorescent, wave guide                         | Waveguide-based optical biosensor | Serum            | —                       | 3.13 μg mL <sup>-1</sup> to 200 μg mL <sup>-1</sup>   | 6.25 μg mL <sup>-1</sup>          | 125  |
| <i>P. aeruginosa</i>  | SERS  | SERS spectra                      | Biological       | AgNPs                   | 0.10–10.0 μmol L <sup>-1</sup>                        | 6.125 ng mL <sup>-1</sup>         | 130  |
| <i>E. coli</i> O55:B5   | SERS  | Raman and SERS spectra            | Biological       | AuNPs                   | 0.1–10 μg mL <sup>-1</sup>                            | $2.6 \pm 0.1$ ng mL <sup>-1</sup> | 131  |
| <i>E. coli</i>  | FRET  | Fluorescence labels/ immunosensor | Clinical         | —                       | $4.91 \times 10^8$ CFU mL <sup>-1</sup>               | 40.0 μM                           | 134  |

<sup>a</sup> Abbreviation: (GFLC): gold nanoparticles/ferrocene/liposome cluster, (GE): graphite electrode, (SAW): surface acoustic wave resonator, (EIS): electrochemical impedance spectroscopy (HCR-HRP): streptavidin-horseradish peroxidase modified hybridization chain reaction, (SPCE): screen-printed carbon electrodes, (NTA): nitrilotriacetic acid complex, (AIE) aggregation induced emission, (CTPY): 1-(4-carboxybenzene)-1,2,2-triphenyl, (SCE): saturated calomel electrode, (QDs): CdTe quantum dots, (Ac-ChLM): *N*-3-hydroxytetradecanoyl chitosan, surface-enhanced Raman spectroscopy (SERS).

signal output components, or molecular recognition components.<sup>139</sup> Also, a sensitive DNA-based biosensor (genosensor) was developed for the rapid and selective detection of LPS of *E. coli*. This system was cost-effective and showed excessive application potential for food safety, environmental monitoring, and real-time diagnosis.<sup>140</sup> Magnetic nanoparticles (MNPs) are imperative in sensing as they offer viable solutions to the continuing challenges related to analytical and nonspecific effects. MNPs are applicable to facilitate electrochemical responses *via* blocking access to an original electrode or after coating to make them conduct.<sup>89</sup> Also, an innovative immunosensor based on MNPs was engineered to determine *S. typhimurium* endotoxin in related samples. The created electrochemical biosensor was selective, specific, and has excellent potential in real-time detection of Gram-negative bacteria LPS.<sup>141</sup> For sensing Gram-negative LPS, Cu<sup>2+</sup>-AuNPs aggregates were appropriately used in the electrochemical platform. The developed biosensor, Fig. 7, showed a high specificity toward LPS in the presence of other common interfering substances and was effortlessly renewed. Moreover, the

invented biosensor presented a good practical application for LPS recognition in human serum samples with satisfactory analytical results (linear range: 0.05 pg mL<sup>-1</sup> to 10 pg mL<sup>-1</sup>, LOD: 0.033 pg mL<sup>-1</sup>).<sup>141</sup>

A sensitive electrochemical platform was established for sensitive determination of LPS based on metal complex immobilized gold electrode. In this research work, electrochemical impedance spectroscopy (EIS) was applied to detect the interaction of nitrilotriacetic acid (NTA)[Cu]<sup>2+</sup> complex with endotoxin. In this work, (0.0001–0.1 ng mL<sup>-1</sup>) and (0.0001 ng mL<sup>-1</sup>) were reported as linear range and LOD, respectively.<sup>142</sup> A three-dimensional cells-in-gels-in-paper miniaturized electrochemical biosensor was fabricated for the rapid and sensitive detection of Gram-negative bacterium LPS. The proposed technique is applicable in food industries with high sensitivity (LOD =  $3.5 \times 10^{-3}$  ng mL<sup>-1</sup>) and low cost construction.<sup>143</sup> Intracellular carbon nanoelectrode was employed as an electrochemical sensor for ultra-sensitive detection (LOD = 1 ng mL<sup>-1</sup>) of hydrogen peroxide generated intracellularly in response to rough and smooth LPS structures. This work



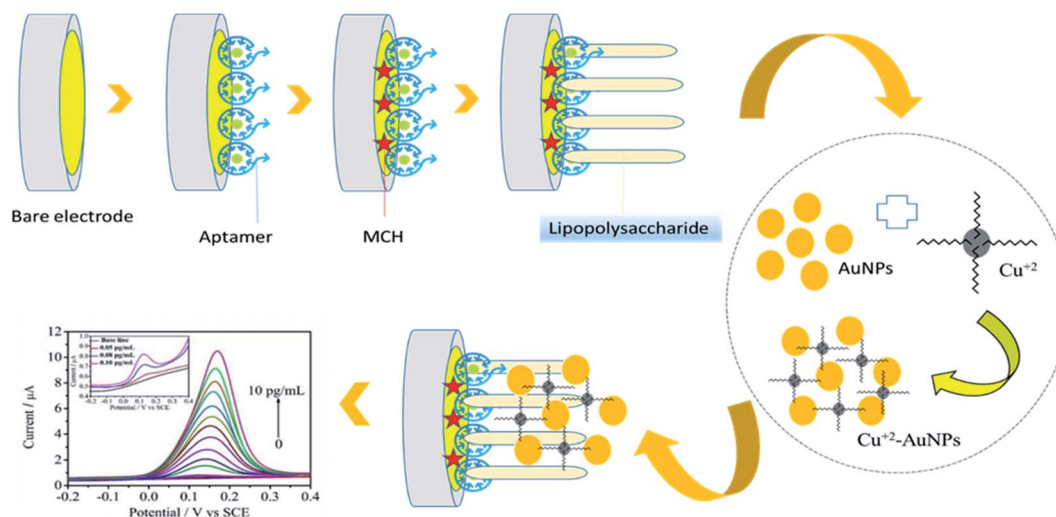


Fig. 7 Graphical illustration of the developed aptasensor for the detection of LPS.<sup>141</sup>

showed different and indirect methods in determining bacterial endotoxin.<sup>144</sup> A peptide was used as the recognition molecule in the electrochemical impedance immunosensor to determine LPS. The developed biosystem was used for real-time detection of endotoxin in real serum samples with acceptable linear range ( $0.01 \text{ pg mL}^{-1}$  to  $1.0 \text{ ng mL}^{-1}$ ) and LOD ( $2.0 \text{ fg mL}^{-1}$ ) (Fig. 8).<sup>145</sup>

A dual electrochemical and mechanical label free lectin platform was developed for highly sensitive detection (LOD = up to 50 cells per mL) of antimicrobial susceptibility and the quantification of bacterial LPS. In this method, a polythiophene interface containing glycosylated fusion quinone units is used to form a carbohydrate platform for immobilization of concanavalin A (Con A), and LPS binding is measured *via* an orthogonal crystal oscillator microbalance and perform an electrochemical read (EQCM).<sup>146</sup> An impedimetric

electrochemical biosensor was developed for the quantification of *E. coli* produced LPS. In the developed system (Fig. 9), concanavalin A (Con A) lectins have been used as a molecular recognition element due to their specific interaction with the carbohydrate groups of LPS. Con A recognizes sigma-bonded mannose as the “core oligosaccharides” of some serum and membrane glycoproteins. The proposed biosensor was constructed based on lectin-functionalized Au-graphene compounds and showed acceptable stability and reproducibility with satisfactory analytical properties (linear range =  $7.03 \times 10^{-2}$  to  $1.41 \times 10^{-1} \text{ pg L}^{-1}$  and LOD =  $600 \text{ pg L}^{-1}$ ). Au and graphene nanoparticles have recently been recognized as efficient and most promising electrochemical and luminescence catalysts.<sup>147</sup>

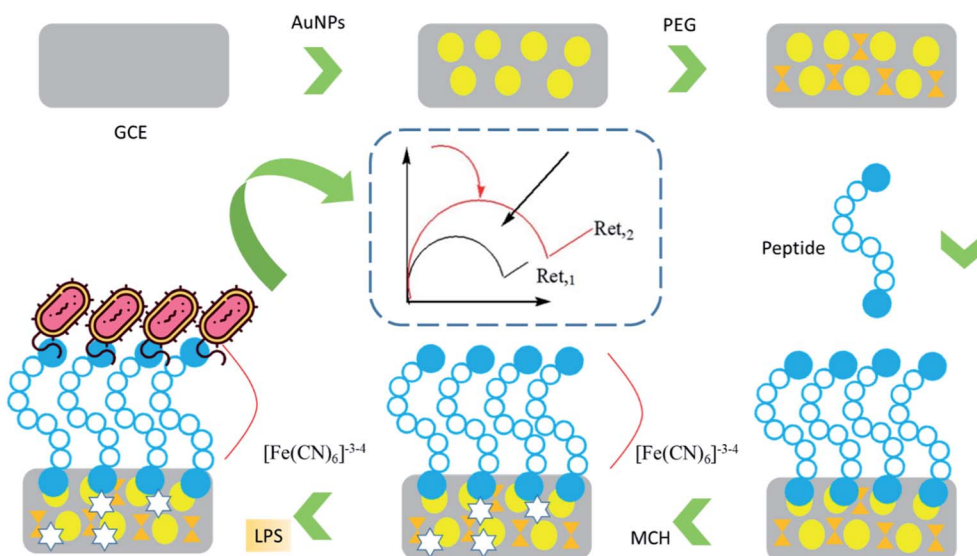


Fig. 8 Graphic illustration of the label-free EIS biosensor for LPS detection.<sup>145</sup>



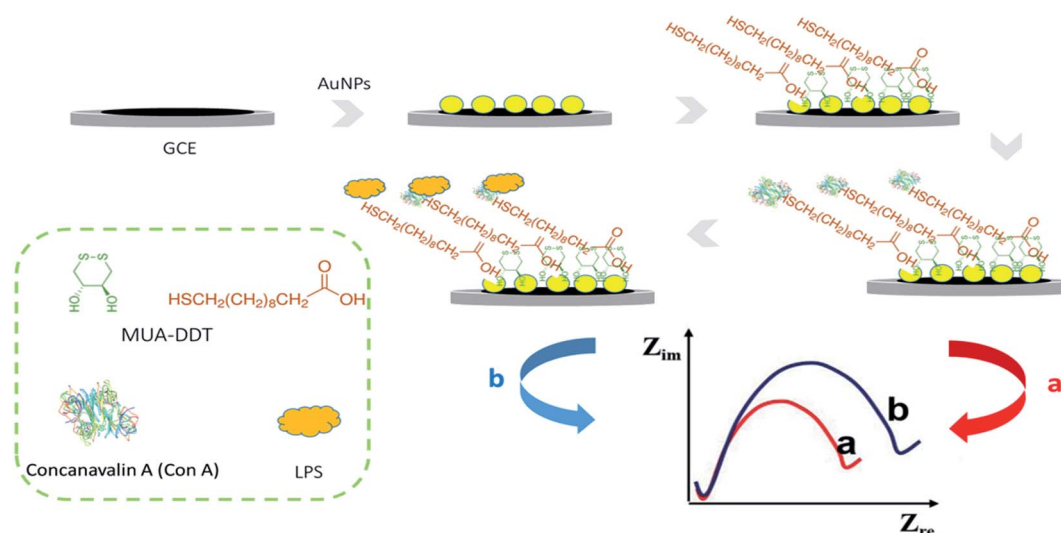


Fig. 9 Representation of impedimetric lectin-based biosensors for bacterial LPS.<sup>147</sup>

DNAzyme-assisted recycling and Ce-based metal-organic structures dual signal amplifiers for sensitive electrochemical detection of LPS were settled. In the planned biosensor, Ce-MOFs were decorated with AuNPs to achieve AuNPs/Ce-MOFs. In this report, AuNPs/Ce-MOFs acted as catalysts to catalyze the oxidation of ascorbic acid (AA) and nanocarrier to capture -SH terminated hairpin probes 2 (HP2) for acquiring HP2/AuNPs/Ce-MOFs signal probes. Projected aptasensor for LPS showed a wide linear range from  $10 \text{ fg mL}^{-1}$  to  $100 \text{ ng mL}^{-1}$  with a  $3.3 \text{ fg mL}^{-1}$  LOD.<sup>148</sup> An innovative electrochemical aptasensing platform based on GCE modified using GO and AuNPs was an assembly for the identification of *E. coli* endotoxin. The obtained results revealed that the modified GCE has a satisfactory selectivity for LPS over other biomolecules and is positively used to investigate serum of healthy persons and patients. In this work, acceptable linear range ( $0.1\text{--}0.9 \text{ pg mL}^{-1}$ ) and LOD ( $30 \text{ fg mL}^{-1}$ ) were reported.<sup>149</sup> An electrochemical impedimetric biosensor based on SAM mixture CramoLL lectin and cysteine-gold nanoparticles (cys-AuNPs) was proposed for bacterial LPS recognition. Findings revealed that LPS from *Serratia marcescens*, *Escherichia coli*, *Salmonella enterica*, and *Klebsiella pneumoniae* are measurable by the settled system with good LOD ( $10 \text{ mM}$ ).<sup>150</sup> A gold atomic cluster was applied as an electrochemical aptasensor for the recognition of LPS. The formed gold atomic clusters make Apt/AuAC/Au show an excellent property ( $\text{LOD} = 7.94 \times 10^{-21} \text{ M}$ ) for the LPS determination in related samples. Also, the designed biosensor demonstrated a simple and easy technique for detecting LPS on the nanoscale amount.<sup>151</sup> To complete our discussion, Table 6 summarizes all EC biosensors for the detection of LPS in different samples and compares their analytical parameters.

Highlights of Table 6 include the following: (A) as demonstrated in Table 6, similar to optical biosensors, despite the presence of various endotoxin-producing bacteria, most studies have been performed on *E. coli*. Therefore, future studies should

be aimed at detecting endotoxins in other bacteria. (B) In most of the studies summarized in the table, the impedimetric technique has been used mainly because of the simplicity of performing and diagnosis in real-time. (C) Good reducibility, high sensitivity, and specificity are critical properties in aptamer-based biosensors. Therefore, this kind of biosensing method has been considered by researchers to date. (D) Gold nanoparticles have been the most used in the development of LPS biosensors, and the results show that their use has increased the sensitivity and specificity of biosensors. Due to the unique properties of carbon-based nanoparticles, such as low cost and high conductivity, it is suggested that their use be further considered in future studies. (E) Analytical results of various studies indicated a linear relationship between  $\mu\text{M}$  to  $\text{nM}$ . Compared to routine methods, these results are acceptable, but their improvement in future studies seems crucial. According to the findings detailed in Table 6, biosensors based on AuNPs show extra sensitivity and specificity.

Electrochemiluminescence (ECL) based sensors combine electrochemistry and quantity of optical luminescence.<sup>153</sup> In this method, when a potential is applied onto an electrode, the electrode surface is excited.<sup>153</sup> Therefore, an electron transfer is done between molecules, and the resulting light is measured. ECL biosensors have been established to recognize chemical and bacteriological contaminants in food products.<sup>153,154</sup> ECL biosensor was advanced for *Escherichia coli* O157:H7 quantitative recognition based on nitrogen-doped graphene quantum dots (N-GQDs) and a polydopamine (PDA) surface imprinted polymer (SIP).<sup>152</sup>

Magnetic resonance elastography (EG) is a valuable noninvasive analytical method of detecting abnormalities, such as liver fibrosis. The method generates tissue deformation by applying external or internal excitation, which causes displacements, and these displacements can be measured using modified MRE pulse sequences. Accordingly, EG as





Table 6 Developed electrochemical biosensors for detection of endotoxin<sup>a</sup>

| Bacterium   | Technique                                | Method                        | Sample         | NPs                     | Linear range  | LOD  | Ref. |
|---|--|-------------------------------|----------------|-------------------------|---|--|------|
| <i>P. aeruginosa</i>  | DPV, FT-IR spectra                       | Fluorescence emission spectra | Food           | GFLC                    | $2 \times 10^{-9} \mu\text{g mL}^{-1}$ to $8 \mu\text{g mL}^{-1}$ | $0.51 \times 10^{-10} \mu\text{g mL}^{-1}$ | 139  |
| <i>E. coli</i>  | Aptasensor                               | Labeled-aptasensor            | Rat serum      | HCR-HRP                 | 1 to 150 ng mL <sup>-1</sup>                                      | 50 pg mL <sup>-1</sup>                     | 152  |
| <i>S. typhimurium</i>   | EIS, impedimetric                        | Immunosensor                  | Food and water | Magnetic                | 0.001–0.1 $\mu\text{g mL}^{-1}$                                   | $10^1 \text{ CFU mL}^{-1}$                 | 141  |
| <i>P. aeruginosa</i>  | CVs                                      | MIP                           | Wastewater     | MIP                     | —   | 16.7 $\mu\text{g mL}^{-1}$                 | 98   |
| <i>S. typhimurium</i>   | EIS                                      | Immunosensor/EDC/NHS          | Clinical       | —                       | $10^4$ and $10^5$ cells per mL                                    | $10^5$ cells per mL                        | 99   |
| <i>E. coli</i>  | EIS                                      | Labeled, aptasensor, DPV      | Serum          | Cu <sup>2+</sup> -AuNPs | 0.05 pg mL <sup>-1</sup> to 10 pg mL <sup>-1</sup>                | 0.033 pg mL <sup>-1</sup>                  | 145  |
| <i>E. coli</i>  | EIS                                      | EIS/CVs                       | Clinical       | Cu <sup>2+</sup> -NTA   | 0.0001–0.1 ng mL <sup>-1</sup>                                    | 1 ng mL <sup>-1</sup>                      | 142  |
| <i>S. enterica</i>  | CVs, DPV                                 | Electrochemical/EIS           | Food           | GO                      | $1 \times 10^1$ to $1 \times 10^4$ ng mL <sup>-1</sup>            | $3.5 \times 10^{-3}$ ng mL <sup>-1</sup>   | 143  |
| <i>E. coli</i> , <i>S. minnesota</i>  | CVs                                      | Labeled electrochemical       | Clinical       | CNTs                    | 1 mg mL <sup>-1</sup>   | 1 ng mL <sup>-1</sup>                      | 144  |
| <i>E. coli</i>  | Impedimetric                             | EIS/immunosensor              | Clinical       | AuNPs                   | 0.01 pg mL <sup>-1</sup> to 1.0 ng mL <sup>-1</sup>               | 2.0 fg mL <sup>-1</sup>                    | 145  |
| <i>E. coli</i>  | Impedimetric                             | Impedimetric/labeled          | Clinical       | AuNPs–graphene          | $1.0 \times 10^{-9}$ to $1.0 \times 10^{-6}$ g L <sup>-1</sup>    | 600 pg L <sup>-1</sup>                     | 132  |
| Gram negative bacterium   | CVs, EIS                                 | Genosensor/labeled            | Serum          | AuNPs/Ce-MOFs           | 10 fg mL <sup>-1</sup> to 100 ng mL <sup>-1</sup>                 | 3.3 fg mL <sup>-1</sup>                    | 148  |
| <i>E. coli</i>  | SWV                                      | Aptasensor/label free         | Serum          | RGO/AuNPs               | 0.1–0.9 pg mL <sup>-1</sup>                                       | 30 fg mL <sup>-1</sup>                     | 149  |
| Gram negative bacterium   | UV-visible absorption spectrum, EIS, DPV | Aptasensor/labeled            | Serum          | Apt/AuAC/Au             | 0.01 attomolar to 1 picomolar                                     | $7.94 \times 10^{-21}$ M                   | 151  |
| <i>S. marcescens</i> , <i>E. coli</i> , <i>S. enterica</i> , <i>K. pneumoniae</i> | Impedimetric, CVs                        | Aptasensor/EIS                | Clinical       | Cys-Au                  | —   | 10 mM                                      | 150  |

<sup>a</sup> Abbreviation: (GFLC): gold nanoparticles/ferrocene/liposome cluster, (GE): graphite electrode, (SAW): surface acoustic wave resonator, (EIS): electrochemical impedance spectroscopy (HCR-HRP): streptavidin-horseradish peroxidase modified hybridization chain reaction, (SPCE): screen-printed carbon electrodes, (NTA): nitrilotriacetic acid complex, (AIE) aggregation induced emission, (CTPY): 1-(4-carboxylbenzene)-1,2,2-triphenyl, (SCE): saturated calomel electrode, (QDs): CdTe quantum dots, (Ac-ChLM): *N*-3-hydroxytetradecanil chitosan.

a flexible analytical method was applied for the detection of coagulation. The rapid detection of LPS is achieved by the EG analysis of endotoxin-induced limulus amebocyte lysate coagulation. This method is better than other methods using the same reagents in not only sensitivity but also detection time.<sup>155</sup>

As mentioned before, various types of biosensors are being developed, among which researchers have given electrochemical and optical biosensors more attention due to their many advantages. In the following, the electrochemical and optical biosensors developed for endotoxin detection are discussed in more detail, respectively. Photoelectrochemical (PEC) biosensors are an imperative branch of electrochemical detection, and PEC sensing has perceived rapid expansion despite the late start.<sup>107</sup> Photoelectrochemistry is a dynamic discipline discovering the effect of light on photoactive materials, which involves the transformation of light into electricity and interconversion of electric energy and chemical energy. A schematic illustration of the PEC sensor is presented in Fig. 10.

In recent years, several PEC biosensors have been developed to detect bacteria. For example, an integrated multifunctional PEC platform for rapid detection of pathogenic bacteria was proposed recently. The platform can offer novel approaches for creating advanced green PEC sensors to recognize various pathogenic bacteria. The developed PEC platform showed satisfactory linearity ( $1 \times 10^2$  to  $1 \times 10^6 \text{ CFU mL}^{-1}$ ) and LOD (46

CFU mL<sup>-1</sup>).<sup>156</sup> A layer-by-layer self-assembly technique was employed to create the working electrode of the PEC aptamer sensor. The planned PEC aptamer-based biosensor displayed good stability, detection specificity, and reproducibility for *Vibrio parahaemolyticus* and provided a simple, cost-saving, and rapid detection approach for screening and controlling food-borne diseases.<sup>157</sup> A signal-off PEC aptasensor was projected for pathogenic *S. aureus* detection based on graphite-like carbon nitride decorated with NiO.<sup>83</sup> Despite the unique features of PEC biosensors, according to the studies conducted in this study, to date, this type of platform for LPS detection has not been developed. Therefore, considering the exceptional advantages of PEC biosensors, their development should be considered in future studies.

## 5. The role of nanoparticles in biosensor development

As reviewed in the present study, various nanoparticles were applied in biosensor development, and some of the presented nanoparticles have been used extensively. The use of NPs, nanotubes, nanowires, etc., in biosensor diagnostic devices is being discovered. With the progression in properties of nanomaterials, with their dimensions at the nanoscale level, smart biosensors that can detect a minute concentration of the



desired analyte are developing. Nanomaterials are generally used as transducer materials that are an important part of biosensor development. The advantages and disadvantages of different nanomaterial-based biosensors are presented in Table 7.

It has been determined that the usage of nanomaterials can also additionally cause extended biosensor overall performance along with extended sensitivities and occasional limit-of-detection of numerous orders of magnitudes. Nanostructured substances display extended surface-to-extent ratio, mechanical strength, chemical activity, electrocatalytic properties, and more advantageous diffusivity. Nanomaterials were anticipated to play a critical role in the high performance of a biosensor. To probe biomolecules along with bacteria, viruses, DNA, *etc.*, the biocompatibility of nanomaterials has been a serious issue in designing a biosensor.

## 6. Discoveries of gaps and future outlook

Biosensors establish an interdisciplinary field that is presently one of the critical parts of investigation in analytical chemistry. Expanding biosensors naturally reduces the need for sample preparation. The biosensor's performance is typically and experimentally assessed based on its LOD, sensitivity, reproducibility, dynamic and linear ranges, or accuracy of the selectivity response and its response to interferences. Additional frequently compared factors include operational and storage stability, sensor reaction time, easy operation, and portability. Ideally, the sensing surface should be reusable for numerous repeated quantities to be made. For various environmental, clinical, and food applications, the sensor should be capable of constantly monitoring the analyte online. Conversely, single-

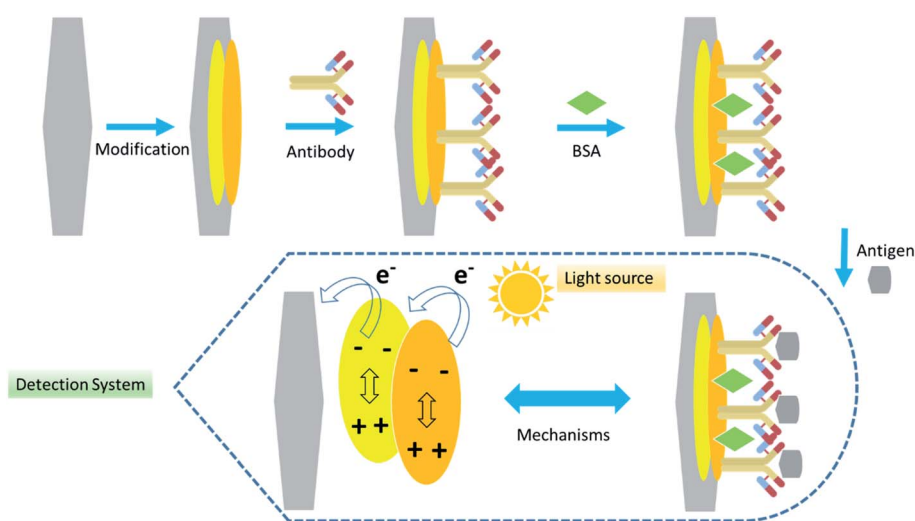


Fig. 10 Schematic illustration of the PEC sensor.

Table 7 Advantages and disadvantages of different nanomaterial-based biosensors

| Nanomaterial | Advantages   | Disadvantages  | Ref.             |
|--------------|--|--|------------------|
| CNTs         | Extremely small and lightweight, plentiful resources, resistant to temperature changes, ability to cross cellular membranes and barriers, high chemical and mechanical stability, strong C-H bonds | Expensive nanotube production process, low yield, low hydrogen storage capacity, purification problems | 158 and 159      |
| GO           | Water dispersibility, polar functionalization, easy workability, low-cost  | Lower electrical and thermal conductivity  | 145, 160 and 161 |
| rGO          | High electrical and thermal conductivity, good control on functionalization, economical and facile technique   | Hydrophobicity, difficult workability, and production methodology are important in their properties    | 88 and 89        |
| MNPs         | Large surface-to-volume ratio, low cytotoxicity to biomass cell, ease of synthesis, capacity to bind several targeted compounds  | High cost of synthesis processes and materials, mobility dependent on environment compatibilities      | 162 and 163      |



use biosensors are acceptable for some main applications, such as personal blood glucose monitoring by diabetics. Therefore, future studies should be in line with developing an ideal biosensor with the mentioned features and capabilities. In addition to the above, the methodology is critical in the development of biosensors; for example: as described in the text, LPS are some intercellular components and cell-based biosensors play a critical role in their determination.

On the other hand, functionalized CNTs can successfully penetrate individual cells and cross biological barriers like cell membranes.<sup>164</sup> This property and the release of CNTs from the cells and mechanism of internalization are of main interest for biological and in particular intracellular biosensing applications. Therefore, the use of CNTs in the development of future biosensors should be given more attention.

## 7. Conclusion

In this review, the up-to-date scientific literature dealing with biosensors for LPS detection was investigated. Different methods advanced by various studies to detect endotoxin are declared. The biosensors have different sample pretreatment requirements, other detection limits for the target organisms, and different degrees of automation evaluation, complexities, and requisite analysis times. Based on the surveyed literature, biosensors have verified the great potential for LPS analysis, but the following points should be considered in future studies. In conclusion, many of the established biosensing systems target *Salmonella*, *E. coli* 0157:H7. At the same time, some of the reported examples are not directed at the main imperative pathogens listed in Table 1. (B) For commercialization, one of the problems still facing the construction of biosensors for direct detection of LPS is the sensitivity of the analyses in the real sample. (C) Specificity is the critical issue in biosensor development and should be improved in future investigations; in other words, a robust biosensor should be able to detect target structures from similar structures in contaminated samples. (D) Simple and low-cost structure, easy to operate and easy interpretation of results, and fast and high sensitivity are the features of LPS biosensors that should be considered in their development. (E) Endotoxins are Gram-negative bacterial wall compounds, so the development of cell-based biosensors is of great importance. (F) As found in reviewing and comparing different studies, various factors play a critical role in constructing an ideal biosensor, the least of which include: the type of the used nanomaterial and techniques. Also, according to the results obtained in this study, the use of appropriate nanomaterials is one of the main factors in achieving improved analytical results. Finally, although biosensors have advantages over routine and older methods, future studies should be developed to address the barriers and weaknesses of biosensors so that researchers should consider the following points: simple and inexpensive structure is one of the strategic goals of biosensor development, so the use of advanced and expensive materials and tools will make the created biosensors not welcome. This may be one of the main reasons for the unsuccessful industrialization and commercialization of biosensors

developed to date. Subsequently, according to studies, labeled biosensors, in addition to high cost, have a time-consuming and complex design process, so the development of label-free biosensors should be considered as much as possible. As shown in Table 4, vulnerabilities to temperature changes and the surrounding environment and pH are one of the main problems and limitations in the development of electrochemical biosensors, especially concerning physiological matrices, so eliminating this vulnerability should be highlighted in the future studies.

## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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