Sensors and Actuators B 197 (2014) 385–404

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Review

Biosensors for pathogen detection: A smart approach towards clinical diagnosis

Renu Singh[a,b,f], Maumita Das Mukherjee[d], Gajjala Sumana[a], Rajinder K. Gupta[b], Seema Sood[e], B.D. Malhotra[c,*]

[a] Department of Science & Technology Centre on Biomolecular Electronics, Biomedical Instrumentation Section, National Physical Laboratory (Council of Scientific & Industrial Research), Dr. K.S. Krishnan Marg, New Delhi 110012, India
[b] School of Biotechnology, Guru Gobind Singh Indraprastha University, Sector -16 C, Dwarka, Delhi 110075, India
[c] Department of Biotechnology, Delhi Technological University, Shahbad Daulatpur, Main Bawana Road, Delhi 110042, India
[d] Amity Institute of Applied Sciences, Amity University, Sector 125, Noida, UP, India
[e] Department of Microbiology, All India Institute of Medical Sciences, New Delhi 110002, India
[f] Sensors & Aerosols Laboratory, School of Mechanical and Nuclear Engineering, Ulsan National Institute of Science and Technology, UNIST-gil 50, Eonyang-eup, Ulju-gun, Ulsan 689-798, South Korea

ARTICLE INFO

Article history:
Received 17 July 2013
Received in revised form 10 February 2014
Accepted 2 March 2014
Available online 12 March 2014

Keywords:
Nucleic acid sensor
Human pathogen
Diseases
Bacteria
Virus
Electrochemical

ABSTRACT

The present review concerns the recent developments of nucleic acid biosensors for detection of the human pathogens as infectious diseases management at an early stage is currently of prime interest so as to circumvent the delay in diagnosis, side effects of drugs and unnecessary health hazards. The advantages of biosensors over existing detection methodologies and the role of various immobilization matrices used for fabrication of nucleic acid sensors are discussed. Besides this, efforts have been made to discuss the various techniques used for biosensor construction, the analytical performance of these biodevices for the bacterial and viral pathogens for their applications to medical diagnosis.

Contents

* Corresponding author. Tel.: +91 11 27294668; fax: +91 11 27871023.
E-mail addresses: bansi.malhotra@gmail.com, bansi.malhotra@dce.ac.in (B.D. Malhotra).

http://dx.doi.org/10.1016/j.snb.2014.03.005
0925-4005/

1. Introduction

Recent years have seen continued growth of the interdisciplinary field of biosensors due to an increased interest between basic and applied sciences researchers for development of novel electronic devices that can be utilized for a variety of applications including human health care and environmental monitoring [1]. A biosensor is an integrated miniaturized analytical device that integrates biological sensing elements like enzymes [2], antibodies [3], nucleic acids [4], cells etc. with transducer equipped with an electronic amplifier [5]. Biosensors offer interesting features such as real-time, on-site, simultaneous detection of multiple pathogenic agents utilizing selectivity of the biomolecules and the processing power of modern nano-electronics [6,7]. They are known to provide fast and accurate results arising due to interaction of an analyte with a given biomolecule and have been found to have applications in various fields including clinical diagnostics [8], environmental monitoring [9], bioprocess monitoring [10], food, water [11] and agricultural product processing [12], etc. According to IUPAC, a biosensor is a self-contained integral device which is capable of providing specific quantitative or semi-quantitative analytical information using a biological element.

There is an increased interest towards the development of nucleic acid biosensors for clinical monitoring [13]. Voltammetry of nucleic acids after the discovery of anodic peak specific for guanine is an important milestone for monitoring of double-stranded and denatured single-stranded DNA. Monitoring change in the DNA redox properties (oxidation of guanine) provides a unique opportunity to detect the hybridization process [14]. Though, the electrochemical detection using guanine oxidation is known to cause irreversible damage to DNA; however, redox indicator-based indirect electrochemistry has been shown to be an important alternative for development of nucleic acid hybridization biosensors. The electrochemical DNA biosensors with redox indicators like methylene blue (MB) [15,16], daunomycin [17], metal complexes such as ruthenium (Ru) complex [18], cobalt complex [19] etc. have been found to be advantageous due to re-usability of the electrode. Besides this, electrochemical biosensors do not require additional labelling step and can be easily integrated with electronics for fabrication of miniaturized devices for diagnosis of infectious diseases and the detection of pathogenic biological species of environmental and clinical interest. Thus, DNA hybridization biosensor technology is at the vanguard of clinical diagnosis which commonly relies on measuring techniques like electrochemical [20], optical [21] and mass-sensitive [22] to recognize the complementary target DNA strand.

For the fabrication of a high performance biosensor, the role of an immobilization matrix is very crucial. Among the various matrices, nanomaterials are being explored due to their exceptional optical and electrical properties owing to electron and phonon confinement, high surface-to-volume ratio, modified surface work function, high surface reaction activity, high catalytic efficiency and strong adsorption ability. In particular, nanomaterials with engineered morphology, size, functionality, adsorption capability and high biomolecule loading capacity have been found to provide enhanced electron transport between a biomolecule and the electrode. The microenvironment provided by a nanomaterial may help a biomolecule to retain its conformation with maximum biological activity, resulting in enhanced signal transduction and biosensor stability. These unique properties of nanomaterials can perhaps be utilized for interfacing biological recognition events with electronic signal transduction and for designing a new generation of bioelectronics devices that may exhibit novel functions.

Infectious diseases, especially those resulting from life-threatening pathogens, have significantly increased over the past few decades, and deaths may occur primarily because of delay in diagnosis, side effects of drugs and commodities necessary for rapid detection, prevention or cure [23]. Pathogens are microbes that are capable of causing illness, remain the world's greatest killer of children and young adults, accounting approximately 13 million deaths a year. Pathogen detection is currently of utmost importance, due to health and safety concern [24–35]. The most common pathogens are bacteria, viruses, fungi and parasites (SI). The increased viral outbreaks e.g., H1N1 flu, H5N1 flu, and SARS etc., recently boost the alarm and raised significant worries as the viruses could rapidly spread and turn into a pandemic. In spite of developments in the diagnostic fields for pathogen detection, there is an urgent need for availability of rapid, portable and accurate diagnostic technique that can be used to control the epidemics and may perhaps have a significant impact on clinical management. This manuscript deals with the recent development of electrochemical, optical biosensing techniques based on nucleic acids for pathogens detection that have implications towards the clinical diagnosis of bacterial and viral infections.

2. Conventional techniques for detection of pathogens

The traditional methods for pathogen detection include microscopy, culture and serology. Microscopy is simple, easy to use and a versatile technique. Culture is the gold standard for diagnosis of many microorganisms, e.g., *Mycobacterium tuberculosis* and serology forms the mainstay of diagnosis in some of the diseases, e.g., syphilis. The traditional methods of detection are inexpensive but protracted methods. Microscopy has limited sensitivity in many settings and the interpretation may be subjective. On the other hand, culture runs the risk of contamination with commensal flora and the possibility of reduced viability during transportation. In addition, choice of culture media and interpretation of culture results requires technical skill. The main disadvantage of serology is the requirement of convalescent sera and the occurrence of false-positive results due to cross-reaction with other organisms. The last two decades have seen a switch to molecular methods that offer a growth independent strategy. It is particularly sited for unculturable organisms, e.g., *Treponema pallidum*; difficult to grow organisms, e.g., HIV, HBV, *Bordetella pertussis*; slow growing organism, e.g., *Mycobacterium tuberculosis*; and dangerous to culture organisms, e.g., *Coxiella burnetii*. Molecular methods include the non-amplification methods, e.g., hybridization using probes as well as amplification based methods. The nucleic acid amplification techniques (NAATs) have taken an irreversible position in diagnostics. These include polymerase chain reaction-PCR, transcription based amplification (target amplification), ligase chain reaction-LCR (probe amplification), branch DNA technology (signal amplification) and loop-mediated isothermal amplification-LAMP (isothermal amplification). These methods are known to be sensitive and yield both qualitative and quantitative information of the tested microorganisms. However, these techniques are expensive and require long assay time (Fig. 1).

Among nucleic acid amplification tests, PCR which is most commonly used, can detect a single copy of a target DNA sequence, and thus, can be used to detect a single pathogenic bacterium. It is promising because it detects the organism by amplifying the target rather than the signal, and is therefore less prone to producing false-positives. Thus, PCR detection of pathogens has distinct advantages over culture and other standard as it offers the advantages of specificity, sensitivity, rapidity, accuracy and capacity to detect small amounts of target nucleic acid in a sample [36–41].