Multiplexed Biosensors for Efficient Diagnosis of the Clinical Conditions toward Health Management

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Abstract

Multiplexed biosensors for detecting and diagnosing several clinical conditions have been advancing rapidly due to the continual research progress. Biosensors are analytical devices that convert or transduce a biological response into a quantifiable signal. Multiplexed biosensors expedite the detection of multiple conditions resulting in a more agile and better disease diagnosis, monitoring, and management. Simultaneous and synchronous detection using multiplexed biosensors provides information beyond what a single sensor or device could render. This chapter describes the potential opportunities and challenges in multiplexed biosensors for the efficient diagnosis of clinical conditions and health management. Some of the major applications covered in this chapter include glucose, drug, and infectious disease monitoring using multiplexed biosensors, along with the most recent advancements and materials considerations in the field of multiplexed biosensors.

Keywords

Multiplexed biosensors · Healthcare · Biosensing materials

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9.1 Introduction

Biosensors and biosensing play a vital role in healthcare diagnostics and monitoring in recent years due to advancements in technology and multidisciplinary research approach. Due to nanotechnology and microtechnology advancements, healthcare monitoring and diagnostic systems have become very compact and portable. On-the-go diagnostic systems are the new methods adopted worldwide. The wearable electronic system can continuously monitor anyone's health when a person is performing his/her day-to-day activity without the need to go to a hospital for routine health inspection. A multiplexed biosensor is the next step in the advanced diagnostic and monitoring technique adopted in healthcare research to give precise information of diagnostic value. Multiple sensors are used to get vital health information on various detections from different bio-analytes, as described in Fig. 9.1. The information from the multiple sensors is multiplexed to get the healthcare information for monitoring and diagnosis.

The biosensor classification is based on paper, array, beads, and microfluidics, as shown in Fig. 9.1. Each of the types has different detection techniques, as listed in Table 9.1. As depicted in Table 9.1 (Vadgama and Crump 1992; Dincer et al. 2017), each biosensor type has different detection schemes such as calorimetric, optical, electrochemical, and amperometry. Some of the systems are commercially available for use in the market, whereas a few systems are under research and development.

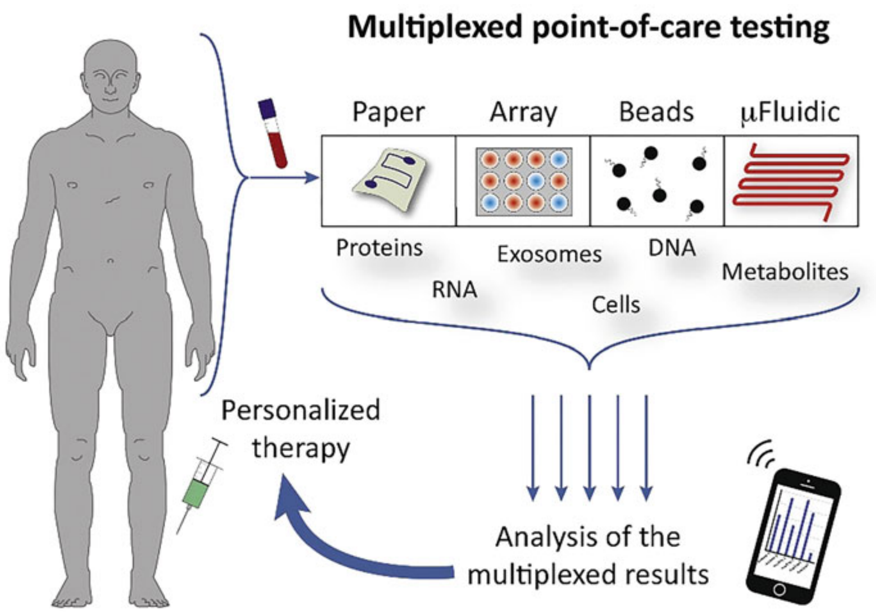

Fig. 9.1 Illustration of the multiplexed biosensor in healthcare

Table 9.1 Biosensor systems classification

Biosensor systems	Detection schemes	
Paper-based systems	Calorimetric detection	Optical detection
Array-based systems	Optical detection	Electrochemical detection
Bead-based systems	Flow calorimetry detection	Real-time PCR detection
Microfluidic-based systems	Calorimetric detection	Amperometry detection

Multiplexed biosensors can be made of more than one of these types of biosensor systems combined for healthcare applications.

The complete system here involves biosensors, bio-analytes, nanomaterials, and bioelectronics. The present chapter gives a detailed insight into the multiplexed biosensor systems and their applications in healthcare. Biosensors get the biological information and convert it into electrical signals to support the bioelectronic system to give healthcare information. The transduction mechanism in the biosensor is the chemical transduction method and physical transduction method. Most of the biological information will be collected from the bio-analytes such as an antibody, DNA, receptor, enzyme, tissue, cell, and organelle. The biosensor transduction system's physiochemical response will be ions, electrons, holes, light, mass, and heat, which will be amplified and communicated to the external system (Vadgama and Crump 1992; Teymourian et al. 2021). Biosensors can be applied on both invasive and non-invasive applications depending on the need of the end-user. As the in vivo applications suggest, these types of biosensors are used on long-term applications such as artificial organs and long-term monitoring of a person's clinical conditions. When the same is applied with multiple sensors in a system for monitoring a person's clinical condition, then the multiplexed biosensors are used. Multiplexed biosensors simultaneously give healthcare information from multiple bio analytes, giving precise information on a person's health condition (Mohanty and Kougianos 2006).

9.2 Multiplexed Biosensors and System Considerations

Multiplexed biosensors are of different types based on the structure and applications such as multiplexed nano-biosensor, soft and fibrous multiplexed biosensor, wearable multiplexed biosensor, multiplexed nano-plasmonic biosensor, multiplexed Point of Case Testing Device (xPOCT), multiplexed label-free biosensor, and Field Effect Transistor (FET)-based multiplexed biosensor. Multiplexed Nano Biosensors use low-dimensional nanostructures such as quantum dots with better photoluminescence property based on the size and the nanoparticle placement on the quantum dots such as Au, Ag, and rare earth materials (Purohit et al. 2019). All these particles have different adhesion with the bio analyte, where it can be used to detect different analytes at the same time. Based on the accurate assessment of the expression of the biomarkers, these type of biosensors offer the flexibility in identifying the disease at the early stage. These types of multiplexed nano biosensors have a single sample that can deliver multiple results simultaneously (Purohit et al. 2019; Mahato et al. 2021). Flexible and helical bundles of functionalized carbon nanotube (CNT)-based biosensors are used as electrochemical sensors for long-term invasive monitoring of disease biomarkers in the human biological systems (Feiner and Dvir 2020). Wearable multiplexed biosensor systems are used to give the in-vitro biomarker information to get insights into the human system's health conditions on a day-to-day basis. These wearable multiplexed sensors work as a biomarker for simultaneous glucose, pH, temperature, and lactate measurement. Since the multiplexed biosensor is made with a flexible substrate, it can be affixed to any part of the human body for continuous monitoring (Yokus et al. 2020).

Nanoplasmonics-based multiplexed biosensors are used to identify more than one bacterial infection related to sexually transmitted diseases (Chlamydia trachomatis and Neisseria gonorrhoeae) from a single bio analyte such as urine. Plasmonic microarray-based gold nanohole sensors are used to identify Chlamydia trachomatis and Neisseria gonorrhoeae (Soler et al. 2017). Point-of-care diagnostics is another critical area in healthcare monitoring. Multiplexed point-of-care devices are becoming increasingly popular in clinical diagnostics to identify the presence or absence of any disease without any need for time to analyze the same. Such devices have multiple detection schemes at the same time. Various multiplexing technologies in the biosensor systems such as paper, bead, array, and microfluidics are used to detect various clinical conditions at the same time by using a single analyte or multiple analytes (Dincer et al. 2017; Park et al. 2016). Label-free biosensors are made of physical-type detections such as mechanical defection due to the antigen–antibody binding on the sensory elements such as nano/microcantilever beams. When these structures are made as matrix structures, they can detect multiple biomarkers simultaneously (Luo and Davis 2013). Hydrogel-based gate materials are used in the FET, where bio-specific receptors are placed to make the bioanalytes settle over the receptor to work as biomarkers (Bay et al. 2019). Similar to the discussed types of multiplexed biosensors, there are yet more multiplexed biosensor types present, based on their applications, such as drug screening, protein profiling, immunology, genomics, proteomics, and metabolomics (Zhao et al. 2013). Figure 9.2 gives a brief insight on the different body fluids used as analytes in biosensors to study the heal condition. Each fluid has unique biomarker nature such as metabolites, proteins, antibodies, lactate, and ions. The same can be applied to multiplexed biosensors for studying health conditions. For example, a multiplexed biosensor can understand the metabolite of tears, saliva, and sweat to get precise health conditions.

Electrochemical multiplexed microfluidic biosensor with CRISPR-powered approach was used to amplify the micro-RNA biomarkers used as a point-of-care diagnostic for identifying the disease in patients. A multiplexed version of the electrochemical microfluidic biosensor was made by making four novel chips for simultaneous quantification of a maximum of eight micro RNAs from pediatric medulloblastoma blood patients for the detection of multiple nucleic acids parallelly (Bruch et al. 2021). Electrochemical-based multiplexed biosensors are used as point-of-care cardiovascular disease biomarkers for C-reactive protein (CRP), troponin I (cTnI), and procalcitonin (PCT). The fabricated point-of-care device is made by a

Fig. 9.2 Biofluids and detection scheme for multiplexed biofluidic sensors

wax printing technique, with multiple detection zones and working electrodes, simultaneously detecting the three cardiovascular biomarkers using a single sample with better sensitivity (Boonkaew et al. 2021). Like cardiovascular disease identification and detection, some diseases are more dangerous, killing the human population. HIV infection and its related diseases are becoming very common in most countries. Array-based sensing is being used for detecting bio analytes recently. A similar approach is adopted for a multiplexed biosensor made of array-based serology assay for detecting HIV and related infections with the waveguide illumination approach (Myatt et al. 2009).

Study of the metabolic change and related health issues is essential in recent years due to the unestimated changes in the chemicals with different age groups due to the lifestyle. Continuous measurement of glucose, lactate, pH, and temperature using a wearable multiplexed biosensor system is much needed to understand the changes in a person's metabolites. In vitro measurement of glucose, lactate, pH, and skin temperature from the sweat gives the metabolites changes (Yokus et al. 2020). Multiplexed glycan microarray biosensor fabricated with glycan probes with 2.6 and 2.3 linkages is used for influenza virus detection based on capturing lectins (carbohydrate-binding proteins) (Zhang et al. 2021). A sepsis biomarker is fabricated with the graphene-based detection from whole blood with the surface chemistry approach. Three sensors were used in multiplexed approach by modifying the approach in a single chip with different capturing probes (Zupančič et al. 2021). As an approach to have multiple detections on the same analyte, different materials have been used in the biosensors. Recent research on the materials has made various materials used for sensing applications with excellent mechanical, electrical, optical, magnetic, and thermal properties. Synthesis methods have made low dimensional structures such as 0-dimension, 1-dimension, and 2-dimension structures with better sensing properties.

9.3 Materials for Multiplexed Biosensors

Different types of materials such as carbon-based, metal-organic, metal oxides, polymer, and composites are used in various sensing applications. Materials used for biosensing are based on the sensing capability and the selectivity of the application. Table 9.2 describes the various biosensing principles and their respective materials. The same materials or different materials can be used in multiplexed biosensing applications based on the device's functional requirement.

The materials used on a particular sensing modality can be used for sensing multiple analytes simultaneously. This can be achieved based on the different nanoparticle distribution over the surface for better adhesion to keep the analyte-binding property better. For instance, any low-dimensional structure can have different metal nanoparticles deposited over it in groups to have sensed on different analytes simultaneously. Hence the materials used in various classes of biosensors can be used in multiplexed biosensors. Materials used in multiplexed biosensors based on their application prospects are discussed in detail in the following section.

Omni dispersible Hedgehog Particles algometry nature has improved surface-enhanced Raman scattering effect with higher intensity than smooth surface colloids. This nature helps the simultaneous detection of multiple targets in complex medium with higher ionic strength. These omni dispersible active colloids help in the multiplexed biosensing capability of biological fluids. Based on multiple sensing, single or multiple biofluids help in precise healthcare monitoring (Montjoy et al. 2018). Quantum dots are the most promising low-dimensional structures beneficial for biosensing application due to the antibody conjugates available for better labeling. Novel bio-functional quantum dots bioconjugate are used to detect the multiple bio-analyte simultaneously and are very useful for multiplexed biosensing applications (Hildebrandt 2011). Nanostructured conductive hydrogel-based multiplexed sensing is another simple and effective approach where the paper substrate can be used as point-of-care diagnostics. Fabrication of the hydrogel-based biosensing is also straightforward, where an inkjet printer is used for depositing the material. A fabricated sensor array and the working electrode in a single page can be used for multiplexed assays to detect glucose, lactate, and triglycerides with outstanding sensitivity. Fabricating a multiplexed sensor device was done with the three rounds of printing on a paper substrate (Valente et al. 2018).

Table 9.2 Biosensor materials

Sensing principle	Materials
Electrochemical biosensing	Magnetic nanoparticles
	Quantum dots
	Polypyrrole nanotubes
	Metal-organic frameworks
	DNA nanomaterials
	Metal nanoparticles
Photoelectrochemical biosensing	TiO_2
	Nano silk
	Quantum dots
Colorimetric biosensing	Metal oxides
	Gold nanoparticles
	Carbon nanomaterials
Fluorescence biosensing	Quantum dots
	Gold nanoparticles
	2D carbon nanomaterials
	Metal oxides
	Zirconium phosphate
Chemiluminescence biosensing	Superparamagnetic iron oxide particles
	CuS nanoparticles
Electrochemiluminescence biosensing	Quantum dots
	Carbon nanomaterials
Surface plasmon resonance biosensing	Titanium nitride nanomaterials
	Gold nanoparticles
	Magnetic nanoparticles
Surface-enhanced Raman scattering biosensing	Metal nanomaterials

ZnO-based sensors have been in research over some time on various bio analyte detection and are used as biomarkers due to the ability of the material's physical and chemical properties. ZnO-based nanoparticles and nanostructures (Nanorods, Nanospheres, Nanoflowers, etc.) were fabricated using different techniques. Due to the better electrical, mechanical, optical, and physical properties, ZnO has been an excellent material for biosensing applications. Nanostructured ZnO can also be used as a multiplexed biosensor to detect cardiac biomarkers cardiac troponin-I, cardiac troponin-T, and B-type natriuretic peptide. The fabricated sensor's response time was recorded better, which can be very useful for quantifying the multiple biomarkers. Sensor response time was also recorded to be very good with a better signal-to-noise ratio and improved signal response using electrochemical detection. High specificity and selectivity were also recorded with the target biomarkers due to the nanostructured ZnO (Shanmugam et al. 2018). Multiplexed immunoassay sensor with modified ZnO nanorods using reduced graphene oxide paper electrode and silver deposition was fabricated with amplification strategy for chorionic gonadotropin to detect prostate-specific antigen and carcinoembryonic antigen (Sun et al. 2015).

Graphene-based biosensors are widely used due to their better physical and chemical properties when used independently or doped with other materials. It is a complex, single-layer, two-dimensional network of carbon atoms having excellent electrical sensing capability. Like hydrogel ink, graphene nano inks are used to fabricate the multiplexed nano array–based biosensors for metabolite detection. These multiplexed biosensors were fabricated with graphene ink on a microfluidic paper to detect the metabolites such as glucose, lactate, xanthine, and cholesterol with better sensitivity. The fabricated biosensor's significant properties include very low measuring time with a broad detection range at a very low sample rate (Labroo and Cui 2014). Similarly, Alzheimer's detection can also be carried out with the graphene oxide material with different bio analytes. A similar approach can be adopted with different bio analyte detection simultaneously with a multiplexed biosensing approach (Wang et al. 2018; Zhou et al. 2018; Jin et al. 2018).

9.4 Bioelectronics for Biosensor Systems

When sensing the bio-analytes, biosensors will have the information about the intermolecular electron transfer found in the biological system in the form of voltage or current with distortion at the output, providing a synergy between the electronics and biology (Szent-Györgyi 1968; Turner 2005). In some cases, the intermolecular interaction may be in some other forms, such as heat and mass. Bioelectronic circuits are used in healthcare systems to perceive and analyze the sensed data. Bioelectronic systems are classified based on the function of micro/nano bioelectronics, plastic bioelectronics, hydrogel bioelectronics, organic bioelectronics, and molecular bioelectronics. Micro and nano bioelectronics use micro and nanostructures such as silicon nanowires, carbon nanotubes, and graphene in conjunction with biology and electronics toward the miniaturized transducers nanoscale with better sensitivity and biocompatibility for healthcare monitoring applications. Table 9.3 lists various nanostructures, nanoparticles, and nanomaterials that are commonly used as bioelectronic components.

The nanostructures and nanomaterials discussed in this section are used in the bioelectronic application as sensors and nanodevices for healthcare monitoring (Zhang and Lieber 2016; Li et al. 2021). Wearable bioelectronic systems are essential due to the dire need for real-time healthcare monitoring. Plastic (polymer and organic)-based wearable and implantable bioelectronic systems are becoming increasingly popular because of the flexible nature of the substrates that could offer better conformity. They are also 3D curved and dynamically mobilizable resulting in a seamless electronic–biological interface that is soft, stretchable, and comfortable. These polymer bioelectronic systems provide outstanding physical, mechanical, electronic, and biological functions such as thermal, acoustic, photonic, chemical interactions, adhesiveness, electrical sensing, and biocompatibility (Someya et al. 2016). Hydrogel-based bioelectronics is used by having hydrogel as a potential

Table 9.3 Nanostructures and Nanoparticles in Bioelectronics

Type	Material structure
Metals/metal oxides	Au nanoparticles
	Ag nanoparticles
	Ag nanospheres
	ZnO nanoflakes
	ZnO nanorods
	Co3N nanowires
	HfO2 nanoparticles
	Ti3C2Tx nanoparticles
	Co3O4 nanoparticles
	NiO nanoparticles
Carbon	Graphene
	Graphene oxide
	Reduced graphene oxide
	Carbon nanotube (CNT)
	Single-walled CNT
	Multi-walled CNT
Inorganic	Si nanowire
	Si nanorods
Nanocomposite	Graphene/Ag NW
	Ag-rGO
	Fe3O4/GO/MIP
	IrO2@NiO core-shell NWs
	CuO/GO/CNF
	Au/rGO/AuPt NP
	rGO-ZnO
Micro-pattern	PANI-Au hybrid nanostructure
	Microneedle
	Microfluidic structure

interface between biology and electronics due to the resemblance as a biological tissue with electrical, mechanical, and bio-functional engineering nature, described in Fig. 9.3.

Due to the tissue–electronics interface and bioelectrical interface, hydrogel-based bioelectronics is more suitable for healthcare applications (Yuk et al. 2019). Nano-scale electronics and biological structures' binding and adhesion are essential for biosensing application. Molecular-level interaction of the electrons and bio-analytes plays a vital role in molecular bioelectronics for healthcare applications (Davis et al. 2005). Organic bioelectronics is similar to another approach where organic compounds and semiconductors such as poly(3,4-ethylene dioxythiophene):polystyrene sulfonate PEDOT:PSS, polypyrrole, horseradish peroxidase (HRP), and glucose oxidase (GOx) are used in bioelectronic applications (Rivnay et al. 2014; Berggren and Richter-Dahlfors 2007). Hence there have been various approaches adopted in bioelectronics based on the materials as discussed.

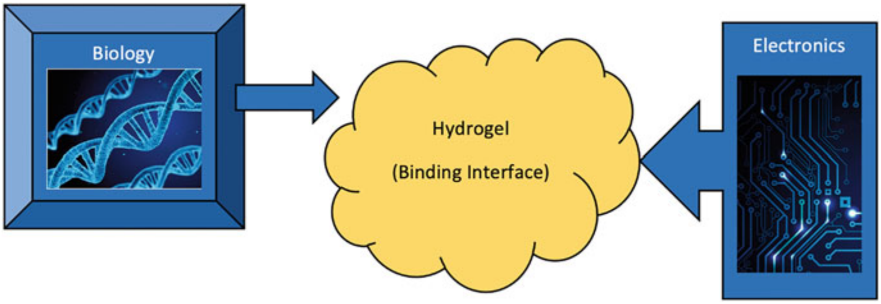

Fig. 9.3 Hydrogel interface

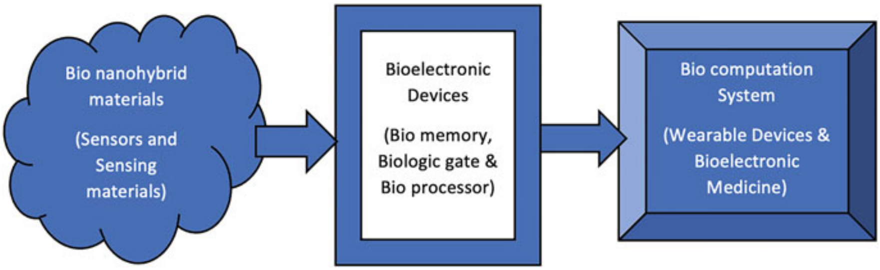

Fig. 9.4 Bioelectronics system

Bioelectronics has wide healthcare applications such as information storage, biosensing, diagnostics, organism mimicking, and neural mimicking, which will have various electronic devices involved (Yoon et al. 2019) (Fig. 9.4).

Figure 9.5 provides insights about the bioelectronic system's detailed information as a block diagram where there are the sensors and sensing materials in contact with the bio analytes on the left-hand side. The next block is the signal conditioning block, where the signal received from the sensor will be conditioned with the bioelectronic devices. The signal conditioning performed here involves analog to digital, digital to analog conversion, filtering, and data conditioning for wired or wireless transmission. Then the information is received by the biocomputation system block for diagnostics and monitoring as in vivo or in vitro. The whole bioelectronic system can be classified concerning the materials used in any of the blocks discussed in the above sections. If the plastic substrate is used on the sensing part, bioelectronic device part, and biocomputation part, it is called a plastic bioelectronic system. Most of the wearable devices used as part of the bioelectronic systems involve plastic electronics due to the device's ability to be mounted or placed on an uneven surface for continuous monitoring. Recent progress on the same can be addressed with tattoo electronics—recent technology development for healthcare monitoring. Similarly, depending on the type of bioelectronic system, it is used for varying healthcare applications.

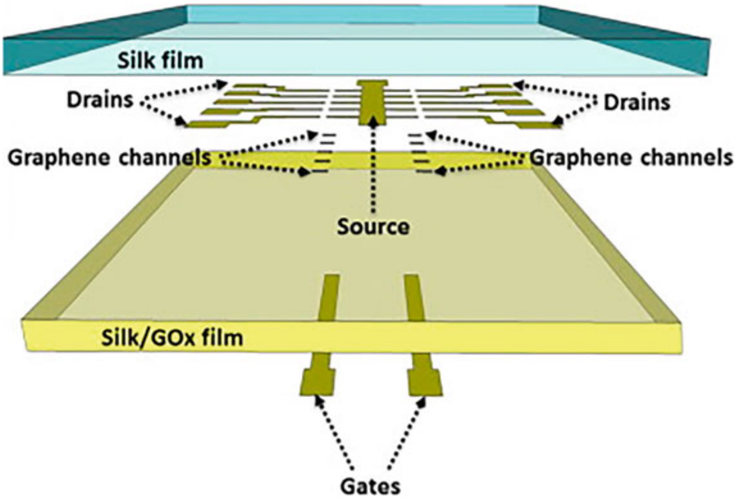

Fig. 9.5 Spatial multiplexed glucose biosensor (You and Pak 2014)

9.5 Multiplexed Biosensor Systems and Applications

Multiplexed biosensing systems have been used for disease detection and healthcare monitoring with various approaches based on the architecture and the materials used. The present chapter will focus more on the metabolites marker and drug delivery approaches using multiplexed biosensing principle. Approaches adopted in sensing includes quantum dots, nanoparticles, microfluidics, lab on a chip, micro/ nanoneedle, and many more. Glucose marker is a significant breakthrough in recent years without invasive approaches, whereas the metabolites of a person are identified through various biosensing methods. Paper-based multiplexed electrochemical and glass fiber strip-based biosensor was fabricated with carbon ink, resulting in a wide range of measurements with reproducibility (Amor-Gutiérrez et al. 2019). Similar to the paper based multiplexed biosensor, microfluidic paper-passed electrochemical biosensor array with eight sensors for detection of more than a few analytes such as glucose, lactate, and uric acid in urine to identify the diabetic level of a person more precisely (Zhao et al. 2013). Like the non-invasive approach for diabetic detection, an invasive approach with microneedles was also adopted in the multiplexed biosensing. Acrylate polymer-based microneedle was fabricated to detect metabolic acids, tumors, and chemistry changes over selective detection of pH, glucose, and lactate with changing physiological conditions (Miller et al. 2012). Carbon-coated, stainless-steel pins were used as three-electrode configurations in the glucose biosensing with enzymatic sensor phase and ferrocyanide electron transfer mediator (Rama et al. 2017). Multiplexed marker array can be achieved by using multiple antibodies placed over the Au nanoparticles, which can detect interleukin-12 and

Table 9.4 Electrochemical glucose sensor progress (2009–2020)

Year	Sensor type	Sensor subtype
2009	Paper-based sensor	Not applicable
2010	Implantable sensor	Not applicable
2011	Nanomaterial-based sensor	Engineered GOx
2012	Wearable non-invasive sensor	Contact lens
2013	Enzymatic sensor	Not applicable
	Self-powered sensor	Self-powered continuous sensor
		Self-powered tear sensor
2014	Flash glucose monitoring	Not applicable
2015	Tattoo ISF sensor	Not applicable
2017	Multiplexed	Fully integrated sensor
2017–2018	Sweat sensor	Drug delivery patch
		Microfluidic sweat patch
		Skin-like ISF sensor
2018	Nanomaterials of wearables-based sensors	Not applicable
2020	Glucose insulin chip sensor	Not applicable
	Flow-through sweat sensor	Not applicable
	On-body MN patch	Not applicable

tumor necrosis factor-a in impedance spectroscopy-based biosensor for detection of diabetes with the information availed from glucose and hemoglobin (La Belle et al. 2011). Glucose and diabetes are very closely related, and hence glucose is used as an effective biomarker for determining diabetes in a biological system. Glucose sensor has been on a progressive arena over the years with different architectures over the decade. Table 9.4 (Amor-Gutiérrez et al. 2019; Miller et al. 2012; Rama et al. 2017; La Belle et al. 2011; Barbee et al. 2010; Ng et al. 2008; You and Pak 2014; Zhu and Trau 2012; Werley et al. 2020; Márquez et al. 2019; Sridara et al. 2020; Wang et al. 2011; Hossain and Park 2017; Shu et al. 2015; Yin et al. 2016; Mei et al. 2016; Bao et al. 2008; Teymourian et al. 2020) illustrates the progress on the electrochemical glucose biosensor over a decade from 2009 to 2020.

Glucose detection with multiplexed biosensing has different approaches adopted recently such as:

- Spatially multiplexed glucose biosensor.
- Reconfigurable multiplexed glucose biosensor.
- Multiplexed biosensor with different materials for glucose sensing.

Spatially multiplexed biosensing is a technique used to detect glucose in a biological system where numerous identical sensors are placed in a coordinate system to record the information simultaneously. Multiplexed sensing systems adopt the spatial approach by pacing the micro/nanoarray/beads in a spatial coordinate on a substrate so that the multiple sensors will give the needed information at the same time simultaneously. This spatial multiplexed sensing system is made more reliable by advancing the micro/nanofabrication techniques available (Barbee et al. 2010; Ng et al. 2008). Spatially multiplexed sensing technique is widely used for various biomarker detection for understanding the biology of a person. Glucose detection using a spatially multiplexed biosensor is one such approach to understanding the change in metabolites in understanding the diabetes condition. Different techniques have been used for the glucose biomarker using the spatial multiplexed sensing approach, such as flexible substrate-based spatial multiplexed system, optical-based spatial multiplexed system, and microfluidic-based spatial multiplexed system. Flexible substrate–based spatial multiplexed glucose sensors have the uniqueness of the sensor's placement on any uneven substrate of a human body, which can function as a wearable system to read the information continuously by using sweat, temperature, and other physiological conditions as bio analytes. Carbon ink on a piece of silk fabric with the thread coated with carbon ink and potassium ferricyanide over which GOx is deposited to detect the bio analyte's glucose is used as a wearable spatial multiplexed glucose biosensor system (You and Pak 2014; Mahato and Wang 2021).

Figure 9.5 shows the structure of the glucose-sensing mechanism of a field-effect transistor (FET)-based sensor. Similar sensor devices are arranged spatially to form multiplexed sensors for glucose sensing. A similar arrangement can be modified with different types of sensors for glucose detection. Spatially encoded microarrays are used for glucose detection with the bio-functional microparticles arrays placed on a gel-based microstructure combined with microfluidics. The proposed mechanism has spatial encoding microbeads with fluorescent dye to identify the tumor using a fluorescence microscope and arrays with enzymes having microparticles to detect glucose (Mahato and Wang 2021). Multiplexed optical biosensor array with similar fluorescent spectra arranged in cells is an approach adopted for detecting glucose. The FLII12Pglu-700μδ6 sensor detects glucose in a cell with an excitation wavelength of 440 nm and an emission wavelength of 480,530 nm (Werley et al. 2020). Reconfigurable point-of-care sensing systems is much in need for the present healthcare monitoring. A reconfigurable, smartphone-interfaced electrochemical sensor for detecting two analytes simultaneously is used for identifying type-1 diabetes. Two electrodes are fabricated to detect two analytes simultaneously by depositing glucose oxidase and lactate oxidase for detecting glucose and lactate. Reconfiguration of the system can be achieved by removal of the membranes by calcium chelator phosphate buffer. Electrodeposition of a new membrane will detect a new analyte of the same type or different type (Márquez et al. 2019).

9.6 Multiplexed Biosensors for Glucose Monitoring

Different materials such as metals, metal oxides, polymers, and composites are used for sensing glucose in multiplexed biosensors approach based on the type of multiplexed sensor. A flexible electrode array-based multiplexed sensor is fabricated using functionalized PDMS film with gold nanoparticles, Prussian blue, GOx, and LOx to detect glucose and lactate as a multiplexed biosensor (Yokus et al. 2020).

Multiplexed paper-based glucose detection is done with paper and carbon ink for electrochemical processes with ferrocyanide used as a mediator. Fabrication of the paper-based multiplexed sensor is cost-effective with the capability of eight simultaneous measurements (Amor-Gutiérrez et al. 2019). Nanostructured conductive hydrogel is an alternative material used as electrodes to fabricate the sensor using an inkjet printer for multiplexed glucose sensing (Li et al. 2018). Graphene ink is used for the fabrication of multiplexed biosensor array for the detection of glucose. The graphene ink's biofunctionalization is done for better sensitivity when used for glucose-sensing applications (Labroo and Cui 2014). Multiplexing capabilities toward glucose sensing can be achieved with the array of sensors having multiple sensing capabilities with different materials having adhesion with the bio analyte to be used as a glucose marker. Different materials with the glucose-sensing capability can be used as a single multiplexed sensor with different arrays.

Electrode materials made of carbon dots and copper oxide composite are used for glucose detection having a three-electrode setup with Ag/AgCl as the reference electrode (Sridara et al. 2020). Similarly, various electrodes made with different materials, as listed below, are used for glucose sensing (Wang et al. 2011; Hossain and Park 2017; Shu et al. 2015; Yin et al. 2016; Mei et al. 2016).

Electrode materials made of carbon dots and copper oxide composite are used for glucose detection having a three-electrode setup with Ag/AgCl as the reference electrode (Bao et al. 2008; Teymourian et al. 2020). Similarly, different types of electrodes made with different materials as listed below are used for glucose sensing (Mei et al. 2016; Bao et al. 2008; Teymourian et al. 2020; Ngoepe et al. 2013; Bian et al. 2020; Garzón et al. 2019; Jarockyte et al. 2020).

- GOx/CdS/Gr on GCE.
- PDDA/Ch/GOx/PtAuNPs/PtZn on Pt.
- Au/GO on GCE.
- Cu/Cu2O/CSs on GCE.
- Nafion/NPC-CB on GCE.
- PDDA/Ch/GOx/PtAuNPs.

Tests such as amperometry and cyclic voltammetry were carried out for the glucose sensors to identify the sensitivity. The sensitivity of the listed glucose sensors ranges from 1.76 to 110 μA mM^{-1} cm^{-2}, in which the PDDA/Ch/GOx/PtAuNPs glucose sensor has a better sensitivity of 110 μA mM^{-1} cm^{-2}. Apart from the electrode-based glucose sensing, other biosensors are made with different materials of bulk and low-dimension nanostructures for better performance (Bao et al. 2008; Teymourian et al. 2020). Some of the materials include CNT-based composites, nanostructured TiO_2, carbon nanofibers/helical carbon nanofibers, Pt-polyaniline (PANI) hydrogel heterostructures, PANI-wrapped boron nitride nanotubes, organic ligand, and metal ions, 3D hybrid graphene–CNT structures, metal dichalcogenides, reduced graphene oxide, MoS_2, WS_2, WSe_2, $MoSe_2$, hollow sphere nanostructured poly(3,4-ethylene dioxythiophene) (PEDOT), AuNP conjugate, Co, Ni, Cu, Nanoporous PtAg, PtCu, Co@Pt core-shell NPs, hollow Ag/Pt

NPs, and Pt/Au nanowires. Different synthesis methods were adopted to manufacture the nanomaterials to get the required low-dimensional structure for improved property to be used as glucose biosensors. Multiplexed glucose biosensors have different architectures to get the multiplexed sensing capability. Micro/nanofabrication techniques such as deposition, lithography, and etching were used to obtain the needed architectures such as micro/nano arrays, microfluidic channels, immune assays, and membranes for multiplexed glucose sensors.

9.7 Multiplexed Biosensor for Drug Monitoring

Drug delivery systems utilize sustainable, responsive, and targeted drug delivery vehicles to offer illness management. Advanced drug delivery systems overcome traditional drug delivery limitations by enhanced bioavailability, therapeutic index, and reduced side effects with improved patient compliance or acceptance (Ngoepe et al. 2013). As the use of therapeutic drugs is constantly increasing, therapeutic drug monitoring (TDM) is clinically practiced for detecting given drug concentrations at fixed intervals in the bloodstream of patients (Bian et al. 2020). Various techniques have been employed in TDM, such as gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and immunoassays. These three techniques have been employed to investigate the minimum effective concentration of drugs in human saliva, urine, blood, plasma, and serum. However, these techniques involve a long time sample process, costly reagents and equipment, trained personnel, and a specialized laboratory for processing them (Garzón et al. 2019). On the other hand, biosensors are the most innovative yet straightforward techniques available today—a single device that meets all the requirements. These biosensors have been used to manage illnesses/diseases such as cardiovascular disorders and diabetes that require glucose and cholesterol levels maintenance (Ngoepe et al. 2013). Standard biosensors are used to detect a single analyte. However, this will not be enough for an early and accurate diagnosis to obtain. In this case, having multiplexed biosensors that involve a multitude of biomarkers capable of performing multiplexed biological detection can be employed (Garzón et al. 2019). Simultaneous detection of several biomarkers minimizes false positives and false negatives during clinical diagnosis, which readily occurs when measuring a single molecule. Hence, multiplexed biosensors capable of simultaneously detecting multiple analytes provide accurate data for therapeutic drug monitoring and diagnosis. Therefore, the multiplexed biosensors emerged as a valued tool for clinical diagnosis (Jarockyte et al. 2020; Yáñez-Sedeño et al. 2017).

9.8 Multiplexed Biosensor Systems for Infectious Diseases Monitoring

Infectious diseases caused by bacteria, virus, parasites, and fungi are the primary concern for the people and governments worldwide as they impact individual and public health. Notable outbreaks of infectious diseases enumerated by the World Health Organization (WHO) include malaria, tuberculosis, AIDS, hepatitis, chikungunya, the Ebola virus, and the recent outbreak of Covid-19 (Rodovalho et al. 2015). Lack of sanitary conditions, the flawed urbanization process, and lack of city planning and water supplies significantly contribute to the spread of infectious diseases (Rodovalho et al. 2015). In this case, a diagnostic test is essential to detect the presence or absence of infection. Standard diagnostic tests rely on laboratory-based techniques, including microscopy and microorganism culture, immunoassays, nucleic acid amplification, Enzyme-linked Immunosorbent Assay (ELISA), and PCR. These techniques lack sensitivity, and the culture process has a significant time delay. Also, techniques such as ELISA and PCR are highly sensitive but have complex sample preparation processes and are challenging to implement multiplex detection (Sin et al. 2014). Hence, the real-time identification of infection offers effective medical treatment and control over epidemic outbreaks. Therefore, appropriate diagnostic tools that are cost-effective, rapid, robust, and sensitive are in great demand (Jain et al. 2021). In recent years, biosensors are widely employed for fast and accurate diagnoses. A biosensor is an analytical device that gives measurable signals via a transducer by converting a target analyte's molecular recognition (Sin et al. 2014). Generally, these biosensors are classified into optical, electrochemical, and piezoelectric devices based on the way they transduce signals (Castillo-Henríquez et al. 2020). Jeong et al. studied bacterial detection using a fluorescent supramolecular biosensor. The pathogen's binding induces conformational changes in supramolecular state that emits fluorescence which can selectively detect E. coli (Jeong et al. 2019). In another study, Mathelie et al. developed a silica NPs-assisted electrochemical biosensor for the specific and sensitive detection of E. coli (Matheli-é-Guinlet et al. 2019). Another type of piezoelectric biosensor refers to the ability of material that generates voltage under mechanical stress. Guo et al. employed a piezoelectric biosensor with antibody-functionalized AuNPs that enhance changes in detection signals. The results demonstrated that the developed biosensor could be a suitable real-time monitoring method for the particular pathogen (Guo et al. 2012). Despite the emerging sensor technology, these next-generation biosensors still need to overcome fundamental challenges in the particular case of infection detection, a fully integrated biosensor capable of multiplexed and label-free detection. The development of multiplexed biosensor is a top priority to address the current issue as it operates by detecting multiple analytes on one device (Soler et al. 2017). Soler et al. studied the multiplexed nanoplasmonic biosensor for the simultaneous detection of Chlamydia trachomatis and Neisseria gonorrhoeae in urine samples. The plasmonic microarray composed of gold nanohole sensor arrays exhibited excellent optical transmission and provided highly sensitive analysis and label-free configuration. Thus, the multiplexed biosensor employed in this study exhibited outstanding sensitivities, with a limit of detection of 300 CFU/mL for C. trachomatis and 1500 CFU/mL for N. gonorrhea. It is successfully employed to identify and quantify the two bacterial levels in a one-step assay (Soler et al. 2017). In another study, Gao et al. investigated the performance of a multiplex electrochemical biosensor for pathogen identification. The study examined the feasibility of rapid diagnosis of bloodstream infections using a multiplexed electrochemical biosensor. The results demonstrated that the sensor successfully identified the bacterial species, such as Klebsiella, Enterobacter, Serratia, Citrobacter, and Enterococcus in spiked blood samples. Thus, the multiplexed biosensor improves clinical management by providing appropriate and timely antimicrobial treatment (Gao et al. 2017).

9.9 Conclusion

Technological growth of science and engineering has made various researchers and scientists work on cross-disciplinary and interdisciplinary research, consenting significant progress in different research areas. One of the promising research areas is biosensing and bioelectronics, where people from medicine, physics, engineering, material science, chemistry, and biology collaborate to give various healthcare application devices. Working on these advanced areas of research always has vast-spread opportunities and challenges. The same is applied to the most recent advancement in bioelectronic systems when more than one sensor is used as multiplexed biosensor systems. Precise diagnostics can be achieved with the multiplexed biosensing approach since multiple bio analytes can be studied to understand the physiological condition of a person. Single bio analytes on multiple sensors can also be applied to better understand the bio analyte nature with varying adhesions and physical properties. Various measurements such as glucose, lactate, pH, and temperature can be done at the same time parallelly. Multiple sensing principles such as optical, electrical, chemical, mechanical, and biological can be applied simultaneously to improve the point-of-care diagnostics. The multiplexed biosensor has significant benefits in healthcare applications due to its low measuring time with a broad detection range at a low sample rate. Overlapping symptoms between the diseases of the human systems have made increasing demand for multiplexed biosensor systems that can detect multiple biomarkers simultaneously. Multiplexed biosensor systems based on membrane-based lateral flow assays limit the biomarkers compared to the existing systems. Multiplexing multiple test lines in lateral flow assays strip has physical limits, which could be overcome with multiple strips. Fabricating multiplexed array for bio analyte detection is tedious due to the cost and fragile nature involved. The use of various nanomaterials such as gold and graphene in multiplexed biosensors has its limits and challenges in their ideal characteristics such as size, shape, and biocompatibility. These challenges have to be adequately addressed when using nanomaterials in multiplexed biosensing applications.

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