Biosensors: Classifications, Medical Applications and Future Prospective

Hani A. Alhadrami[1,2*]

[1]Faculty of Applied Medical Sciences, Department of Medical Laboratory Technology, King Abdulaziz University, P. O. Box 80402, Jeddah, 21589, Kingdom of Saudi Arabia.

[2]Special Infectious Agent Unit, King Fahd Medical Research Centre, King Abdulaziz University, P. O. Box 80402, Jeddah, 21589, Kingdom of Saudi Arabia.

[*]Crossponding author: hanialhadrami@kau.edu.sa

Abstract

Biosensors are devices that combine a biological material with a suitable platform for detection of pathogenic organisms, carcinogenic, mutagenic and/or toxic chemicals or for reporting a biological effect. In recent years, enormous number of different types of biosensors have been constructed and developed for several medical applications. The reason for that was primarily due to the numerous advantages and applications that can be offered by biosensors. This review article has been started with demonstrating the powerful of biosensor technologies versus analytical and conventional techniques. Subsequently, more emphasis has been added on the classification and the role of biosensors in several medical applications such as detection and monitoring of carcinogenic and mutagenic chemicals, reporting of endocrine disrupting compounds and detection of pathogenic organisms. The most common reporter genes used in biosensors engineering and construction have

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been also summarized. Prospective strategies and recommendations for the future construction of biosensors have been highlighted.

1. Introduction

Modern technologies and industrialization have caused serious health challenges although they have created a more convenient life for humans. Substantial amounts of carcinogenic, mutagenic and toxic materials are annually generated and released into our environments. Serious diseases like cancer can be generated as carcinogenic materials are continuously released from the extensive usage of petroleum products and various industrial activities [1]. Therefore, a permanent need of early detection and monitoring are required for carcinogenic, mutagenic and toxic materials. In parallel with the development in conventional methods for monitoring of different carcinogens and toxins, still there is a need for bioassays that not only detect carcinogenic and toxic substances, but additionally report its bioavailability and judge its impacts on human. As a forward step to fulfil this necessitate, biosensors technologies that relay on genetically engineered microorganisms have been rapidly expanded. The field of bioreporters or biosensors has been massively developed since 1990s when researchers in Gary Sayler's laboratory engineered the first genetically modified microbial biosensor [2]. The term biosensor is used to describe the wide range of biological materials producing a measurable signal through a suitable transducer when exposed to carcinogenic and toxic substances [3,4]. Biosensors have been given various definition in the literature however, van

der Meer and Belkin have precisely defined biosensor as a device which combines a biological material with a suitable platform for the detection of a chemical or a biological effect [5]. The biological material can be whole cell (i.e. microbial biosensors), antibody (i.e. immunosensors), or nucleic acid (i.e. DNA aptamer based sensors). Biosensors based on enzymes and DNA aptamer are more specific and sensitive than whole cell biosensors. Whole cell biosensors may sometimes lack specificity for targets, but are generally more stable, cheaper and able to report bioavailability. The most widely used whole cell biosensor is microbial biosensor which reports carcinogenicity and toxicity using genetically modified microorganisms by measuring various factors, such as growth inhibition, enzyme inhibition and cell viability. For toxicity biosensors, the biological reaction measured can be, for instance, the enzyme activity, the induction of gene expression or even cell death [6,7]. The transducer used for detecting these biological reactions can be colorimetric, optical, electrochemical, mass sensitive or thermal [8].

Whole cell microbial biosensor should comprise two correlated genetic components: a sensing element and a reporter [9] (Figure 1). The reporter element has been selected from number of genes or gene cassettes, such as the *lux* gene, or sets of genes coding for protein(s) which has certain functions by itself or it catalyses a biochemical reaction to generate an easily detectable signal [10]. A wide range of reporter genes is now available for use in the construction of genetically modified microbial biosensors (Table 1). For instance, the *lux* genes have become commonly employed for medical applications in recent years due to the ease of measuring real-time light production without disruption of the bacterial cells [11]. The sensing element varies between microbial biosensors, and its selection relies on the specificity and the distinctiveness of the final sensor. The sensing element is basically a promoter which marks the point at which a gene or a set of genes transcription should start [11]. A reporter gene from one microorganism can be genetically merged to a promoter

sequence from a second microorganism and transferred into a third microorganism (a bacterial host) either in plasmid form or through integration into the bacterial chromosome [12].

In this review article, the attractive features of biosensors versus bioanalytical techniques have been discussed, followed by biosensors classification based on biorecognition elements and signal transduction. Thus, enzymatic biosensors, protein receptor based biosensors, immunosensors, DNA aptamer based biosensors and whole cell sensors have been addressed. Further, the most common medical applications of biosensors and the future prospective in biosensors construction and fabrication have been emphasized.

2. Biosensors and Bioanalytical Techniques

Biosensors are easily differentiated from bioanalytical techniques and bioassays. Bioassays usually involve multiple processing steps like the addition of an external reagent (i.e. enzymes or substrates). Although most of biosensors detect single analyte and not a mix of many analytes, the development of cutting edge technologies like array configurations has proved the ability of biosensors to detect multianalytes in a given sample. In general, the use of biosensors does not compete with bioanalytical techniques. Biosensors are mostly designed as the first filter of pre-screening of samples by governing authorities. Therefore, the main target is reducing the cost of monitoring programs [13].

Although cytotoxicity tests using animals and higher organisms provide a greater human relevance than biosensors, there are several limitations coupled to their use for toxicity and mutagenicity assessments. These limitations include time consumption as well as cost and space requirements [14]. Among the test organisms, microorganisms appear to have many advantages that make them more attractive option to study toxicity and mutagenicity than other bioassays. These advantages include their low cost, rapid growth rates, and their enormous population sizes. Furthermore, many

bacteria can be easily genetically modified, which makes the detection of their responses to any changes in their environmental conditions detectable by producing a certain signal [15].

3. Classification of Biosensors

Biosensors have been classified based upon several perspectives however, the most commonly used classification relies on two factors: the biorecognition element and the signal transduction. Table 2 illustrates different types of biosensors based upon these two perspectives.

3.1 Signal Transduction Perspective

Biosensors can be categorized based upon methods of signal transduction into four categories: electrochemical, thermal sensors, optical and mass sensitive sensors. Electrochemical biosensors are cost effective, portable, highly sensitive and compatible with modern microfabrication technologies [1]. The most widespread biosensors after the electrochemical-based systems are optical sensors. These are characterized by their high detection speed, sensitivity, robustness, and their ability to detect multiple analyte [16]. An example of an optical technique usually employed in biosensors construction is surface plasmon resonance (SPR) which able to determine the presence of a chemical with no need for labelled molecules [17]. Mass sensitive biosensors offer various advantages such as real-time operation and monitoring in liquid, vacuum and air environments [18]. Nevertheless, most of these biosensors are less frequently used because they comprise piezoelectric materials as the signal transducers and thus, the sensitivity tends to be lower than SPR [19,20]. Microcantilever biosensors are examples of mass sensitive biosensors. The main attractive features of microcantilever biosensors are their lower detection limits compared with classical methods, and their miniscule dimensions (less than 10^{-3} mm^2), which means a minute amount of both receptors and analyte is required for the assay [21]. Finally, thermometric biosensors have mainly been employed for monitoring of clinical and industrial processes [22].

3.2 Biorecognition Perspective

Based on the biological recognition element, biosensors have been classified into: enzymatic, protein receptor-based, immunosensors, DNA biosensors and whole-cell biosensors. The principle of each biosensor, with an example of its application have been addressed in the following sections.

3.2.1 Enzymatic Biosensors

They are based on the principle of the enzyme catalysed alteration of undetectable substrate into electrochemically measureable outcome or vice versa [23]. Many enzyme catalysed reactions comprise the release or utilization of a measureable outcome (i.e. O_2, CO_2, and ions), which can be measured using a suitable transducer coupled to an immobilized enzyme [24]. The key analytical enzymes that have been employed in these types of biosensors are oxidoreductases and hydrolases [25]. *gusA* gene, for instance, has been used as a marker gene to construct several enzymatic biosensors. The marker gene *gusA* obtained from *E. coli* encodes for enzyme β-glucuronidase which catalyses the hydrolysis of a wide range of β-glucuronides. The detection of microorganism marked by this gene is carried out by growing the bacteria on medium containing the chromogenic substrate X-gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide). Consequently, the colourless colonies will turn into blue colonies [26].

3.2.2 Protein Receptor-Based Biosensors

Protein receptor-based biosensors or non-catalytic proteins biosensors rely on the ability of cell membrane proteins to act as receptors. These receptors permit the transduction of the binding signal across the membrane either by metabotropic receptors (i.e. enzyme secretion) or by ionotropic receptors [121]. Hock *et al*., studied the attachment of human oestrogen receptors α to recombinant oestrogen receptors using an optical sensor BIA core 2000. *gfp* gene (the gene encodes

for green fluorescence protein) was used to chromosomally marked *Pseudomonas fluorescens* A506 using Tn5-based transposon delivery systems [27]. Furthermore, it has been demonstrated that the expression of a single *gfp* gene was enough to visualize different bacterial strains by confocal microscopy and epifluorescence, and the detection was based on flow cytometry [28].

3.2.3 Immunosensors

The construction of most immunosensors uses the concept of solid-phase immunoassays, where antigens and antibodies are immobilized on a solid support. Consequently, antigen-antibody interaction occurs at the solid-liquid interface. The main attractive features of immunosensors involve the substantial sensitivity and selectivity, in addition to the possibility of developing their affinity and selectivity by designing new recombinant antibodies [29].

3.2.4 DNA-Aptamers Biosensors

DNA-aptamers based biosensors have been developed as alternatives to antibodies due to their high stability, specificity and very low cost [30]. Aptamers are defined as miniscule single stranded DNA (ssDNA) or RNA sequences with approximately 100 nucleotides or less [31]. The unique intramolecular interactions between these nucleotides makes the aptamers fold into a distinctive 3D assembly. DNA-aptamers based biosensors can selectively bind with superior specificity and affinity to a specific bacteria, viruses, proteins, hormones, analyte and even small molecules and ions [32]. The types of bonds used in this binding are mainly hydrogen bonds and Van der Waals forces. Development of aptamers allowed fabrication of innovative biosensor devices with great stability and specificity, lower cost and much simpler detection strategies compared with immunosensors [33].

Shin *et al*., 2016 developed DNA aptamer-based electrochemical biosensor integrated with a microfluidic platform for monitoring a minute concentration (< 1 ng ml^{-1}) of creatine kinase (CK)-MB as a biomarker for human cardiac injury [109]. The sensitivity of the biosensor was compared with antibody-based immunosensors. Additionally, the overall performance of the biosensor was evaluated in combination with heart-on-a-chip microfluidic platform fabricated from human embryonic stem cell cardiomyocytes [109]. The developed biosensor is a promising tool with substantial applications in organ-on-a-chip platforms, and for detection and reporting of several cell secreted biomarkers at minuscule concentrations.

Alhadrami *et al*., 2017 constructed DNA aptamer fluorescence-based biosensor for detection of progesterone (P4) using truncated mechanism [33]. The aptasensor illustrated superior sensitivity for monitoring of progesterone with detection limit of 100 pg ml^{-1}. The truncated technique used to construct the aptasensor has significantly enhanced the affinity of the DNA aptamer and thus, the overall sensitivity of the fluorescence bioassay [33]. In a similar study, DNA aptamer based label free biosensors was constructed using a random library of 1015 single strand DNA sequences via the *in vitro* selection method for detection of progesterone [110]. Characterization of aptamer affinity to progesterone, sensitivity, and cross-reactive were all evaluated and reported [110]. The developed aptasensor showed high sensitivity for monitoring of progesterone with a detection limit of 0.90 ng ml^{-1}, which is promising for several applications in therapeutic biosensing and clinical samples.

3.2.5 Whole Cell Biosensors

Microbial biosensors, which based on luminescence reporter genes, have been extensively used for several medical applications. Genetically engineered whole cell microbial biosensor uses either prokaryotic or eukaryotic cells to report chemicals composition, toxicity, carcinogenicity and mutagenicity in real time and cost-effective manner [102]. Whole cell biosensors have been

constructed to not only report the presence or absence of chemical under investigation, but also to precisely measure the sublethal concentration which causes toxic or mutagenic effect [103]. Additionally, whole cell biosensors provide information with high specificity about the bioavailable and bioaccessible fraction of chemicals and/or drugs under-investigation. This information is important to determine the maximum amount of analyte concentration that can be absorbed by human gastrointestinal tract and reach to the blood circulation [102,104]. Hence, they provide more biologically relevant evidence than analytical techniques. Bioluminescence-based biosensors generally comprise the *luxCDABE* operon from the donor marine microorganism *Vibrio fischeri*, and the *luxAB* genes which encodes luciferase from *Vibrio harveyi* [34,35]. These genes are commonly employed as reporter genes in the construction of several biosensors [35]. Light emission in bioluminescence-based biosensors is generated by a series of reactions encoded by the *luxCDABE*. The principle of these reactions involves the production of the luciferase enzyme (coded by *luxAB* gene), which catalyses the oxidation of the luciferin substrate (coded by *luxCDE* gene) flavin mononucleotide ($FMNH_2$) and fatty acid aldehyde (RCHO) in the presence of O_2. Consequently, the aldehyde and the long-chain fatty acid are rejuvenated to FMN and RCOOH, leading to the emission of blue-green light which can be measured at a wavelength of 490 nm [16,35].

Using the *luxCDABE* reporter cassette does not require addition of substrate for the luciferase enzyme and thus, light emission can be measured *in vivo*. On the other hand, *luxAB* requires adding a fatty aldehyde, for instance *n*-decanal, as an enzyme substrate [3]. Bioluminescence-based biosensors are of three main types: non-specific toxicity biosensors (constitutive), stress-induced or semi-specific biosensors, and analyte specific biosensors (Figure 1 A & B).

4. Medical Applications of Biosensors:

4.1 Applications of Biosensors for Detection of Endocrine Disrupting Compounds (EDCs)

EDCs can simulate or antagonise the properties of oestrogens and androgens. The synthesis and metabolism of several hormones and hormone receptors can be disrupted by EDCs [8]. Thus, detection and monitoring of EDCs should be a priority for regulatory agencies. EDCs represent a considerable number of chemicals used in industrial and household products. Examples of these chemicals are polycyclic aromatic hydrocarbons (PAHs), dioxins and polychlorinated biphenyls (PCBs). The major exposure route to EDCs is direct contact with contaminated surfaces, which leads to several diseases related to human reproductive health such as birth defects, testicular, breast cancer, and deterioration in sperm numbers. Biosensors have been constructed and classified into two types to monitor EDCs. The first type are biosensors that evaluate the endocrine impacts, and the second are biosensors that detect and report the occurrence of a specific EDC or group of EDCs. Table 3 summarizes different examples of biosensors employed for monitoring of EDCs.

4.2 Applications of Biosensors for Detection of Genotoxicity

Human health can be negatively affected by genotoxic substances. Genotoxic chemicals are widespread which demands the construction of biosensors to screen large number of samples for harmful properties like genotoxicity and carcinogenicity [36,37]. Biosensors for genotoxicity assessment have shown great consistency with traditional bioassays, and are attractive due to their ability to detect the presence of multiple genotoxic compounds with only modest requirements for laboratory equipment and space [38]. Although there is a debate surrounding the predictability of genotoxicity and carcinogenicity by microbial biosensors, a strong correlation has been widely reported between the carcinogenic impact of compounds on microbial biosensors and their genotoxic and tumour-inducing characteristics in mammals [36-41]. Microbial biosensors are attractive for detection and screening of genotoxic and carcinogenic chemicals in pharmaceutical

and medical research due to their simplicity, specificity and sensitivity [36,38]. They have been used world-wide in research laboratories and by regulatory agencies for more than two decades.

Alhadrami & Paton have compared the sensitivity, specificity and the performance of the SOS-*lux* biosensors for detection and screening of mutagenicity against the Ames assay (the traditional *Salmonella* assay) [41]. They reported that, SOS-*lux* based microbial biosensors were reliable, sensitive and practically had many advantages over the Ames assay. SOS-*lux* biosensors were faster than any other bioassay for mutagenicity assessment, as the test results were ready within 1 to 2 h [41]. *In vivo* analysis is offered by the bioluminescence reporter system without causing any cell disruption, because the bioluminescence signal is reported from the living cells and the measurement can be repeated several times. Furthermore, the time kinetics of SOS cell induction is monitored and reported as the bioluminescence signal is constantly measured and monitored during the incubation period. SOS-*lux* biosensors allow the differentiation between the cytotoxic and genotoxic effect of the substance under investigation. Nevertheless, the direct measurements of carcinogenic effects in animals cannot be replaced by SOS *lux* biosensor. Instead, SOS *lux* biosensor can be used as a first line of screening for genotoxicity and carcinogenicity, and for predicting where and when animal tests should be implemented [38,41].

The SOS-*LUX*-TEST in the microplate format has been developed by Rettberg *et al*. [42]. *E. coli* K12 C600 was transformed by *E. coli* plasmid pPLS-1. An exposure to genotoxic agents, ionising or UV-radiation increases the level of luciferase and thus, increases the bioluminescence [42]. Consequently, the concentration of the genotoxic agent is proportional to the intensity of bioluminescence [43].

Ptitsyn *et al*., constructed a biosensor based on SOS DNA repair system for detection of genotoxins DNA-damaging agents. The construction of the biosensor was initiated by introducing pPLS-1

plasmid to *E. coli* C600 strain. The recombinant *E. coli* C600 conveys the plasmid with the promoterless *lux* operon (*luxCDABFE*) of *Photobacterium leiognathi* govern by a strong SOS promoter. This SOS *lux* biosensor was sensitive for detection of different genotoxins such as mitomycin C (MMC), hydrogen peroxide (H_2O_2), formaldehyde (CH_2O), UV and γ radiation, MNNG and dimethylsulfate (DMS). The sensitivity of the biosensors was comparable with other genotoxic assays such as the *umu* test and the SOS-chromotest [44].

Two commercially available SOS biosensors were developed and known as VITOTOX® test and GenoTox [105]. The VITOTOX® test uses *E. coli* and *S. typhimurium* strains that comprise a fusion of the *recN* promoter to *A. fischeri luxCDABE* gene cassette [106]. The VITOTOX® test has been employed for genotoxicity assessment of several drugs during the early phases of drugs development [106]. GenoTox uses the colicin D gene *cda* (a constituent of the ColD plasmid) and *gfp* as a report gene [107]. The signal green fluorescent was measured using flow cytometry to improve the overall sensitivity of the fluorescent biosensors. The sensitivity of GenoTox to detect genotoxicity was comparable to the *umu* test, the Ames assay and the SOS chromotest [107,105].

4.3 Applications of Biosensors for Detection of Pathogenic Organisms

Microorganisms, such as bacteria and viruses, can be potentially harmful for humans. They spread widely in food and the environment, and they gain access to human gastrointestinal tract via the consumption of contaminated food. Many of these microorganisms play a fundamental role in life however; some of them are pathogenic and have negative impacts on human health, because they can cause infections and several diseases [19]. Some of these microorganisms tolerate harsh environmental conditions and thus, they cause sever diseases with high fatality rate [45]. Enormously selective methodology is required for monitoring of human pathogenic microorganism

due to the presence of these pathogens among many other non-pathogenic microorganisms in a complex biological environment. Several conventional techniques (i.e. biochemical and serological screening, selective enrichment, morphological evaluation etc.) have been used for detection, monitoring and identification of pathogenic microorganisms. Most of these techniques are feasible, sensitive and offer quantitative and qualitative data on the number of the tested microorganisms [46]. Nevertheless, conventional methods are inappropriate to monitor, isolate and identify viable but non-culturable microorganisms which form the majority of human pathogenic microorganism. On the other hand, biosensors are attractive for monitoring and identification of a broad spectrum of pathogenic microorganisms and for clinical diagnostics due to their high sensitivity and specificity [19]. Building up biosensors for identification of pathogenic microorganisms is based on a biological recognition element (i.e. nucleic acids) connected with an appropriate transducer. Construction of biosensors using the stat-of-art of nanobiotechnology has several advantages over the conventional methods for detection of pathogenic microorganism. For instance, large number of clinical samples can be screened in a short period of time with a superior sensitivity and small sample volume [111]. Several biosensors have been constructed for detection of pathogenic microorganisms using optical, mechanical, chemical, electrochemical and nuclear magnetic resonance (NMR) methods [112-116]. Among these methods, optical biosensors (especially colorimetric) are more attractive as they are sensitive, portable, cost effective, rapid, provide high throughput screening for large number of clinical samples and can be integrated with microfluidics. The incorporation of optical biosensors with microfluidics allows the fabrication of lab-on-a-chip system which offers sample delivery, separation, analysis and detection all in one device with minimum reagents volume and lowest cost. Many optical biosensors for detection of pathogenic microorganisms are now commercially available, and have been recently reviewed by Yoo and Lee [111]. Biosensors based on

bioluminescent have been extensively employed for identification, detection and monitoring of several pathogenic microorganisms [47-49].

4.3.1 Reports Phage

Applications of bioluminescent biosensors for identification and detection of pathogenic microorganisms have been initiated by Ulitzur and Kuhn when they constructed the first luciferase reporter phage. The genes encoding luciferase has been infused into the genome of bacteriophage [50]. A bioluminescent phenotype can then be awarded to previously non-bioluminescent microorganism if that virus infects a host bacteria. Reporter phages are considered as a rapid tool for detection and identification of microbial host cells post phage attack. The number of detected cells using reporter phage system is as lower as 10 *E. coli* and enterobacterial cells and 100 *Salmonella typhimurium* cells [51]. L5 bacteriophage was constructed by Sarkis *et al*., to identify *Mycobacterium segmantis*. L5 was able to measure 100 cells of *Mycobacterium segmantis* in few hours post infection [52]. The same approach was used to monitor and detect different species of *Salmonella* and *Listeria* [53,54].

Listeria monocytogenes is the causative agent of human listeriosis due to ingestion of contaminated food, and is one of the most common opportunistic food-born pathogen. *Listeria monocytogenes* is detected conventionally by growing on selective media, followed by biochemical identification. Loessner *et al*. developed the reporter bacteriophage A511**::***luxAB* for identification of various strains of *Listeria*. It was reported that the recombinant phage A511**::***luxAB* was feasible, rapid, infective and simple tool for effective and sensitive detection of viable *Listeria* cells in contaminated food [51]. Reporter bacteriophage A511**::***luxAB* can be used in enormous clinical applications. For instance, it can measure low number of *Listeria* cells even in the existence of high number of other microorganisms. Further, it can be employed for the quantitative determination of viable *Listeria*

cells on the basis of RLUs reading or by using a microplate luminometer format. The detection limit of this assay is comparable to or better than the results obtained from polymerase chain reaction (PCR) methods or nucleic acid hybridization [51]. Despite the several advantages of the reporter bacteriophage A511::*luxAB*, this system has some limitations such as, the false-negative response especially with *Listeria monocytogenes*, which has been found to cause most of outbreaks listeriosis. Furthermore, species discrimination is necessary while using this technology as other *Listeria* species such as *L. welshimeri. L. innocua, L. seeligeri and L. ivanovii* can also be infected by A511 which allows luciferase expression [55].

Blasco *et al*., developed biosensor bacteriophage for identification and detection of *E. coli and Salmonella newport*. Once the bacteriophage infects the target strain, ATP bioluminescence is induced and measured as a signal for the release of cell contents. The sensitivity of this biosensor was enhanced by focusing on adenylate kinase as the cell marker instead of ATP. The detection limit of this biosensor was up to 10^3 cells/0.1 ml of sample volume, and the cell count was proportional to bioluminescence induction [56].

Biosensor bacteriophage has been extensively used due to its potential applications in monitoring and detection of opportunistic pathogens [47]. Several bacteriophage-based biosensors have been constructed and employed for detection, identification and monitoring of pathogenic microorganisms as well as toxic and carcinogenic chemicals [57-65]. As an attempt to overcome some limitations associated with the previously reporter biosensor bacteriophage-based, Birmele *et al*., developed bacteriophage-based bioluminescent sensor for identification and detection of *E. coli*. Their bacteriophage biosensor comprised *luxI* incorporated to lambda phage and a *lux*-based bioluminescent reporter cell which sensitively responsed to the infection [47]. Although the physiology of the target pathogen had an influence on the detection limit and signal response of the

bioreporter system, results of Birmele's study showed a potential application of this bioreporter system for remote sensing of pathogens [47].

4.3.2 Biosensors for Detection of *E. coli* O157:H7

E. coli O157:H7 is one of the most dangerous food borne pathogens. This strain secretes Shiga 1 and 2 (Stx1 and Stx2) toxins in the lining of human intestine which causes dangerous diseases such as bloody diarrhoea, hemorrhagic colitis and haemolytic uremic syndrome (one of the major cause of death especially in young children) [45]. An enormous number of school kids and elderly people died in Japan annually because of *E. coli* O157:H7 infection since 1996 [62]. One of the most virulent feature of *E. coli* O157:H7 is its ability to become viable but non-culturable microorganism. The natural reservoirs of *E. coli* O157:H7 are cattle and other domestic animals [64]. The most common mode of transmission of *E. coli* O157:H7 to human is consumption of contaminated meat, infection through direct contact and swimming in contaminated water [66]. Sensitive and rapid identification of *E. coli* O157:H7 is crucial for minimizing the outbreak of the infection and for hygienic supervision. The three most common methods available for identification of *E. coli* O157:H7 are immunoassay, PCR and enrichment culturing. These methods have several downsides: for instance, conventional culture methods require at least three days to be performed and it is non-applicable for *E. coli* O157:H7 once it enters the viable but non-culturable (VBNC) state. PCR for detection of *E. coli* O157:H7 is based upon targeting the genes encoding for Stx1 and Stx2 which is applicable for the human samples however, it is useless to identify *E. coli* O157:H7 from environmental samples due to the presence of these genes in non-pathogenic environmental microorganisms [67]. A virulent phage (PP01) was able to infect *E. coli* O157:H7 with a substantial specificity [68], which made it attractive for sensitive and effective detection of *E. coli* O157:H7 [62]. The detection assay relied on the expression of green fluorescence protein (GFP) in the host cell by incorporation of PP01 phage

genome with genes encoding the marker protein (GFP). GFP-labelled recombinant PP01 phage was employed for the identification of *E. coli* O157:H7 in both culturable and viable but non-culturable state [62]. Furthermore, optical biosensor was constructed using Surface Plasmon Resonance (SPR) for detection of *E. coli* O157:H7 [117]. T4 and BP14 bacteriophages were used in this biosensor as capturing elements, and the biosensor was sensitive enough to detect 10^3 CFU/ml in only 20 min [118]. In another study, electrochemical biosensor was constructed to recognize carbohydrate α-mannoside for detection of *E. coli* ORN178 which is a substitute strain for *E. coli* O157:H7 [119]. The detection limit of the biosensor was as few as 10^2 CFU/ml. Recently, Zhang *et al.,* 2017 developed multichannel SPR based biosensor for detection of *E. coli* O157:H7 simultaneously with other two vital foodborne pathogens: *Listeria monocytogenes* and *Salmonella enteritidis*. The biosensor was able to detect 14, 28 and 6 CFU/ml of *E. coli* O157:H7, *L. monocytogenes* and *S. enteritidis* respectively [120].

4.3.3 Biosensors for Detection of Biofilms

Development in biosensors technology has facilitated investigation on species metabolic cross-talk, and has been successfully used to investigate the *in vivo* manifestation of virulent factors [69]. Additionally, the recent advances in biosensors technology have enormously enhance our understanding of biofilm development and formation. For instance, *gfp* marker gene was employed as a reporter of *gtfB* expression which encodes glucosyltransferase of *Streptococcus mutans* during biofilm formation [70]. In another study, the physiological status of *Pseudomonas putida* R1 was monitored throughout the formation of biofilm by insertion of a fusion between rRNA promoter *rrnBP1* and *gfp* [71]. Rice *et al*., reported that it was possible to track individual GFP-tagged cells

during the formation of biofilms [72]. Table 4 summarizes some of the medical applications for different types of biosensors.

5. Future Prospective

It is clearly illustrated that biosensors play a vital role in medical and biomedical applications compared to conventional techniques. Biosensors work as a first detection filter for mutagenicity, carcinogenicity and toxicity of drugs and chemicals of concerns. Biosensors have several attractive features such as:

- They offer powerful techniques to critically determine, measure and evaluate the bioavailable and bioaccessible fractions of a mutagenic and/or carcinogenic compound that might reach human blood circulation and cause diseases.
- They offer cost effective techniques which provide a simple way of identifying mutagenic, carcinogenic and toxic elements.
- The stability of assay conditions due to the use of intact living microorganisms provides information on the toxicology and mutagenicity of different compounds/drugs.
- Biosensors comprise a substantial potential for equipment miniaturization because of their minute size.
- They are able to detect groups of mutagenic and/or toxic elements rather than single element. This is a remarkable feature when particular compounds of a group have the same toxic and/or mutagenic characteristics.

- Biosensors can be integrated in microfluidics to establish a lab-on-a-chip platform which has substantial medical and biomedical applications.

Although biosensors play an essential role in medical and biomedical applications, future improvements and further investment in biosensors engineering should be considered in order to avoid the limitations of this technology. Recommendations that can be considered in future studies to improve the role of biosensors in medical applications are: construction of biosensors suitable to work in anaerobic environments, insertion of reporter genes into viable but non-culturable microorganisms (VBNC), development of intrinsic production of luciferin for eukaryotic luciferase reporters, development of protein engineering of existing biosensors to enhance expression level, reduction of the cost of signal detection and optimization of the biosensor's performance in terms of sensitivity, detection limit, and rapidity of reporting mutagenic and toxic chemicals/drugs.

One of the primary concerns in the selection of a mutagenicity testing protocol is that the test should reflect potential mutagenic effect in humans. In order to accomplish this goal, the use of mammalian cells has been developed recently, since they offer a more realistic simulation of human response. Further research is required to investigate and construct biosensors to assess not only the occurrence but also the effect of endocrine-disrupting compounds (EDCs).

Table 1. **Examples of reporter genes used in the construction of some microbial biosensors.**

Target receptor	Reporter gene	Host organism	Induced by	Donor organism	Construction	Reference
*cop*A promoter/CueR	*lux* gene cluster.	*E. coli* W3110.	Copper & sliver.	*Vibrio fischeri*.	pUA615.	[73]
*mer*B3 with *mer*B_3O/P.	*lux*AB.	*E. coli* DH5α	Inorganic mercurials, organomercurials Inorganic mercurials.	*Bacillus megaterium* starin MB1, *Vibrio harveyi.*	pHYB3*lux* pHYΔB3*lux*	[74]
*znt*R, *znt*A promoter	*luc*	*E. coli* MC1061	Zn^{2+}, Cd^{2+}, Hg^{2+}	*Photinus pyralis*	pzntRluc	[75]
*mer*R, *mer* operon operator/promoter	*luc*	*E. coli* MC1061	Hg^{2+}, Zn^{2+}, Cd^{2+}	*Photinus pyralis*	pmerBRBSluc	
*chr*B, *chr*A promoter.	*luc*	*Ralstonia eutropha* AE104.	Cr^{3+}.	*Photinus pyralis*	pchrBluc	
P *mer mer*R (from Tn21) promoter.	*luc*FF	*E. coli* MC1061	Mercury & cadmium.	*P. pyralis*	pTOO11 (7.8 kb).	[76]
*cnr*YXH	*lux*CEABE	*Ralstonia eutropha* CH34	Co^{2+}, Ni^{2+}	*R. eutropha* CH34	pMOL1550	[77]
ZntR ZntA	*lux*	*E. coli* MG1655	Zn^{2+}, Cd^{2+}, Hg^{2+} Pb.	*V. fischeri*	pZNT *lux*, pCOP *lux*	[78]

cup 1 promter	gfp_{uv}	*Saccharomyces cerevisiae* DY150	Copper ions	*Aequorea victoria*	pYEX-GFPuv	[79]
Promoter *Pars* *ars*R(from *S.aureus* pI258)	*luc*FF	*S. aureus* RN4220	Arsenite & antimonite	*P. pyralis*	pTOO21 artificial plasmid cloned from pCSS810	[80]

Table 2. Biosensors classification according to signal transduction and biorecognition perspectives

Recognizing Biomolecule	Example	Signal Transduction	Example
Antibodies (Immunosensors)	*Monoclonal* *Polyclonal*	Electrochemical	*Amperometric* *Conductimetric* *Impedimetric* *Potentiometric*
Protein Receptors	*Metabotropic Receptors* *Ionotropic Receptors*	Optical	*Absorbance* *Fluorescence* *Phosphorescence* *Bio/Chemiluminescence* *Reflectance* *Raman Scattering* *Refractive Index*
Enzymes	*β-glucuronidase (encoded by gusA marker gene).* *β-galactosidase (encoded by lacZ marker gene).*	Mass Sensitive	*Surface Acoustic Wave Biosensor* *Cantilever Biosensors*
Whole Cells	*Microbial Sensors* *Mammalian Cells Tissue*	Optical	*E. coli* K12C600 *E. coli* HB101 *E. coli* DPD1718
Nucleic Acids	*Hybridization* *DNA-aptamers based biosensors*	Optical (*Fluorescence)*	Fluorescence-based aptasensor

Table 3. **Biosensors for detection of EDCs.**

Transducing element	Biorecognition element	Analyte	Reference
Electrochemical	Immunoglobulins	Atrazine	[13]
Electrochemical	Immunoglobulins	Surfactants	[81]
Electrochemical	Immunoglobulins	Estradiol	[82]
Electrochemical	Enzyme (AChE)	Paraoxon and pesticides	[83]
Electrochemical	Enzyme	Phenols	[13]
Electrochemical	Photosystem II	Diuron	[84]
Electrochemical (amperometric)	*Pseudomonas* and *Achromobacter*	Surfactants	[85,86]
Electrochemical	*Trichosporon cutaneum*	LAS	[87]
Electrochemical	DNA	Daunomicyn	[88]
Optical	Immunoglobulins	Pesticides	[89,90]
Fibre optic	Enzyme	Chlorophenols	[91]
Fluorescence	*Chlorella vulgaris*	Isoproturon & simazine	[92]

Table 4. Medical Applications of Biosensors.

Reporter Gene	Host strain	Application	Reference
luxCDABE	*E. coli* K12C600	Detection of mutagenic chemicals such as mitomycin-C (MMC), benzo (a) anthracene (B[a]A), chrysene and benzo (a) pyrene (B[a]P).	[38]
luxI	*Bacteriophage PP01-luxI*	Detection of *E. coli* O157: H7.	[93]
gfp	*Pseudomonas putida KT2440*	Detection the microbial antifungal agent phenylacetic acid.	[94]
gfp	*Streptococcus mutans*	Monitoring of biofilm formation.	[72]
luxCDABE	*Acinetobacter DF4/PUTK2*	Used as a real-time indicator of the bacteriostatic actions of the antimicrobial agents, and as a rapid screening tool for active antimicrobial agents.	[95]
mer-lux	*E. coli HMS174*	Mercury accumulation in *E. coli* HMS174, and the effect of pH on the toxicity of mercury.	[96]
luxCDABE	*Acinetobacter DF4/PUTK2*	Toxicity assessment of phenols.	[97]
lux	*Acinetobacter DF4/PUTK2*	Monitoring the toxicity of heavy metals.	[98]
gfp	*Pseudomonas fluorescens*	Detection of benzene derivatives toxic compounds.	[99]
luxCDABE	*E. coli* DPD1718	Detection of mutagenic chemicals such as sodium azide, MMC, acridine, B[a]A, chrysene and B[a]P	[41]
gfp	----------	Fluorescence-based aptasensor for the detection of P4 (Progesterone).	[33]
gfp	*Pseudomonas syringae & E. coli*	Measure water availability to bacterial cells.	[100]
GFPuv gene	*E. coli*	Monitoring the cellular growth phase and the elevated concentrations of ethanol and NaCl.	[101]

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Figure 1. General principle and construction of whole cell microbial biosensors. (A) construction and genetic engineering of analyte specific biosensor for detection of mutagenic chemicals/drugs. (B) “light on” and“light off” examples of non-specific (constitutively marked) whole cell microbial biosensor for toxicityassessment.