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Metal Oxide Nanostructure-modified Electrode for Glucose Biosensor

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Outline

Introduction to Glucose Biosensor

Diabetes mellitus is a chronic disease that occurs when the pancreas fails to produce sufficient insulin to regulate blood sugar or when the body is unable to use effectively the insulin produced. According to the statistics stated by the World Health Organization, 9% of adults aged 18 years and older had diabetes in 2014 [1]. In 2012, diabetes was the direct cause of 1.5 million deaths [2]. Diabetes mellitus has become the major health problem in most developed societies worldwide and has encouraged the development of glucose biosensors. Scholars have focused on producing fast, precise, and low-cost biosensors for monitoring blood glucose at home. Glucose biosensors have been the subject of concern not only in medical science but also in the food industry. The detection signal of glucose biosensors originates directly from glucose or by promoting the conversion of glucose to other determinable electroactive species [3].

Basic Principle and Challenges in Glucose Biosensors

New glucose biosensors have been developed through numerous processes and methodologies, such as electrochemical method, colorimetry, conductometry, optical methods, and fluorescent spectroscopy. Glucose biosensors fabricated through electrochemical method have gained the most attention over the last 40 years because of their sensitivity and selectivity. Moreover, electrochemical techniques exhibit low detection limit, fast response time, improved long-term stability, and low price. Both enzyme-based and non-enzymatic strategies have been employed for glucose detection. In particular, detection systems based on immobilized glucose oxidase (GOx) are the most popular because of their high specificity. Enzyme activity is affected by temperature, pH, humidity, and toxic chemicals. To solve the limitations of enzyme systems, scholars have investigated non-enzymatic sensors with improved electrocatalytic activity and selectivity toward glucose oxidation.

Clark and Lyons [4], who are the fathers of the biosensor concept, were the first to perform a research on glucose quantification through GOx entrapment with a dialysis membrane on the oxygen electrode surface; glucose amount was estimated based on the reduction in the dissolved oxygen concentration [2]. Since then, studies have investigated amperometric, potentiometric, impedimetric, or conductometric glucose biosensors based on GOx, which catalyzes the oxidation of glucose into gluconic acid, as shown in Equation (1):

$$D-Glucose + O_2 + H_2O \xrightarrow{GOx} D-gluconic\ acid + H_2O_2 \quad (1)$$

The most important factors that should be considered in the development of glucose biosensors include good electron transduction capability, bioactivity, easy accessibility toward the analyte, and large surface area for enzyme immobilization. Nanostructured materials offer significant advantages toward the biosensor performance. Nanostructured materials (1–100 nm length scale) offer high surface-to-volume ratio for high loading for enzyme immobilization and responsive micro environment for stabilization of the immobilized enzyme from leakage [5]. Nanostructured materials promote efficient electron transfer from the enzyme to the electrode, and this process requires additional catalytic step for fast electron transfer, resulting enhanced sensitivity.

Roles of Nanostructured Materials in Glucose Biosensors

Scholars have investigated a range of nanostructured materials, including nanoparticles, nanotubes, and nanorods, which are prepared from metals, semiconductors, and carbon-based materials, the fabricate glucose biosensors with improved performance. Moreover, the electrical contact between the redox biomolecules and the electrode surface is important for construction of electrochemical sensors. However, biomolecules are not directly in contact with the electrode surface because of the thick insulating protein shells surrounding the active sites of biomolecules and the blocked electron transfer between the two moieties, resulting in problems in electrochemical measurements. In this context, different kinds of nanostructured materials with diversified roles have been developed to improve glucose biosensor performance. Figure 3.1 summarizes the possible functions of the nanostructured materials when integrated in glucose biosensors.

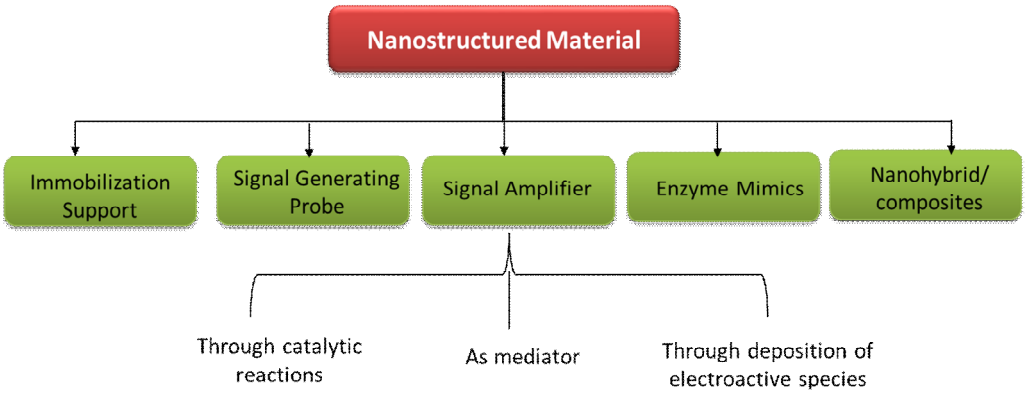

FIGURE 3.1
Summary of the possible functions of nanostructured materials when integrated in glucose biosensors

Nanostructured material surface has a strong tendency to adsorb biomolecules and act as an immobilization support for biomolecules during biosensor construction. The direct adsorption of biomolecules onto bulk materials may commonly result in denaturation and loss of bioactivity, whereas nanosized materials retain the bioactivity of biomolecules. Several nanoparticles carry charges and can provide electrostatic surface to attach the biomolecules with different charges. Introduction of appropriate surface functional groups into nanostructured materials can contribute to strong binding of the biomolecules to the materials. Hence, the high-conductivity properties of nanostructured materials render them to function as a signal-generating probe and a signal amplifier.

Nanostructured materials can act as enzyme mimetic in a glucose biosensor due to their distinct properties, such as high surface area-to-volume ratio, abundant reactive groups on their surface, unique optical properties, and fascinating catalytic activity. Various types of nanostructured materials exhibit catalase-, oxidase-, peroxidase-, or oxidase-like activities. Metallic nanoparticles act as electron transfer mediators or electrical wires to replace commonly used mediators in the construction of electrochemical biosensors. Metallic nanomaterials, such as gold (Au) [6, 7], platinum (Pt) [8, 9], palladium (Pd) [10, 11], and nickel (Ni) [12, 13], are used as modifiers of electrodes because they can amplify the electrochemical signals of biosensors. According to Katz et

al. [14], metallic nanomaterial amplified the electrochemical detection, and the conductivity properties of the metallic nanomaterials at the nanoscale dimension allowed the electrical contact of the redox center in enzymes with the electrode surface. Metallic nanomaterials offer excellent catalytic properties, ensuring their electrochemical reversibility for redox reactions, which are irreversible in bulk metal electrodes [14, 15]. However, metallic materials are expensive and exhibit low selectivity due to their small current response to target molecules [16].

Carbon-based nanomaterials, especially carbon nanotubes (CNT) and graphene, are also commonly used nanostructured materials for modifying glucose biosensor electrodes. In particular, CNTs show excellent properties in electrochemical biosensors due to their high surface area for sensing interaction and amazing conductivity for electron transfer. However, CNTs suffer from some limitations, such as high hydrophobicity, inability to be wetted by liquids, and surface tension higher than approx. 100 or 200 mNm^{-1}. Thus, CNTs require surface modification or functionalization using metals or metal nanoparticles to adhere onto the CNT surface. For example, Shrestha et al. [17] fabricated high-performance glucose biosensor by using polypyrrole (PPy)-Nafion-functionalized multi-walled carbon nanotubes (MWCNTs) nanocomposites to modify the glassy carbon electrode (GCE) through a facile one-step electrochemical polymerization, followed by chitosan–glucose oxidase immobilization on its surface.

Nanostructured metal oxides have gained much interest as matrices for developing glucose biosensors; these oxides can immobilize enzymes due to their good optical and electrical characteristics [18]. Moreover, nanostructured metal oxides are suitable for constructing miniaturized glucose biosensors with satisfactory performance and thus must be explored. This chapter focuses mainly on the development of different nanostructured metal oxides, namely, nanoparticles, nanorods, nanowires, and nanotubes of iron oxide and zinc oxide (ZnO), to modify electrodes for glucose biosensor applications.

Metal Oxide Nanostructures in Glucose Biosensor

Metal oxide nanostructures exhibit functional biocompatibility and non-toxic properties but are relatively inexpensive to produce. Meanwhile, nanostructured metal oxides possess a high surface area and isoelectric point (IEP), which provide a suitable and reliable surface for immobilization of low IEP enzymes. Several high IEP metal oxide nanomaterials used in glucose detection are zinc oxide (ZnO) [18, 19], iron oxide (Fe_3O_4) [20], titanium dioxide (TiO_2) [13, 21, 22], copper oxide (CuO) [23-25], cerium oxide (CeO_2) [26, 27], and zirconia (ZrO_2) [28, 29]. Figure 3.2 summarizes the properties and applications of common metal oxide nanostructures. This chapter reviews iron oxide and zinc oxide nanostructure-modified electrodes for glucose biosensors.

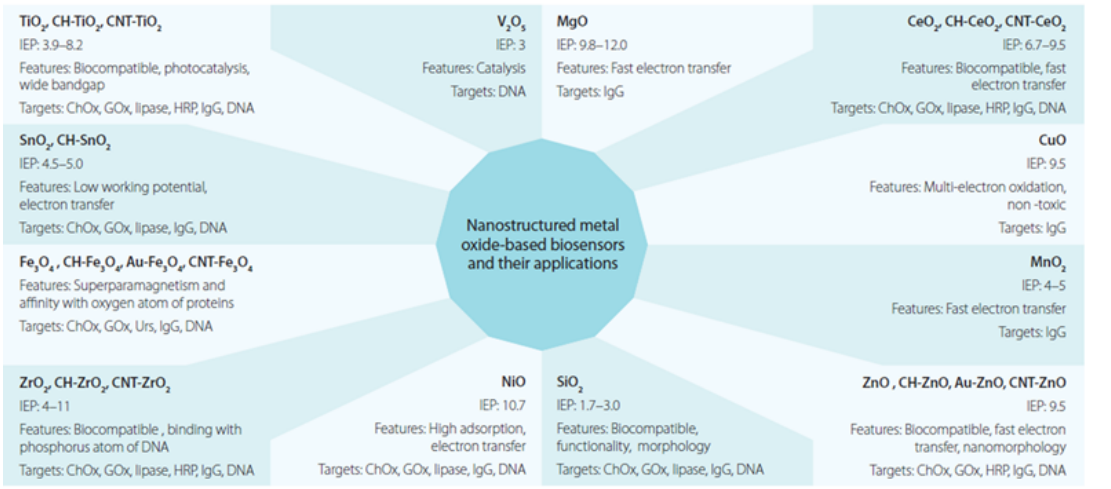

FIGURE 3.2
Summary of the properties and applications of common metal oxide nanostructures [152]

Iron Oxide Nanostructures-based Glucose Biosensors

Iron oxide nanostructures have gained wide interest for development of glucose biosensors because of their outstanding properties that contribute to enhanced sensor performance. Iron oxide has several advantages, such as chemical and biological stability, low toxicity, super paramagnetic property, and low cost for large-scale production. Iron oxide nanostructures also offer high conductivity and catalytic properties, which render them as suitable electronic wires to enhance electron transfer. However, iron oxides tend to be attracted together, thereby reducing their high surface energies. This agglomeration can be prevented by functionalization of iron oxide with organic, inorganic, and biopolymeric materials, such as chitosan, silica, polymers, and carbon.

Properties of Iron Oxide Nanostructures

Iron oxide can either be useful or undesirable depending on their phases, compositions, and functions [30]. Among all iron oxide compounds, hydroxide and oxide–hydroxide and magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) phases are the most important. Magnetite and maghemite have been synthesized in the laboratory for various applications because of their polymorphism involving temperature-induced phase transition. Magnetite and maghemite exhibit nontoxicity (at low dosage), excellent magnetic and catalytic properties, biodegradability, and biocompatibility and thus can be potentially applied in glucose biosensors [31]. Magnetite possesses a spinel structure with face-centered cubic that contains both divalent (Fe^{2+}) and trivalent (Fe^{3+}) ions and 32 O^{2-} ions, which are distributed regularly in close packing [Figure 3.3 (a)]. Fe^{2+} ions occupy half of the octahedral sites, and Fe^{3+} ions are evenly distributed across tetrahedral and octrahedral sites stacking along the [111] direction. Magnetite is black in color and has the highest magnetic saturation compared with other iron oxides. However, magnetite is more thermodynamically unstable in the presence of oxygen compared with hematite (α-Fe_2O_3). Magnetite slowly oxidizes to maghemite at room temperature and hematite at high temperatures in the presence of oxygen.

Magnetite can be both an n- and p-type semiconductor. However, magnetite has the lowest resistivity among iron oxides due to its small bandgap (0.1 eV) [32].
Maghemite has a structure that is isostructural with magnetite and contains most of the cations in the trivalent state. Maghemite has the cubic unit cell with 32 O^{2-} ions, 21⅓ Fe^{3+}, and 2⅓ vacancies [Figure 3.3 (b)]. O^{2-} ions form a cubic close-packed array, whereas Fe^{3+} ions are distributed in tetrahedral and octahedral sites. The cation vacancies are attributed to the oxidation of Fe^{2+} ions into Fe^{3+} ions. Maghemite has a red-brown color and occurs in soils as the weathering product of magnetite or the product of heating other iron oxides [33]. Therefore, maghemite can be considered as fully oxidized magnetite. Maghemite has lower magnetic saturation than magnetite but also exhibits lower toxicity and higher chemical stability, which make it as an interesting material for biomedical applications. With regard to electrical properties, maghemite is an n-type semiconductor with bandgap of 2.0 eV [34].

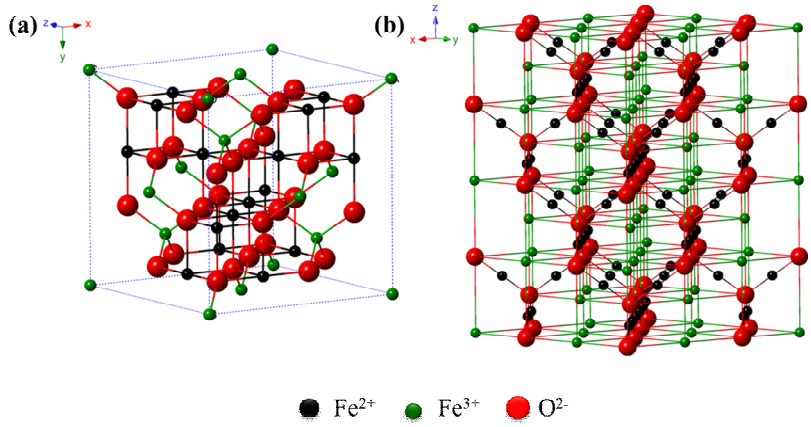

FIGURE 3.3
Crystal structure and crystallographic data: (a) magnetite and (b) maghemite [34]

Synthesis of Iron Oxide Nanostructures for Glucose Biosensor Electrodes

The synthesis method influences the properties of iron oxide because the synthesis parameters determine the particle size, shape, crystallinity, particle distribution, and stabilization of the resulting iron oxide nanostructures. Iron oxide nanostructures are used in modified electrodes for glucose biosensors for electrochemical sensors. Studies on electrode modification for glucose sensing used conventional fabrication method to separate between the synthesis of the iron oxide nanostructures and the attachment of the iron oxide nanostructures to the conductive electrode [35]. This method exhibits some drawbacks, such as reduced electrocatalytic activity, low stability, and poor reproducibility, because the biosensor performance depends on the distribution of the iron oxide nanostructures on the electrode and the strength of binding between the iron oxide nanostructure and the electrode surface. To improve the contact between the iron oxide nanostructures and the electrode surface, scholars have used electrospinning, electrochemical anodization, and inkjet-printing methods for direct deposition of iron oxide nanostructures on the electrode surface [36]. However, these methods require high temperatures, thereby increasing the cost to produce glucose biosensors.
Various physical and chemical routes have been used to synthesize iron oxide nanostructures. Commonly used chemical methods include co-precipitation, hydrothermal/solvothermal,

electrochemical, thermal decomposition, and sonochemical techniques, and physical methods include vapor deposition, ball milling, laser pyrolysis, and gas phase deposition. Physical methods always require subdivision from bulk materials into nanometer size nanostructured that requires high energy consumption and complicated procedures, resulting in non-uniform particle size. Chemical methods are commonly applied to produce small and uniform iron oxide nanostructures. Table 3.1 summarizes the comparison of methods for synthesis of iron oxide nanostructures and their advantages and disadvantages in glucose biosensor applications.

Co-precipitation is commonly used to synthesize iron oxide nanoparticles because this method offers a simple synthesis process, which can be scaled up to produce hydrophilic particles at low temperatures. However, this method leads to large particle size distribution and cannot control the shape of iron oxide nanoparticles. Yang et al. [37] synthesized water-stable iron oxide nanoparticles through co-precipitation and used these particles to modify platinum electrodes for enzymatic glucose biosensors. In their work, Fe^{2+} and Fe^{3+} ions in the ratio of 1:2 were mixed in hydrochloric acid (HCl) and precipitated in sodium hydroxide (NaOH) solution under inert condition with vigorous stirring. The produced iron oxide nanoparticles were functionalized with chitosan and dispersed in water for storage [38]. The iron oxide nanoparticles obtained are composed of well-dispersed spherical shaped particles, with an average diameter of 20 nm. A similar approach was used by Sanaeifar et al. [39] to synthesize iron oxide nanoparticles for modification of tin (Sn) electrode for enzymatic glucose biosensor. Based on the X-ray diffraction (XRD) pattern, the synthesized iron oxide nanoparticles are in magnetite phases and have size of 3 nm. The produced iron oxide nanoparticles were functionalized with polyvinyl alcohol (PVA) solution to overcome aggregation and rapid degradation. Godarzi et al. [40] prepared iron oxide nanoparticles decorated on multi-walled carbon nanotubes (MWCNTs) through ultrasonic-assisted co-precipitation to modify a glassy carbon electrode (GCE) for highly stable and selective non-enzymatic glucose biosensor. Iron oxide nanoparticles with size of around 10 nm were produced and agglomerated on the surface of MWCNTs. Other studies employed reverse precipitation to synthesize iron oxide nanoparticles [41, 42]. This method produces narrow particle size distribution, uniform shape, and pure magnetite phase [43, 44] because it only uses ferrous ions to precipitate in basic solutions. Therefore, the molar ratio of Fe^{2+}: Fe^{3+} should not exceed 1:2 when oxidizing Fe^{2+} ions [45].

Nanotubes, nanorods, and nanowires have been increasingly used to modify electrodes for glucose biosensors because their one dimensional (1D)-nanostructure provides a direct electrical channel for electron transport and does not undergo irreversible aggregation. Zhang et al. [46] fabricated a non-enzymatic glucose biosensor by using iron oxide nanorod arrays prepared by electrochemical anodization of iron foil, followed by *in situ* annealing under hydrogen flow. The iron oxide nanotubes were produced through anodization of iron foil in ethylene glycol solution containing Fe^{2+} ions and iron oxide nanorods, followed by annealing. Based on the XRD patterns, the iron oxide nanorods possess a cubic structure of magnetite. The scanning electron microscopy (SEM) image shows the uniform distribution of the iron oxide nanorods with vertical distribution of $\sim$2.5 μm in length and 70 nm in diameter. Chen et al. [47] reported direct growth of iron oxide nanotubes on fluorine-doped tin oxide (FTO) electrode for non-enzymatic glucose biosensor applications. Iron oxide nanotubes were fabricated using the grown ZnO nanowires through hydrothermal method. The ZnO nanowire structures were then transformed into tube-like morphology of iron oxides after immersion in the Fe precursor and annealing under air and inert condition. The mechanisms of the iron oxide nanotube formation are the ion exchange process that occurs spontaneously on the zinc oxide nanowire surface and the etching of the nanowire due to acidity of the Fe precursor, resulting in the hollow structure of iron oxide. After annealing, the phase transition of maghemite into magnetite occurred, forming iron oxide nanotubes. Similarly, Ahmad et al. [35] developed non-

enzymatic glucose biosensor by using vertically oriented zinc oxide nanorods modified with iron oxide (Iron oxide–ZnO nanorods). The nanorods were grown on the electrodes through hydrothermal method and modified with iron oxide. The rough surface of the iron oxide–ZnO nanorods were observed on vertically oriented and uniformly distributed ZnO nanorods, with length of ~2.15 nm and diameter of 80–90 nm. Many innovative techniques and improvement have been explored for synthesis of iron oxide nanostructures. However, cost effectiveness and repeatability are the most important factors to consider in the synthesis of iron oxide nanostructures for glucose biosensor application to obtain accurate results.

TABLE 3.1
Comparison of the synthesis method of the iron oxide nanostructures for glucose biosensor applications

Method	Description of Method	Advantages	Drawbacks	References
Co-precipitation	Involves mixing of ferric and ferrous ions in a 1:2 molar ratios in a very basic solution at room temperature or at elevated temperature	▪ High yield ▪ Conducted at low temperature ▪ Suitable to produce colloidal stable nanostructures	▪ Requires size selection process ▪ Broad particle size distribution ▪ Low control on the particle shape formation	[20, 37, 39, 40, 48-50]
Reverse precipitation	Involves ferrous ion precipitate in basic solution at room or elevated temperature	▪ Narrow particle size distribution ▪ Uniform shapes ▪ Obtains pure magnetite phase	▪ Suitable to produce smaller size iron oxide nanoparticles	[41, 42]
Hydrothermal/ Solvothermal	Chemical reaction in solvent performs in a sealed vessel in which the temperature of solvent can be brought to around their critical points via heating concurrently with autogenous pressures	▪ Uniformly distributed ▪ High crystallinity ▪ Monodispersed nanostructured ▪ Produce various shapes of iron oxide	▪ Requires high temperature and pressure ▪ Complicated process (requires seed layer and annealing process)	[35, 47, 51, 52]
Sonochemical	Involves acoustic cavitation creation, growth formation, and implosive collapse of bubbles in ultrasonically irradiated liquid	▪ Narrow particle size distribution ▪ Simple and quick process	▪ High energy consumption ▪ Low control on particle shape ▪ Low yield	[53]

Functionalization of Iron Oxide Nanostructures for Glucose Biosensors Electrodes

The hydrophobic interaction between large surface area-to-volume ratio of iron oxide nanoparticles and the magnetic forces from the external magnetic field induced the iron oxide nanoparticles to form aggregates by dipole–dipole interaction. Therefore, surface modifications or functionalization of iron oxide nanoparticles must be conducted during or after synthesis. Surface functionalization iron oxide nanoparticles are beneficial to improving dispersibility, biocompatibility, prepare environment suitable for biomolecule attachment and transfer the hydrophobic iron oxide nanoparticle surface into hydrophilic. Several types of organic, inorganic, and biopolymeric materials are commonly used for surface functionalization of iron oxide nanoparticles. The summary of the materials used and the properties of the materials in functionalization of iron oxide nanoparticles for glucose biosensor applications are listed in Table 3.2.

In a simple method, iron oxide nanoparticles can be functionalized by surfactant and small molecules, such as citric acid, oleic acid, 3-aminopropyltriethyloxysilane (APTES), and mercaptopropyltriethoxysilane agents. This functionalization introduces the functional group of amino (-NH_2), carboxyl (-COOH), hydroxyl (-OH), and silane, which are suitable to make the water particles dispersible and allow further modification with enzyme for glucose biosensor applications. Recently, Vallabani et al. [54] reported the functionalization of iron oxide nanoparticles using citric acid for glucose detection application. The functionalization was performed in post-synthesis process. The stable colloidal suspension of iron oxide nanoparticles was produced, wherein the average zeta potential value of particles dispersed in PBS at pH 7.4 was found to be −39.8 ± 0.6 mV with an average particle size of 13 nm. A few other works reported on the introduction of citrate, amine, and silane functional group to the iron oxide nanoparticles to be applied in glucose biosensor applications [24, 55, 56].

Natural polymers, such as chitosan, dextran, starch, albumin, and liposomes, have been extensively employed for coating purposes. Among all natural polymers, chitosan is commonly used to functionalized iron oxide nanoparticles owing to chitosan unique properties, such as excellent biocompatibility and contains multiple functional groups that are suitable for enzyme immobilization. The enzyme allows to covalently attach, self-assemble, and organize on the surfaces of chitosan-iron oxide nanoparticles, making them promising in the development of a glucose biosensor. Kaushik et al. [20] utilized iron oxide nanoparticles embedded in chitosan matrix and immobilized with GOx to modify the indium–tin oxide (ITO) glass electrode. The functionalization of chitosan to iron oxide nanoparticles was performed through post synthesis, wherein the –NH_2/OH groups of chitosan interacted with surface charge of iron oxide nanoparticles through electrostatic interaction and hydrogen bonding. Chitosan prevented aggregation of iron oxide nanoparticles without changing the optical and electrical properties. Similar approach has been reported by Khun et al. [57], wherein chitosan–iron oxide nanoparticle-modified gold-coated glass substrate was fabricated for enzymatic potentiometric glucose biosensor. They found that even without using any crosslinker and nafion, high-sensitivity and high-specificity glucose biosensor can be developed.

Several synthetic polymers, such as polyethylene glycol (PEG), Ppy, polyvinylpyrrolidone (PVP), polyaniline (PANI), and PVA, have also been used recently to functionalize the iron oxide nanoparticles to be applied in glucose biosensor applications. Sanaeifar et al. [39] reported the iron oxide nanoparticles functionalized with PVA for enzymatic glucose biosensor applications. PVA solution was added after the synthesis process of iron oxide nanoparticles. PVA matrix reduced the aggregation between the iron oxide nanoparticles and thus increased the surface-to-volume ratio

for biomolecule immobilization. From the SEM image, the enzyme molecules were absorbed uniformly on the surface of PVA-iron oxide nanoparticles, thereby improving the performance of the modified electrode in glucose detection. Conducting polymers, such as PPy and PANI, were also used to functionalize iron oxide nanoparticles [58, 59]. PPy and PANI have good redox property and contain numerous functional groups on their backbone. These functional groups can form coordination interaction with metal ions, which effectively protect the metal nanoparticles from aggregations and can act as linker. Yang et al. [60] reported potentiometric enzymatic glucose biosensor based on iron oxide nanoparticles functionalized with PPy. Iron oxide nanoparticles were synthesized through co-precipitation and crosslink to GOx by using carbodiimide hydrochloride. Then, GOx immobilized onto iron oxide nanoparticles was encapsulated by polymerization of PPy. The core shell of the PPy produced well-dispersed iron oxide nanoparticles that have excellent magnetic and electrical conductivity. Al-Mokaram et al. [61] reported the ITO electrode modified with nanocomposite of iron oxide nanoparticles–chitosan–PPy for application in non-enzymatic glucose biosensors. The successful incorporation of iron oxide nanoparticles into Ppy–chitosan molecules produced rapid amperometric response with high selectivity and wide linearity to detect glucose non-enzymatically.

In glucose biosensor applications, iron oxide nanoparticles normally functionalize with single inorganic coating or the combination of a few inorganic coating that can be called as hybrid materials. Inorganic materials coating to iron oxide nanoparticles, such as gold (Au), silver (Ag), platinum (Pt), silica (SiO_2), and zirconia (ZrO_2) not only provide stability to the iron oxide nanoparticles in solution but also provide improvement in semiconductor efficiency, optoelectronics, catalysis, and biological attachment properties. Baby et al. [48] reported the modification of magnetic glassy carbon electrode (MGCE) with iron oxide nanoparticles coated with SiO_2 decorated on MWCNT. Iron oxide nanoparticles have been uniformly coated with biocompatible SiO_2 using a simple chemical reduction method in core–shell structures. SiO_2 layer functionalized iron oxide nanoparticles have considerably enhanced the antioxidation properties for naked iron oxide nanoparticles and improved the loading of enzyme molecules by providing silanol functional group. He et al. [42] utilized the composite of iron oxide nanoparticles conjugate to bovine serum albumin (BSA) and decorated with Au nanoparticles to modify the Pt electrode (Au nanoparticles/BSA-iron oxide nanoparticles/Pt) for enzymatic glucose biosensor application. They prepared the composite by first co-precipitation of the iron oxide nanoparticles, adsorption of BSA, and *in situ* reduction of Au chloride to form Au nanoparticles. The morphology observation showed that the produced composite composed of cubic iron oxide nanoparticles with uniform size and the Au nanoparticles distributed evenly on the surface of iron oxide nanoparticles. Uniform Au nanoparticle distribution was obtained due to excellent colloidal stability of iron oxide nanoparticles during the Au nanoparticle adsorption process. The size of iron oxide nanoparticles produced is $\sim$ 25 nm and the size of Au nanoparticles is $\sim$3 nm with ratio of the number of Au nanoparticles to iron oxide nanoparticles estimated to be 10:1, indicating the presence of many Au nanoparticles on the surface of BSA–iron oxide nanoparticles. The BSA protein shell provides a suitable environment for enzyme adsorption and contributes to long-term bioactivity, whereas that of Au nanoparticles promotes electron transfer. Wang et al. [52] used iron oxide nanoparticles completely covered with a uniform transparent silica layer and coated with Au nanoparticles. Many Au nanoparticles with an average size of 15 nm are decorated on the surface of SiO_2-iron oxide nanoparticles. Silica layer was prepared on iron oxide nanoparticles through modified Stöber's method and Au nanoparticles through citrate reduction method. Silica coating is beneficial to prevent aggregation between iron oxide nanoparticles, and the Au nanoparticle coating is beneficial to enhance enzyme absorption and increase the electron transfer.

TABLE 3.2
Summary of the materials used and the properties of the materials in functionalization of iron oxide nanoparticles for glucose biosensor applications

Materials used	Properties	Functional Group	Reference
Chitosan	High mechanical strength, biocompatibility, high water permeability, excellent film-forming ability, non-toxicity, susceptibility to chemical modifications, cost-effectiveness	$-NH_2$	[20, 37, 49, 57, 61]
Polyvinyl Alcohol (PVA)	Contains large hydroxyl group, improves biocompatibility, induce water stability, good chemical and thermal stability and non-toxicity	–OH	[39]
Polyaniline (PANI)	High conductivity, good environment, electrochemical stability, electro-optical properties, strong bimolecular interaction, biocompatibility, favorable storage ability, film-forming ability, and size exclusion properties	-	[62-65]
Polypyrole (PPy)	High conductivity, easy synthesis, comprises functional group on the backbone, enables the growth of film from aqueous solutions and avoid interference	-	[58, 60, 61, 66]
Citrate	Induces high water stability, easy functionalization process, provides biocompatibility, cheaper and non-toxic	-COOH	[24, 54, 55]
Silane	Improves the biocompatibility, easy to functionalize, prevent agglomeration	silane	[67, 68]
Silica (SiO_2)	Prevents aggregation, provides functional group, allows secondary surface-mediated reaction, improves biocompatibility	silanol	[48, 52, 69, 70]
Gold	High electrical conductivity, good biocompatibility, high catalytic activity, strong absorption ability, and well suitability	-	[42, 52, 71]

Performance of Iron Oxide Nanostructures in Glucose Biosensors

In current research, the high sensitivity, high stability, and high detection accuracy of glucose biosensor are on demand. Exploiting iron oxide nanostructures can be used to modify electrodes for glucose biosensors. In this chapter, both enzymatic and non-enzymatic strategies for glucose biosensor applications are reviewed. Despite all the techniques conducted to improve the glucose biosensor performance, the technique must meet the criteria of sensor-to-sensor reproducibility and cost effectiveness to be applied industrially.

In glucose biosensor fabrication, the modification of electrode commonly conducted through self-assembly or layer-by-layer assembly of the iron oxide nanoparticle solution and their composite onto the electrode surface. Self-assembly refers to the process by which nanoparticles or other discrete components spontaneously organize to direct specific interactions [69]. In ensuring the

occurrence of self-assembly, the molecules must be mobile, because as nanoparticles become larger than molecules, gravity and friction control the Brownian motion [70]. The main advantage of self-assembly method is that large various nanomaterial shapes and functions can be developed on the electrode. The main disadvantage of self-assembly method is difficulty to control the morphology. Another common approach to modify surface of electrodes with nanoparticles is through electrochemical deposition, wherein a thin and tightly adherent desired coating of metal, oxide, or salt can be deposited onto the surface of a conductor substrate by a simple electrolysis of a solution containing the desired metal ion or its chemical complex. The main advantage of this technique is better control morphology of the developed nanostructure. However, this method has limitation in functionalization of the developed nanostructure and suffers from mass transport limitations.

Enzymatic Glucose Biosensors based on Iron Oxide Nanostructures

In enzymatic glucose biosensor, glucose detection based on GOx enzyme is widely explored due to easy handling and has a potential for industrial applications due to high sensitivity and specificity. The immobilization of enzymes to matrix of nanostructure is important to prevent enzyme leakage, retain enzyme bioactivity, and prolong the shelf life of the glucose biosensor. Various techniques of enzyme immobilization have been reported in literature; these techniques include physical adsorption, electrostatic interaction, layer by layer deposition, electrode position, crosslinking, covalence immobilization and polymer entrapment, as shown schematically in Figure 3.4. The immobilization of GOx can be performed to functionalize iron oxide nanostructure alone or in nanocomposite form. Table 3.3 lists the performance of the enzymatic glucose biosensor based on iron oxide nanostructure measured using amperometric technique.

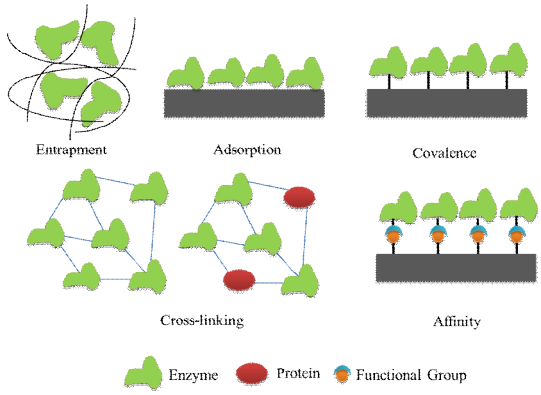

FIGURE 3.4
Schematic of the main methods for enzyme immobilization. E: enzyme, P: inert protein [72]

Physical adsorption consists of simple deposition of an enzyme onto a surface and its attachment through weak bonds. This immobilization strategy has been widely used to develop enzymatic glucose biosensors. Sanaeifar et al. [39] reported the self-assembly of GOx-PVA-iron oxide nanoparticles on the tin (Sn) electrode for glucose biosensor applications. First, GOx was physically absorbed on the nanocomposite (PVA-iron oxide nanoparticles) before the mixture of GOx-PVA-iron oxide nanoparticles was drop-casted on the Sn electrode. The dispersion of iron oxide

nanoparticles in PVA matrix not only promotes great electron transfer between enzyme and electrode but also provides a biocompatible environment for enzyme immobilization. The performance of GOx/PVA-iron oxide nanoparticles/Sn bioelectrode could measure glucose in wide linear range from 5 μM to 30 mM, high sensitivity of 9.36 μAmM^{-1} and exhibited a low detection limit of 8 μM. A more complex nanocomposite has been developed by Baby and Ramaprabhu [1]. The nanocomposites of iron oxide nanoparticles@SiO_2-MWCNT, GOx enzyme, and nafion layer were immobilized layer by layer on the glassy carbon electrode (GCE). MWCNT was synthesized through CVD method and was further functionalized. The functionalized MWCNT was then decorated with iron oxide nanoparticles and uniformly coated with SiO_2 layer. Surface functionalization MWCNT with iron oxide nanoparticles enhanced the sensitivity in glucose sensing due to large surface area and the high porosity and reactivity that made them as an ideal candidate for the storage of neutral species as well as electron donors when used as electrodes in electrochemical reactions. The role of SiO_2 coating is to improve the biocompatibility and solubility of the nanocomposite. GOx enzyme can be effectively immobilized on the iron oxide nanoparticles@SiO_2-MWNTs/GCE through physical absorption to produce a fast direct electron transfer. The immobilized GOx maintained its bioactivity and native structure. The resulting glucose biosensor displayed a high sensitivity (58.9 $\mu AmM^{-1}cm^{-2}$) and a wide linear range from 1 μM to 30 mM for glucose determination and can efficiently exclude the interference of commonly coexisting uric acid and ascorbic acid.

The combination of self-assembly method and electrode position method was reported by Li et al. to modify the electrode with nanocomposite for glucose detection [73]. The magnetic nanocomposite of iron oxide nanoparticles-MWCNT-chitosan was prepared and self-assembled on GCE. Then, Pt nanoparticles were electrodeposited followed by adsorption of GOx enzyme at the surface of the electrode by physical absorption and cover up with nafion layer. A sensitive and selective amperometric glucose biosensor was obtained, because iron oxide nanoparticles-MWCNTs act as efficient conductor for electron and effective support for Pt nanoparticles. The combination of iron oxide nanoparticles-MWCNTs and Pt nanoparticles in the biosensor results in the improvement of analytical performance, characterized by broad linear range (6 μM–6.2 mM) and lower detection limit (2 μM) for glucose determination, as compared to those MWCNTs and iron oxide nanoparticle-based glucose biosensors. Li et al. [49] developed a glucose biosensor based on iron oxide nanoparticles-Au nanoparticles-chitosan composite on the Au electrode through one-step electrode position as a novel matrix for the immobilization of GOx. The electrode position method has the advantage of controlling the film thickness of the nanocomposite at the moderate condition and in a facile manner. The modified electrode showed high stability and excellent electrocatalytic activity of glucose detection with linear response in the range between 3 μM–0.57 mM and detection limit of 1.2 μM. The simple electrode fabrication methodology and cheap materials made the biosensor low-cost and promising for mass production.

Enzyme immobilization through cross-linking bonding formed strong chemical binding between biomolecules. The enzyme can be either cross-linked with each other or in the presence of a functionally inert protein, such as BSA. However, the main drawback is the possibility of activity losses due to the distortion of the active enzyme conformation and the chemical alterations of the active site during crosslinking between enzyme/cross-linker. Yang et al., 2009 [37] have employed this method by crosslinking GOx with chitosan medium using glutaraldehyde. The biosensor of Nafion/Chitosan-iron oxide nanoparticles-GOx/Pt was developed through layer–by-layer assembly. The electrode performance in glucose detection showed high sensitivity of 11.54 $\mu Acm^{-2}mM^{-1}$, low detection limit (6 μM), and good storage stability. A linear calibration plot was obtained in the wide concentration range from 6 μM to 2.2 mM. The wide detection range and high sensitivity may be

assigned to the amplification of the magnitude of current response because iron oxide nanoparticles could catalyze the reaction of H_2O_2. The modified electrode could virtually eliminate the interference during the detection of glucose and was successfully applied to detect glucose in human serum sample. Chen et al. [74] have reported the bienzyme glucose biosensor based on three-layer Au nanoparticles-iron oxide nanoparticles@SiO_2 magnetic nanocomposite crosslink to the GOx enzyme and horseradish peroxidase (HRP) for ITO electrode modification. Nafion and glutaraldehyde have been used as the crosslinker. The homogenous mixture of Nafion-GOx-HRP/Au nanoparticles–iron oxide nanoparticles@SiO_2 were magnetic-assisted self-assembled on the ITO electrode. The proposed method, wherein a mediator transfers electrons between the enzyme and electrode, showed remarkably enhanced sensitivity and selectivity for glucose biosensing in the presence of excess ascorbic acid and uric acid as inference. The high-sensitivity low-detection limit glucose biosensor was developed recently by He et al. [42]. Au nanoparticles/BSA/iron oxide nanoparticle nanocomposite was synthesized and further used to modify the electrodes for glucose detection. BSA protein shell can provide a suitable environment for enzyme adsorption and contribute to its long time bioactivity, whereas Au nanoparticles and iron oxide nanoparticles can promote electron transfer. The enzyme electrode has a fast response time as quick as 0.8 s, excellent sensitivity at 115.3 $\mu AmM^{-1}cm^{-2}$, a high affinity for glucose with a wide linear detection range, and excellent anti-interference property against uric acid and ascorbic acid. The simple fabrication procedure leads to excellent reproducibility among different biosensors prepared under similar conditions.

Enzymes can also be immobilized in three-dimensional matrices, such as an electropolymerized film, an amphiphilic network, a photopolymer, a silica gel, a polysaccharide, or a carbon paste. This immobilization is easy to perform. Enzyme, mediators, and additives can be simultaneously deposited in the same sensing layer. No modification of the biological element was performed so that the activity of the enzyme is preserved during the immobilization process. Biosensors based on physically entrapped enzymes have several advantages, such as improved stability and robustness. However, physically entrapped enzyme in glucose biosensor fabrication has some limitations, such as leaching of biocomponent and possible diffusion barriers that can restrict the performance of glucose biosensor. Peng et al. [75] have employed the physically entrapped GOx enzyme in polymerized nanocomposite of GOx-Au nanoparticles-polydopamine-iron oxide nanoparticles for the glassy carbon electrode modification. The modified electrode not only has the magnetism of iron oxide nanoparticles that makes them easily manipulated by an external magnetic field but also has the excellent biocompatibility of polydopamine to maintain the native structure of the GOx and good conductivity of Au nanoparticles, which can facilitate the direct electrochemistry of GOx in the biofilm. Hence, the presence of GOx–Au nanoparticles–polydopamine–iron oxide nanoparticles biofilm displays good linear amperometric response to glucose concentration ranging from 0.02 mM to 1.875 mM.

Non-enzymatic Glucose Biosensors based on Iron oxide Nanostructures

Non-enzymatic glucose biosensors with excellent stability, low cost, and ease of fabrication have gained increasing attention for glucose biosensor applications because enzymatic glucose biosensor constantly suffer with several limitations, such as instability, expensiveness, and loss of activity during reuse because of washing and drying steps. Among all metal oxide nanostructure materials that have been used to develop non-enzymatic glucose biosensor, iron oxide nanostructure offers considerable attention, because non-enzymatic glucose biosensor based on iron oxide nanostructure exhibited wide linear range, highly reproducible response, and long-term stability.

However, the performance of iron oxide nanostructure glucose biosensors is usually compromised by the poor conductivity of iron oxide and irreversible aggregation of the dispersed nanomaterials. Table 3.4 lists the performance of the non-enzymatic glucose biosensor based on iron oxide nanostructure measured using amperometric techniques.

The mechanism of the non-enzymatic glucose biosensor based on iron oxide nanostructure is explained by electron hopping between Fe^{3+} and Fe^{2+} at specific structure of iron oxide that aids in accelerating the electron transfer reaction. The reactions 2–4 below describe the glucose catalytic reaction. First, glucose is catalytically oxidized by the 2 Fe(III) species and produces gluconolactone. Gluconolactone is converted into gluconic acid, and Fe(II) is electrochemically oxidized into Fe(III) species:

$$2Fe(III) + Glucose \rightarrow 2Fe(II) + Gluconolactone + H_2O \quad (2)$$

$$Gluconolactone + H_2O \rightarrow H^+ + gluconate \quad (3)$$

$$2Fe(II) \rightarrow 2Fe(III) + 2e^- \quad (4)$$

Recently, the 1D nanostructures of iron oxides, such as nanotube arrays, nanorods array, and nanowire arrays, have been developed in modification of electrode for glucose detection. These 1D nanostructures can provide a direct electrical channel for electron transport and do not suffer from the irreversible aggregation. Zhang et al. [51] reported the non-enzymatic glucose biosensor based on iron oxide nanorod arrays prepared by electrochemical anodization of iron foil, followed by *in situ* annealing under hydrogen flow. The high performance of the array is attributed to the improved electron transfer pathway and the cooperative electrochemical oxidation of glucose by Fe(III) and Fe(II) species. The high sensitivity of the glucose biosensor has been developed with linear range of 0.5–3.7 mM and detection limit of 0.1 μM. The sensor is stable and can be applied to real sample analysis with minimum interferences. Chen et al. [47] reported recently on the non-enzymatic glucose biosensor based on iron oxide nanotubes developed on the FTO electrode. The nanotube arrays are indexed to the cubic phase of Fe_3O_4 that has high conductivity. The directly grown iron oxide nanotube array on FTO electrode is mechanically stable and provides excellent electrochemical sensing performance due to many transport channel of nanometer scale provided by iron oxide nanotubes for glucose accessibility thus enhanced the electron transfer between glucose and iron oxide nanotubes. The iron oxide nanotube-modified electrode displayed a high sensitivity of 673.3 $\mu AmM^{-1}cm^{-2}$.

Baby and Ramaprabhu [1] reported the non-enzymatic amperometric glucose biosensor based on SiO_2-coated iron oxide nanoparticles dispersed on MWCNT. The glucose biosensor showed high sensitivity to glucose detection with linearity from 3 μM to 14 mM and high specificity where no interference from uric and ascorbic acids. In improving the selectivity of the electrode, thin nafion layer (0.5%) was coated on the iron oxide nanoparticles@SiO_2/MWNT/Glassy carbon electrode. The electrons generated from the biochemical reactions would transfer to the iron oxide nanoparticles@SiO_2/MWNT/ Glassy carbon electrode through the Fe^{2+}/Fe^{3+} couples. The iron oxide nanoparticles-SiO_2/MWNT acts as electron-transfer mediator that helps to enhance the current response of electrode and decrease the redox potential for glucose oxidation/reduction reaction. The following section discusses on the applications of ZnO nanostructure in the modification of electrode for glucose biosensor applications.

TABLE 3.3

Performance comparison of enzymatic glucose biosensors based on iron oxide nanostructure-modified electrodes

Electrode Modification	Nanoparticles Assembly Method/ Enzyme Assembly Method	Applied potential/ Reference Electrode	Linearity	Sensitivity ($\mu AmM^{-1}cm^{-2}$)	LOD	Km	Detection time	Reference
GOx-PVA-IONPs/Sn	Self-assembly/ Physical absorption GOx	-0.19 V vs Ag/AgCl	5 µM–30 mM	9.36	8 µM	1.42 mM	10 s	[39]
Nf/GOx/Pt/ IONPs-MWCNTs-CS/MGCE	Self-assembly & Electrode position/ Physical absorption GOx	+0.30 V vs SCE	6 µM–6.2 mM	-	2 µM	9 mM	8 s	[73]
Nf/GOD/Nf-IONPs@SiO2-MWCNT/GCE	Layer by layer assembly/ Physical absorption GOD	- Ag/AgCl	1 µM–30 mM	-	0.8 µM	13 mM	3 s	[48]
GOx/IONPs-AuNPs-CS/Au	Electrochemical deposition/ Physical absorption GOx	-0.40 V vs SCE	3 µM–0.57 mM	-	1.2 µM	-	6 s	[76]
Nf/CS-IONPs-GOD/Pt	Layer by layer assembly/ Crosslinking GOx-GA	+0.4 V vs Ag/AgCl	6 µM–2.2 mM	11.54	6 µM	-	-	[37]
Nf-GOD-HRP/AuNPs-IONPs@SiO_2/ ITO	MA self-assembly/ Crosslinking GOx-GA	-0.20 V vs SCE	0.05–1.0 mM 1.0–8.0 mM	92.14 15.00	10 µM	-	5 s	[70]
GOx/AuNPs/ BSA-IONPs/Pt	Self-Assembly/ Affinity & crosslinking GOx	+0.40 V vs Ag/AgCl	0.25–7.0 mM	115.13	3.54 µM	15 mM	0.8 s	[42]
GOx/IONPs/CS-Graphene/Pt	Layer by layer assembly/ Crosslinking GOD	+0.5 V vs Ag/AgCl	0–26 mM	5.658	16 µM	-	-	[77]
GOx-Au-PDA-IONPs/MGCE	Self-assembly/ Co-polymerizatio	-0.50 V vs SCE	0.02–1.875 mM	-	6.5 µM	1.67 mM	-	[75]

	n GOx							
GOx/rGO-IONPs/MSPCE	Layer by layer assembly/ Electrostatic interaction GOx	-0.45 V vs Ag/AgCl	0.05–1.0 mM	5.90	0.1 µM	0.16 mM	3 s	[56]
GOx-IONPs@AuNP / MnO_2/SPCE	MA self-assembly/ Chemisorption GOx	+0.38 V vs Ag/AgCl	0.2–9.0 mM	2.52	13.2 µM	-	-	[71]

Abbreviations: CS, chitosan; IONPs, iron oxide nanoparticles; GOD and GOx, glucose oxidase; Pt, platinum electrode; Au, gold nanoparticles; HRP, Horseradish peroxidase; GS, graphene sheet; SPCE, screen-printed carbon electrode; MnO_2, manganese oxide, GA; glutaraldehyde, MWCNT; multiwalled carbon nanotube, PDA; polydopomine, r-GO; reduced graphene oxide, MA; magnetic assisted, MGCE; magnetic glassy carbon electrode, GCE; glassy carbon electrode

TABLE 3.4

Performance comparison of non-enzymatic glucose biosensors based on iron oxide nanostructure-modified electrodes

Electrode Modification	Nanoparticles Assembly Method/	Applied potential/ Reference Electrode	Linearity	Sensitivity ($\mu AmM^{-1}cm^{-2}$)	LOD	Detection time	Reference
Nf/IONPs@ SiO_2/MWNT/ GCE	Layer by Layer assembly	Ag/AgCl	3 µM–14 mM	54.42	1 µM	7s	[1]
1DIONRs-Array/foil	Electrochemical anodization	+0.6 V vs SCE	0.5 µM–0.77 mM 0.76–3.67 mM	406.9 134.1	0.1 µM	8 s	[51]
IO-ZNRs/FET	Hydrothermal Growth	-	0.05–22.0 mM	105.75	12 µM	-	[35]
IONTs-Array/FTO	Hydrothermal Growth	+0.6 V vs Ag/AgCl	0.1 µM–0.125 mM 0.125–1.0 mM 1.0–5.0 mM	673.3 71.2 9.58	0.1 µM	-	[47]

Abbreviations: IO, iron oxide; IONPs, iron oxide nanoparticles; IONRs, iron oxide nanorods array; ZNRs, zinc oxide nanorods; SiO2, silica; MWCNT, multiwalled carbon nanotube; GCE, glassy carbon electrode; FET, field effect transistor; FTO, fluorine-doped tin oxide

Zinc Oxide-based Glucose Biosensors

Synthesis and Properties of Zinc Oxide

Significant advances have been achieved in using metal oxide nanomaterials as alternative matrices for glucose biosensor development, particularly zinc oxide nanomaterial owing to its outstanding properties. Zinc oxide is classified as a semiconductor in groups II–VI and crystallizes in either rock salt, zinc blende (cubic structure), or wurtzite (hexagonal structure), and normally crystallize in wurtzite structure under normal conditions (Fig. 3.5) [78-80]. ZnO structure is composed of two interconnecting sub lattices of Zn^{2+} ions surrounded by tetrahedral O^{2-} ions and vice versa. At room temperature, ZnO exhibits a direct band gap of 3.37 eV with a large exciton energy of 60 meV [80, 81]. As an oxide semiconductor, zinc oxide is highly interesting for a range of sensors – from gas sensors to biological sensors. In addition, ZnO has the high ability to react with oxygen that makes ZnO one of the best candidates to be used in electrochemical sensors [82]. Nanostructure ZnO also possesses a good chemical stability, low toxicity, intrinsic hydrophilicity, and high electron mobility [83-86]. Ultimately high isoelectric point (IEP) approximately pI∼9.5 of ZnO results in a unique ability for absorption of enzymes with low IEP, particularly glucose oxidase (GOx) (pI∼4.2) [86]. With low IEP, enzyme immobilization will be driven by simple physical adsorption and electrostatic interaction [85, 86].

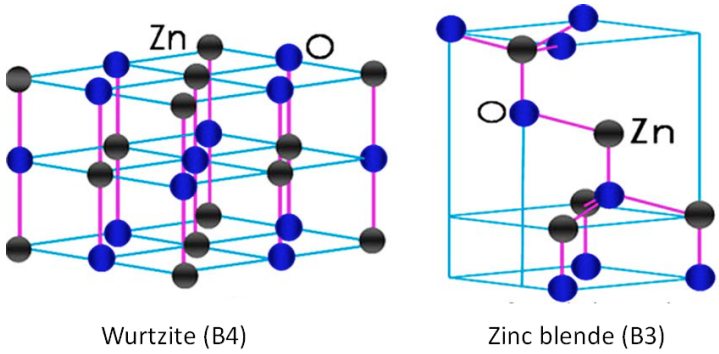

FIGURE 3.5
Schematic of ZnO crystal structure; wurtzite and zincblende. The Zn and O atoms are marked as well with ash and blue circles in the schematic, respectively [87]

Owing to its unique properties, ZnO nanostructures have gained increasing attention for fundamental research and for potential device application with various structures of nanometric. ZnO can occur in 1D, 2D, and 3D structures by modifying the growth conditions. Hence, numerous methods have been conducted on synthesizing ZnO nanostructure. Yang et al. [88] classified the ZnO fabrication methods into two groups: wet-chemical/solution and physical methods [88]. The former approach included hydrothermal/solvothermal process and capping agent/surfactant-assisted synthesis. These methods offer a low-temperature pathway and simple process compared to the physical technique, which usually requires high temperature, high pressure, and low-product yield. Several physical methods include template-directed synthesis, vapor-liquid-solid (VLS) and vapor-solid (VS) process [88]. Table 3.5 summarizes the method in obtaining ZnO nanostructure. Solution-based approach has gained much interest for ZnO nanostructure growth and normally

uses dominant zinc sources, such as zinc acetate dihydrate, zinc acetate, zinc nitrate, and zinc chloride dissolved in alcoholic or other organic solvents as starting solutions [89]. Basic solution, such as sodium hydroxide (NaOH) or potassium hydroxide (KOH), is normally added under vigorous stirring where refluxing of the mixture is optional [90-93]. Several authors have utilized this method in producing ZnO nanoparticle, which is further used as matrices in glucose biosensor construction due to its simple and low temperature (<200 °C) process in producing ZnO [90-95]. Vijayaprasath et al. [93] prepared cobalt-doped ZnO by co-precipitation aqueous solution from zinc acetate dehydrate, cobalt acetate dehydrate, and sodium hydroxide. They observed a transformation of flower-like morphology obtained from pristine ZnO to spherical particles as the concentration of Co increases. For Co-doped ZnO, the incorporated Co ion into Zn lattice sites caused the flower-like morphology converted to agglomerated small particles, which then bound to one another to form spherical shape to lower the surface energy [93]. Kamaldeep et al. [90] and Kumar et al. [91] have prepared ZnO nanoparticles with similar precursor but different in reaction temperature, which resulted in different sizes of ZnO nanoparticles produced, namely, 120 and 21.6 nm. This apparent differences owing to a higher reaction temperature increases the rate of decomposition of zinc-hydroxo-complex to form ZnO, resulting in the growth and coarsening of the end product [96]. Sol-gel method offers a simple, low cost, and relatively mild conditions for ZnO films and colloidal structure [97]. In general, the formation of ZnO nanostructure through sol-gel process involves transformation of molecular precursor into an oxide network by hydrolysis and condensation reaction [98]. The significant advantage of sol-gel method is that high purity products can be produced as the reactant used is available in high purity materials. In addition, homogeneous compositions of sol and gel can be prepared at low temperatures, which is economic [99]. Using this approach, Anusha et al. [15] produced ZnO thin film using zinc acetate dihydrate and 2-methoxyethanol as precursor and solvent, respectively. They obtained a high crystallinity of fabricated film with hexagonal wurtzite phase. Meanwhile, Xia et al. [100] produced ZnO inverse opal structure (IOs) by sol-gel method using polymethylmethacrylate as a template [100]. XRD analysis showed that produced ZnO matched well to the hexagonal ZnO phase.

Hydrothermal method is commonly used to synthesize ZnO because it features low cost, lack of sophisticated equipment requirement, and environment friendliness [101, 102]. Hydrothermal reaction is normally performed under relatively lower temperatures (<100 °C), and therefore this method is suitable for ZnO synthesis on flexible/soft plastic and paper substrates [101, 102]. Fung et al. [103] has grown ZnO nanowires through hydrothermal on flexible plastic substrate. ZnO seed layer was printed onto a plastic substrate through flexographic printing-assisted growth of nanowires. Prior to hydrothermal growth, hydrothermal solution and a beaker of deionized water was heated up to 70 °C for 30 min. After 30 min, the substrate was immersed in deionized water for 5 to 10 s to warm the substrate. Afterward, the substrate was immediately transferred into the growth solution. This additional step ensures that nanowires were only grown inside the printed seed region [103]. Karuppiah et al. [104] demonstrated the growth of ZnO nanorod and CuO nanosphere composites by hydrothermal reaction. Uniform formation of both oxides was assisted by the presence of polyethyleneimine (PEI). PEI is a cationic polymer that acts as stabilizing and/or reducing agent for nanocomposite growth. Chu and co-workers [105] had introduced Ni into ZnO lattice as a method to improve the catalytic activity of sensor performance. In their work, the effect of different mole ratio of nickel (II) nitrate hexahydrate use as precursor solution was investigated. The hydrothermal solution was sealed in Teflon reactor and stored for 10 h at 119.85 °C. The diameter rods decreased with increasing doped NiO in ZnO as the dopant ions inhibited the growth of ZnO crystal. Different morphologies and properties can be attained by varying and controlled synthesis parameters. To control the aspect ratio (AR) of the produced ZnO nanorods, Ahmad et al.

[106] varied the hydrothermal growth duration from 2 h to 14 h. The value of AR was calculated by dividing the average length with average diameter of individual ZnO nanorods. In general, a few parameters exist, which significantly affect the morphology and aspect ratio of ZnO, including concentration and pH of precursor, growth temperature, growth time, and substrates [107]
Through physical technique, ZnO can be synthesized in different morphologies, for instance, using thermal evaporation method. In general, thermal evaporation is a simple process that involves heating a solid material to a certain temperature sufficient to turn the solid material into vapor phase. This evaporated material travels along the furnace and deposited onto desired substrate[80]. The process is normally performed in a horizontal tube furnace. Different morphologies of ZnO can be obtained by modifying the process of thermal evaporation: temperature, pressure, substrate, carrier gas (gas type and flow rate), and evaporation time [80]. Gallay et al. [108] produced ZnO nanowires with an average length of 2–6 µm at a reaction temperature of 1050 °C. Wang et al. [109] produced a ZnO nanocomb with an average stem length of 10 µm at 900 °C. Growth temperature determines the kinetic energy and quantity of the reactive vapor generated and the surface diffusion length of the adsorbed vapor types. Evaporation temperature has an effect on various degrees of supersaturation of ZnO in its gaseous state [110]. Thus, different structures of ZnO can be produced by varying evaporation temperature, evaporation time, carrier gas, and pressure used [80].
Different morphologies considerably affect the properties of nanostructures themselves. Therefore, gaining the control over the size and morphology of the grown ZnO is important. Synthesis methods must be simple, economically effective and high yield, and compatible to work well in industrial scale. The summary of the most frequently used methods for ZnO synthesis for glucose biosensor fabrication are discussed in the following section.

TABLE 3.5
Summary of methods for obtaining zinc oxide as matrices in glucose biosensors

Method	Precursor	Synthesis condition	Morphology/Properties	Ref
Aqueous solution / Chemical bath deposition	$Zn(NO_3)_2{\cdot}4H_2O$ + NaOH	Reaction temperature: 55 °C	Spherical nanoparticle/ Ave size of 120 nm	[90]
		Room temperature reaction	Spherical nanoparticle / Ave size of 21.6 nm	[91]
	$Zn(CH_3COO)_2.2H_2O$ + NaOH $Co(CH_3COO)_2.2H_2O$ (doping precursor)	Reaction temperature: 80 °C	Flowerlike morphology for pure ZnO Transformation to spherical shape as doping concentration increase	[93]
Sol-gel	$Zn(CH_3COO)_2{\cdot}2H_2O$ + MEA + $C_3H_8O_2$	Stirred for 1 h at 65 °C / aging 5 h	Thin film Average particle size of 48 nm	[15]
	$Zn(NO_3)_2.6H_2O$ + C_2H_5OH + $C_6H_8O_7$ +TEOS	Stirred until becoming the colorless transparent solution	Inverse opal structure Wall thickness of 200 nm	[100]
Hydrothermal	$Zn(NO_3)_2{\cdot}6H_2O$ + HMT	Reaction : 4 h / 90 °C	Nanoflake morphology with wall thickness of 200	[111]
	$Zn(NO_3)_2.6H_2O$ + $Ni(NO_3)_2.6H_2O$ (doping precursor) + C_2H_6O	Ni doping concentration Reaction: 10 h / 119.85 °C	Nanorods Ave. length: 1–1.4 µm / Ave. diameter: 20–100 nm Increase Ni doping decrease diameter size	[105]

	$Zn(NO_3)_2.6H_2O$ + HMT NaOH (dissolution solution)	Seeded substrate Reaction: 2 h / 90 °C Dissolution reaction: 1.5 h / 35–95 °C	Nanorods Average diameter: 395±15 nm Nanotube Perfect hexagonal tubular structure obtain at 65 °C	[86]
	$Zn(NO_3)2.6H_2O$ + $Cu(NO_3)_2{\cdot}3H_2O$ + NH_4OH polyethyleneimine (PEI)	PEI as stabilizing or reducing agent for nanocomposite growth Reaction: 2 h / 120 °C / Annealed: 2h / 300 °C	Nanorods (ZnO) Ave. length: 100–530 nm/Ave. diameter: 60 nm Nanosphere (CuO) Average diameter : 150–550 nm	[104]
	$Zn(NO_3)2.6H_2O$ + deionized water + HMT	Seeded substrate Growth solution was ultrasonic and filtered before used Reaction: 4 h / 85 °C / Dried: 15 min / 300 °C	Nanorods High density and good alignment of the as-grown nanorods	[112]
Hydrothermal	$Zn(CH_3COO)_2{\cdot}2H_2O$ + NH_3	Sol-gel seeded substrate Precursor concentration: 0.02–0.05 M and 1.4–5.8 ml, respectively Reaction: 2 h / 80 °C	Nanowires Average length: 650 nm Average diameter: 100–150 nm	[83]
	$Zn(CH_3COO)_2{\cdot}2H_2O$ + HMT	Printed seed layer using zinc acetate precursor ink Preheating substrate in DI water at 70 °C before hydrothermal Reaction: 6 h / 70 °C	Patterned growth of nanowires Average length: 2 µm	[103]
	$Zn(CH_3COO)_2{\cdot}2H_2O$ + HMT	Spray coated seed solution by air brush with substrate temperature at 80 °C Reaction: 1 h / 90 °C	Nanorods Average length: 150 nm Average diameter: 30 nm	[85]
Solvothermal	$Zn(NO_3)_2.6H_2O$ + $AgNO_3$ + C_2H_5OH + NaOH	Different Ag concentration Stir in Teflon-lined stainless steel autoclave for 10 min Reaction: 12 h / 120 °C / Dried for 12 h / 80 °C	Nanorods Increase $AgNO_3$, rods become shorter, Ag nanoparticle become larger and inhomogeneous	[113]
Electrode position	$Zn(NO_3)_2.6H_2O$ + KNO_3	Reaction: -0.6 V / 200 sec	Spherical nanoparticle Average diameter: 80±11	[114]
Electrode position	$Zn(NO_3)_2.6H_2O$ + HMT	Reaction: -0.8 V / 7000 sec / 80 °C	Nanorods Average length: 2–3 µm / Average diameter: 200 nm	[115]
	$Zn(NO_3)_2.6H_2O$	Firstly ZnO	Hexagonal prisms (ZnO)	[116]

	+ HMT $NiSO_4$ (doping precursor)	Reaction: −0.9 / 4000 sec / 70 ℃ Second Ni doping Reaction: −0.9 / 60 s / 70 °C	Average diameter: 1 µm Nanoparticle (Ni) Average diameter: 100 nm	
	$ZnCl_2$ + KCl	Nanorods reaction: -1.0 V / 2000 sec / 85 ℃ / continuous O_2 Nanotube reaction: 1.1 V / 1 h / 85 °C	Nanotube Well defined hexagonal Average diameter: 300 nm	[117]
Thermal evaporation	ZnO powder + graphite flake	Reaction: 1050 °C / 40 min / under a steady flow Ar + O_2 gas	Nanowires Average length: 2–6 µm Average diameter: 65 nm	[108]
		Reaction: 900 °C / 30 min / under a steady flow Ar + O_2 gas	Nanocombs Thickness stems of nanocombs: 50 nm Length of main stem: 10 µm	[109]
Pulsed laser deposition (PLD)	Target pellet (1 in. diameter) ZnO powder + 3% of KFCN	Ablating with fourth harmonic of Nd-YAG laser (λ= 266 nm) / fluence of 1.2 J cm^{-2} / 50mT oxygen pressure	Composite thin film Thickness: 80 nm	[118]
Electrospinning	$Zn(CH_3COO)_2{\cdot}2H_2O$ + PVP	Distance between precursor needle and collector = 10 cm Power: 15 kV Calcination: 5h / 700 °C	Single nanofiber Average diameter: 195 nm	[119]

Abbreviations: ZnO, Zinc Oxide; $Zn(NO_3)_2{\cdot}4H_2O$, Zinc nitrate tetrahydrate; $Zn(NO_3)_2{\cdot}6H_2O$, Zinc nitrate hexahydrate; $Zn(CH_3COO)_2.2H_2O$, Zinc acetate dehydrate; $ZnCl_2$, Zinc chloride; NaOH, Sodium hydroxide; $Co(CH_3COO)_2.2H_2O$, Cobalt(II) acetate dehydrate; $Ni(NO_3)_2.6H_2O$, Nickel (II) nitrate hexahydrate; $Cu(NO_3)_2{\cdot}3H_2O$, Copper (II) nitrate trihydrate; $AgNO_3$; Silver nitrate; $NiSO_4$, Nickel (II) sulfate hexahydrate; MEA, monoethanolamine; $C_3H_8O_2$, 2-Methoxyethanol; C_2H_5OH, Ethanol; $C_6H_8O_7$, Citric Acid; TEOS, Tetraethoxysilane; HMT, Hexamethylenetetramine; PEI, Polyethyleneimine; KCl, Potassium chloride; KFCN, Potassium ferrocyanide; PVP, Polyvinylpyrrolidone

Zinc Oxide Nanostructure in Glucose Biosensor

ZnO nanostructure plays an important role by providing a solid support and method for enzyme immobilization. A difference in nanostructure morphology significantly affects the glucose detection performance, therefore various morphologies of ZnO have been investigated as matrices for application in glucose biosensor [120]. Table 3.6 summarizes the difference in ZnO nanostructure morphology on glucose biosensor performance. For example, ZnO nanotetrapods were used as matrices for GOx immobilization in glucose biosensor [121, 122]. ZnO nanotetrapod is a quasi 1D nanostructure that possesses four crystalline legs. In both works by Lei et al. [122] and Loan et al. [121], ZnO nanotetrapods were produced through vapor phase transport method, then transferred onto standard Au electrode conventionally used in electrochemistry for glucose biosensor fabrication. Lei et al. [122] obtained a high sensitivity of 25.3 µA/mMcm^2 with LOD of 4 µM of glucose biosensor performance. This result was attributed to the larger specific area of ZnO

tetrapods provided for GOx immobilization while maintaining the affinity of GOx and the direct electrochemistry path provided by multiterminal structure of individual ZnO tetrapods. Hashim et al. [123] prepared a simple and direct thin ZnO nanofilm on Si substrate through RF sputtering and investigated the amperometric direct responses to glucose. High current response was due to sputtered ZnO film that is highly matched to the Si substrate, which accelerates the electron transfer. In comparison, Saha et al. [118] used a thin film of ZnO–potassium ferricyanide (ZnO–KFCN) composite film and ZnO film as matrices for glucose detection. Pure ZnO film and composite film of ZnO-KFCN were prepared by pulsed laser deposition (PLD) on ITO glass substrate. The authors suggested that ZnO–KFCN composite thin film proven a promising matrix for glucose detection as electron communication enhanced with the presence of redox mediator in the matrices itself. The modified composite film electrode exhibited a quasi-reversible system owing a KFCN presence in matrix itself.

You et al. [124] and Xia et al. [100] employed an inverse opal structure in glucose biosensor fabrication. In general, inverse opal is a 2D structure with periodical porous structures on purpose of obtaining the larger effective surface area, which are beneficial for molecule diffusion and mass transport in sensor materials [125]. You et al. [124] showed that the inverse opal structure would reduce more the glucose diffusivity compared with nanowires structure. The structure would allow diffusion-limited glucose oxidation on ZnO even at high bulk glucose concentration thus increasing the linear detection range of the sensor. In addition, a small size of GOx can traverse transverse through the pore canal and immobilized on the wall of ZnO inverse opal structure [100] due to larger size of macroporous pore. A different 2D structure has been employed as matrices by Fulati and co-workers [111]. They have grown ZnO nanoflake (wall thickness around 200 nm) with a honeycomb-like structure and on the tip of aluminum-coated glass capillary. The resulting matrix exhibits a fast response time of 4 s with a sensitivity of -65.2 mV/decade. This result is due to the honeycomb structure that provides a larger and wide area for GOx to immobilize and a different distribution of reactants on ZnO surface [111]. Recently, Ahmad and colleagues used hierarchically assembled ZnO nanosheet microspheres in biosensor assembly, which showed a remarkably high sensitivity (210.8 $\mu A/mMcm^2$) in the wide linear response range of 0.05–23 mM.

Nanotubes, nanowires, and nanorods from 1D nanostructures feature a considerable research interest as glucose biosensor matrix due to their unique properties and easy fabrication. Lei et al. [126] stated that directly grown matrix on a substrate effectively and efficiently increased the sensitivity of glucose detection. Their work demonstrated that well-aligned nanorods ensure a more direct electron communication path between transducer and electrode than the transferred nanorods, which appeared randomly distributed and stacked with each other. Randomly stacked nanorod is slowing the electron transfer process as electrons must travel and jump between rods before transfer to the electrode. Ahmad et al. [106] and Kim et al. [127] demonstrated the influence of nanorods surface area on the amount of enzyme immobilization and the performance of glucose detection. They showed that by increasing the surface area of rods, a better performance of glucose biosensor was obtained due to the higher surface of rods for GOx to immobilize. Increasing the degree of GOx immobilization is important as it ensures a wide linear range of glucose detection, as well as for high selectivity and fast sensitivity [106]. Besides nanorod structures, nanotubes also become a desirable option as matrix for glucose biosensor construction. In general, nanotubes are a more hollow structure with high porosity and low mass density compared with nanorods due to the extremely large surface area over volume [128]. This unique morphology is expected to efficiently enhance the number of GOx to be immobilized. Several published works [86, 117, 129] employed ZnO nanotubes to immobilize GOx and demonstrated a better performance in glucose biosensor. Zhou et al. [86] compared the performance between

nanorods and nanotubes structure. They found that ZnO nanotube structures provided a better performance due to better hydrophilicity of ZnO nanotubes compared to nanorods that ensure more GOx immobilization and large solid–liquid interface between ZnO nanotube and GOx solution.

TABLE 3.6
Summary of glucose biosensors based on ZnO nanostructure

ZnO morphology	Electrode matrix	Immobilization mode	Sensitivity ($\mu A/mM^1.cm^2$) / Linear range (M)	LOD (M) / Response time (s)	Ref
TPSP	Nafion/GOx/TPSP-ZnO/GCE	Adsorption	- / 0.05 – 8.2 m	0.01 m / -	[130]
Nanoparticle	ZnO-BCA/Au	-	38.133 / 1 – 10 m	- / < 5	[95]
Nanoparticle	ZnO/CPEs	Crosslink	- / 0.02 – 16 m	9.1 µ / -	[131]
Inverse opal	GOx/ZnO-IOP/Au/Glass	Adsorption	22.5 / 0.01 –18 m	-	[124]
Inverse opal	Nafion/GOx/ ZnO IOP/FTO	Adsorption	8.62 / 0–10 m	-	[100]
Nanosheets in microspheres	Nafion/GOx/ HAZNMs/Ag/Si	Adsorption	210.8 / 0.05 –23 m	50 µ / -	[132]
Nanoflake in honeycomb structure	Nafion/GOx/ZnO/ Ti/Al/Borosilicate glass capillaries	Crosslink	−65.2 mV/decade / 500 n – 10m	- / 4	[111]
Nanofilm	GOx/ZnO/Si	Adsorption	50 / 500 µ – 21 µ	-	[123]
Single nanofiber	L-Cys/GOx/PVA/ ZnO/Au	Adsorption	70.2 / 0.25 – 19 m	1 µ / <4	[119]
Nanotube	Nafion/GOx/ ZnO/ITO	Adsorption	30.85 / 10 µ – 4.2 m	10 µ / <6	[117]
Nanotube	Nafion/GOx/ZnO/ Au	Crosslink	21.7 / 50 µ – 12 m	1 µ / 3	[129]
Nanotube	GOx/ZnO/AuCS	Adsorption	2.63 / 0– 6.5 m	8 µ / -	[86]
Nanotube	Nafion/GOx/ ZnO/glass coated Au	Adsorption	69.12 mV/decade / 0.5 µ– 12 m	- / <4	[133]
Nanowire	Nafion/GOx/ ZnO/Si	Crosslink	17.2 / 0– 1 m	<0.02 m / <12	[134]
Nanowire	GOx/ZnO/Ag IDE	Crosslink	-	1.66 m / <3	[135]
Nanowire	GOx/ZnO/SiNW	Adsorption	129 / 0– 30 m	12 µ / 5	[83]
Nanorod	Nafion/GOx/ ZnO/Au/SiO_2/Si	Adsorption	315 / 1– 10 m	166.6 µ / 2	[112]
Nanorod	GOx/ZnONWs/ Au/PET	Adsorption	19.5 / 0.2– 2 m	<50 µ / <5	[136]
Nanorod	Nafion/GOx/ ZnO/Ag/Si	Adsorption	110.76 / 0.01– 23 m	0.1 µ / 2	[106]
Nanocomb	Nafion/GOx/ ZnO/Au	Adsorption	15.33 / 0.02– 4.5 m	0.02 m / <10	[109]
Nanotetrapod	Nafion/GOx/ ZnO/Au	Adsorption	25.3 / 0.005–6.5 m	4 µ / <6	[122]
Nanotetrapod	PS mixed with ZnO/GO*x* /ZnO/Au	Adsorption	- / 1–6 m	0.5 m / -	[121]

Abbreviations: GOx, Glucose oxidase; TPSP, Tetragonal pyramid-shaped porous; ZnO, Zinc oxide; GCE, Glassy carbon electrode; BCA, Butyl carbitol acetate; CPE, Carbon paste electrode; IOP, Inverse opal; FTO, Fluorine-doped Tin Oxide; IDE, Interdigitated electrode; AuCS, Au cylindrical spiral; SiNW, Silicon nanowire; PET, Polyethylene terephyhlalate; SiO_2, Silicon Oxide; HAZNM, Hierarchically assembled ZnO nanosheet microspheres; Au, Gold; Ag, Silver; Si, Silicon; Al, Aluminum; Ti, Titanium; PVA, Polyvinyl alcohol; PS, Polystyrene

Zinc Oxide Nanostructure/Hybrid in Glucose Bionsensors

Important advances have been achieved for combining and integrating nano-composites as individual component to enhance electron communication and improve the performance of glucose biosensor. The relatively poor conductivity of ZnO affects the glucose biosensor performance, thus the integration. Hybridization and doping of ZnO have been investigated. Several studies on ZnO hybrid with carbon-based materials [137-139], metal-based materials [115, 140-142], and metal transition-based materials [18, 109, 116] have been conducted. Karuppiah et al. [114] and Gallay et al. [108] reported the incorporation of ZnO nanomaterial with graphite by electrode position and vapor transport method, respectively. Graphite is an anisotropic material with a highly reactive large edge planes with insert basal plane defects, which can be highly reactive after the electrochemical pre-treatment and excellent as a matrix for the incorporation of nanoparticles [138]. In their work, ZnO nanoparticles with average size of 80±11 nm were incorporated onto graphite nanosheet through electrode position. ZnO nanoparticles are homogeneously distributed on graphite nanosheets, resulting in a high surface coverage concentration of GOx and high heterogeneous electron transfer rate of 3.75 s^{-1}. Meanwhile, Gallay et al. [108] employed a self-sustained film of entangled ZnO nanowire grown on compacted graphite flakes using vapor transport method without metal catalyst required. The authors claimed that a high surface area of ZnO nanowire embedded on graphite sheet was suitable for immobilization of GOx. However, Gallay et al. [108] reported a lower sensitivity of 17 $\mu A/mM^{-1}$ cm^{-2} compared with Karuppiah et al. [114] that reported a sensitivity of 30.07 $\mu A/mM^{-1}$ cm^{-2}. A lower sensitivity was recorded by Gallay et al. [108] compared with Karuppiah et al. [114] that could be due to the loss of GOx/ZnO-graphite composition during transfer to Pt electrode. Palanisamy et al. [143] reported the difference of carbon-based material use as a hybrid with ZnO nanostructure. In their first work, ZnO microflowers were electrode posited onto reduced graphene oxide (rGO) and later they used ZnO microsponge deposited on multiwalled carbon nanotubes (MWCNT). ZnO/rGO electrode exhibited a sensitivity of 18.97 $\mu A/mM^{-1}$ cm^{-2} (range between 0.02 and 6.24 mM), whereas ZnO/MWCNT electrode exhibited a sensitivity of 4.18 $\mu A/mM^{-1}$ cm^{-2} (range between 0.2 and 27.2 mM). The high sensitivity of ZnO/rGO compared with ZnO/MWCNT was due to 2D structure of rGO. Unlike MWCNT, rGO monolayer structure has its whole volume exposed to the glucose environment resulted in maximum glucose detection.

Metal nanoparticle (MNP) incorporation with ZnO for matrix can significantly increase glucose biosensor performance, due to the excellent catalytic properties of metal nanoparticles to more efficiently catalyze the glucose electrode [144]. One of the most preferable metal nanoparticles to be hybrid with ZnO was gold nanoparticles (AuNPs) due to the unique properties of AuNPs, such as catalytically active property, stability, and good biocompatibility [140, 145, 146]. Wei et al. [146] incorporated AuNPs onto ZnO NRs surface through hydrothermal method. Hybrid matrix was then subjected to GOx immobilization, wherein crosslink method was employed. The produced AuNPs/ZnONRs electrode exhibited low Michaelis–Menten constant (K_m) of 0.41 mM. This result showed that AuNPs/ZnONRs electrode possesses a good biocompatibility with GOx enzyme to be

immobilized and retain its properties. K_m value shows how compatible matrix is used with a given enzyme. High K_m values indicate that the enzyme does not bind efficiently with the matrix [147]. Zhao et al. [145] incorporated AuNPs synthesized by photo-reduction method onto ZnO NRs/FTO matrix that was prepared through hydrothermal method. In their work, ZnO NRs were uniformly distributed on FTO electrode with an average length of 2.5 µm, and the average diameter of AuNPs was 8–10 nm. AuNPs/ZnO/FTO electrode showed a good sensitivity of 43.7 µA/mM^{-1} cm^{-2} compared with ZnO/FTO electrode with 24.3 µA/mM^{-1} cm^{-2} due to the fine electrocatalytic ability of AuNPs to facilitate the enzyme catalytic reaction induced by decorated AuNPs on ZnO NRs surface [145]. Anusha et al. [15] reported a simple construction of glucose biosensor by dispersing platinum (Pt) nanoparticles over ZnO nanopores. The modified electrodes were then subjected to chitosan (CS) coating as protective layer and as bio-adhesion material to promote the electron transfer kinetics [15]. The modified electrode was denoted as GOx/CS/Pt/ZnO/FTO. The presence of PtNPs increased the magnitude and background current response compared with the absence of PtNPs. This result is due to the behavior of PtNPs, which accepted more electrons during reoxidation of GOx that resulted in the formation of electron-transfer accelerating layer [15]. High-electron transfer process eventually led to a high sensitivity of 62.14 µA/mM^{-1} cm^{-2} and low detection limit of 16.6 µM for GOx/CS/Pt/ZnO/FTO electrode. Li et al. [113] demonstrated the effect of silver (Ag) content-doped ZnO toward glucose biosensor performance. Ag content was varied from 1.05 wt% to 27.5 wt%. The optimum 11.26 wt% Ag-doped ZnO produced the best electrochemical response. In their work, pristine ZnO formed in nanorod structure, and after the incorporation of Ag, the formed Ag-ZnO retained the morphology of nanorods, whereas AgNPs were attached to the surface of ZnO NRs. Other materials employed for hybridization in the construction of glucose biosensor are presented in Table 3.7 along with their associated sensitivity and LODs.

TABLE 3.7
Summary of glucose biosensors based on ZnO nanostructure hybrid

Hybrid composition	Electrode matrix	Immobilization mode	Sensitivity (µA/mM^1.cm^2) / Linear range (M)	LOD (M) / Response time (s)	Ref
Incorporation of ZnONPs within graphene–CNT networks from zinc acetate reduction	GOx/ZnO/ GR-CNT/ GCE	Adsorption	5.362 ±0.072 / 10 µ –6.5 m	4.5±0.08 µ / -	[137]
Incorporation of ZnO flower like by electrode position over reduce graphene oxide	GOx/ZnO/ rGO/GCE	Adsorption	18.97 / 0.02– 6.24 m	0.02 m / -	[139]
Incorporation of ZnO tetragonal pyramid within reduce graphene oxide	1. GOx/rGO-ZnO/Nafion/GCE GOx/rGO-ZnO/Nafion/SPE	Adsorption	13.7±0.1 / 0.2–6.6 m	0.2 µ / 4	[148]
Incorporation ZnONPs by electrode position over graphite	GOx/ZnO/ GR/SPE	Adsorption	30.07 / 0.3– 4.5 m	0.07 m / -	[114]

Incorporation of ZnONWs directly grown on graphite flake via vapor transport method	GOx-ZnO-graphite-GCE	Adsorption	17.0 / 0.03– 1.52 m	1.52 m / -	[108]
Incorporation of ZnO microsponge by elecctrodeposition over MWCNT	GOx/ZnO/ MWCNT/ GCE	Adsorption	4.18 / 0.2– 27.2 m	20 µ / -	[149]
Composite thin films of ZnO–$K_3(Fe(CN)_6)$	GOx/ZnO–KFCN/ITO	Adsorption	0.078 / 2.78–11.11 m	0.23 m / 10	[118]
Composite of ZnONRs grow on ferocenyl-alkanethiol monolayer	Nafion/GOx/ZnO/ $FeC_{11}SH$/Au/Ti/Si	Adsorption	27.8 / 0.05– 1 m	20 µ / -	[18]
CuNPs deposited over ZnO film by electrode position	Cu/ZnO/ GCE	-	0.16 ± 0.02 / 1 µ – 1.53 m	0.2 µ / 3	[150]
CuNPs deposited on ZnONRs by electrode position	Cu/ZnO/ FTO	-	609.8 / 5 µ – 1.1 m	0.3 µ / 3	[151]
Ni doped ZnO NRs via hydrothermal method	GOx/Ni-ZnO/Pt	Adsorption	61.78 / 0.5– 8 m	2.5 µ / <5	[105]
Commercial Pt NPs dispersed over ZnO porous film	GOx/CS/Pt/ZnO/FTO	Adsorption	62.14 / 100 µ– 2 m	-	[15]
Decorated AuNPs over plant-like ZnO	Nafion/GOx/ AuNPs/ZnO/ITO	Adsorption	3.12 / 2.77 – 22.2 m	-	[142]
Decorated AuNPs over ZnO nanosheet in flower structure via *in situ* reduction of $HAuCl_4$	GOx/AuNPs/ZnO/GCE	Adsorption	1.409 / 1– 20 m	0.02 m /	[141]
Deposited AuNP over ZnONRs by self-assembled method	GOx/AuNPs/ZnO/ITO	Adsorption	1.44 / 1.38– 22.22 m	3.51 /	[140]
Coated Au thin film over ZnONRs using precision etching coating system (PECS)	Nafion/Au/ZnO/ITO	Crosslink	20.19 / 0– 24 m	0.5 m / 3	[115]
AuNPs directly grow on ZnONRs by photo-reduction of $HAuCl_4$	Nafion/GOx/ AuNPs/ZnO/FTO	Adsorption	0.0437 / 3 µ– 3 m	3µ / -	[145]
AuNPs decorated on ZnONRS via hydrothermal	Nafion/GOx/ AuNPs/ZnO/GCE	Crosslink	1492 / 0.1–33 µ	10 n / 5	[146]
Ag doped ZnO NRs via hydrothermal	Nafion/GOx/ Ag-ZnO/GCE	Adsorption	0.0187 / 0.01– 1.5 m	0.007 m / -	[113]

Abbreviations: GOx, Glucose oxidase; ZnO, Zinc oxide; GR, Graphene; rGO, Reduced graphene oxide; CNT, Carbon nanotube; MWCNT, Multiwall carbon nanotube; $FeC_{11}SH$, Ferocenyl-alkanethiol; GCE, Glassy carbon electrode; SPE, Screen printed electrode; ITO, Indium tin oxide; FTO, Fluorine tin oxide; KFCN, Potassium ferrocyanide; CS, Chitosan; AuNPs, Gold nanoparticle

Conclusion and Future Prospect

Tremendous efforts and numerous publications have been made for the construction of nanostructured metal oxide-based biosensors, particularly iron oxide and zinc oxide materials. This study highlights the importance of electrochemical glucose biosensor in clinical and non-clinical diagnostic. Glucose biosensor should possess excellent performance, which include high sensitivity and selectivity, wide and linear range of detection and fast response with reliable stability and repeatability. Although considerable development and success of glucose detection has been achieved in the laboratory level, challenges persist in practical applications. To the best of the authors' knowledge, no commercial enzymatic biosensors based on metal oxide, particularly iron oxide- and ZnO-based electrodes, have been reported until recently. To implement the metal oxide as glucose biosensor electrode in practical application, several points of consideration must be controlled. First, morphologies of glucose biosensor matrix must be controlled as it influences the properties and performance of the produced biosensors. Second, the fabrication method of metal oxide must be efficient, can be produced in large scale and consistent repeatability. Third, the efficient method for enzyme immobilization can be used for large-scale production. Glucose biosensors must retain their long-life bioactive enzyme properties. Finally yet importantly, glucose biosensor should be able to avoid any interference and false results. In laboratory level, glucose biosensor could perform at its best performance. However, all of the abovementioned points should be overcame and improved significantly prior to commercialization.

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