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ORIGINAL ARTICLE

Applications of Nanomaterials for Biosensor Fabrication Based on Redox Enzyme and Protein: A Short Review

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ABSTRACT – Redox enzyme and protein modified biosensors are commercially triumphant bioelectronic devices used in the point-of-care analysis. The use of nanotechnology derived nanomaterials during enzyme immobilization creates a synergistic effect by integrating enzyme's recognition and catalytic properties with the electronic properties of nanomaterials. This synergy improves the biosensor's sensitivity, conductivity stability, surface-to-volume ratio, selectivity, detection limit and other analytical features. This critical review focuses on the redox enzymes and proteins most frequently used in glucose and hydrogen peroxide sensing, such as horseradish peroxidase (HRP), glucose oxidase (GOx), hemoglobin (HB), and cytochrome C (Cyt c). Besides, we evaluate the state of art of this approach, selection of nanomaterials, preparation and immobilization mechanisms, their role and sensing applications. Besides advantages, the discussions have discussed on the pressing challenges of developing these sensors. This review will guide the research community to develop rational and highly efficient nanomaterial immobilized biosensors.

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INTRODUCTION

Biosensor is an advanced bioelectronic device used to monitor various chemical and biological compounds in which an electrochemically active biological material is connected to a physicochemical transducer or transducing microsystem [1]. In 1962, Clark and Lyons were developed a glucose-sensing enzyme electrode and is considered the forerunner of biosensing era [2]. Biosensors are presently a hot topic and are frequently used to detect different varieties of chemicals, biomolecules, microbes, and many more [3-6].

Hydrogen peroxide (H_2O_2) and glucose are two important detectable chemicals that are intimately involved in various intracellular and extracellular processes. H_2O_2 is a versatile molecules used to sterilize equipment's and to preserve milk and juice in food industry [7, 8]. Intracellular H_2O_2 regulates immune cell activation, proliferation, differentiation and acts as a cancer marker [9, 10]. Besides its usefulness, exposure to uncontrolled H_2O_2 advances Alzheimer's, cancer inflammation, arteriosclerosis, and neurodegeneration [10-12]. Glucose is also an important compound that contributes to diabetes mellitus, one of the leading causes of death and disability. Diabetics are at increased risk for several health problems, including cardiovascular disease, kidney failure, neuropathy, vision loss etc. [13]. So far, various analytical methods have been introduced to detect H_2O_2 [14-16] and glucose [17-19]. Compared to electrochemical detection, the others are sophisticated, hard to automate, time-consuming, less sensitive, and expensive [16, 18]. Electrochemical biosensor have evolved as an effective and sensitive alternative for detecting H_2O_2 and glucose [20].

Electrochemical biosensors are potentiometric, amperometric/voltammetric, conductometric, and impede-metric based on their bio-recognition signals [21, 22]. This review concentrates on amperometric/voltam-metric techniques used in H_2O_2 and glucose biosensor development. HRP, GOx, Hb, and Cyt c are highly catalytically active redox molecules and vindicate catalysts for biosensing. Redox protein and enzyme-based biosensor offer direct electron transfer between recognition centers and electrodes during sensing [23]. But the electroactive prosthetic groups of these redox molecules are deeply embedded in their protein structure, making direct electron transfer sluggish. Besides structural features and unfavourable orientations of the proteins at the electrode surface also lingering the electron transfer [24, 25]. Furthermore, redox enzymes/proteins are too large to make direct contact with electrode without being denatured [26]. This consequently affects the stability, sensitivity, signal strength of the biosensor.

The inclusion of nanomaterials with biocatalysts during electrode fabrication has emerged as a convenient way that aids in stabilizing their intrinsic properties as well as improves the direct electron transfer. Nanomaterial-based biosensors combine electrical properties with enzyme recognition and catalytic properties to create synergistic properties [23]. These hybrid composites alleviate challenges posed by biocatalyst and improve biosensor features such as sensitivity, catalytic activity retention, redox current increment and conductivity [27, 28]. In light of this, different nanomaterials, including metals, metal oxides, carbon nanotubes and graphene have been introduced for immobilizing of redox enzymes and protein. Nanomaterials are attractive to scientists because of their large amount of available surface area, unusual chemical and physical capabilities, regular structure, and excellent chemical and thermal durability [26, 29]. In this review, we are

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devoted to the biosensor that are fabricated using metals, metal oxides, carbon nanotubes and graphene-based nanomaterials. Besides, the way they accelerate direct electron transfer, it also enhances the sensitivity and catalytic activity.

METHODOLOGY

The articles were gathered from widely used scientific databases, including Web of Science, ScienceDirect, Scopus, PubMed, and Springer Nature, from 2019 to 2022. The period of time has been decided for more focus and recent development of the biosensors. The articles search also limited to biosensors made using amperometric and voltammetric methods.

APPLICATION OF NANOMATERIALS IN GLUCOSE AND H_2O_2 BIOSENSOR

Nanmaterials in horseradish peroxidase based biosensor

HRP is a heme group containing enzyme and widely used to develop electrochemical H_2O_2 biosensors because of their specificity and catalytic property. But the redox active sites of HRP are surrounded by polypeptide chain that hinders direct electrochemistry and affects sensing performances [30]. It has been reported that biosensor based on only HRP had low sensitivity and could only detect 0.3 mM of H_2O_2 [31]. In recent years gold, graphene and carbon nanotube derivatives have been used to immobilize HRP in biosensor because of their large surface area, high catalytic efficiency and biocompatibility. A spherical shape gold–nickel (Au_2Ni_1) alloy nanoparticles was synthesized to immobilize HRP on glassy carbon (GC) electrode for H_2O_2 sensing. The small size alloy NPs possess large surface area and high catalytic activity that increase the electroactive area and charge transfer rate of HRP modified electrode. The fabricated electrode displayed higher sensitivity, stability and LOD of 0.021 µM [32]. Narayanan et al. prepared a Au NPs decorated Au microwire electrode for HRP immobilization in order to detect H_2O_2. Au NPs decoration enhance the HRP electron kinetics and prevents the desorption during repeated usage [33]. Ariffin et al. reported a HRP/MSP-NAS/Au NPs/SP electrode for H_2O_2 sensing [34]. A nanoconjugates was synthesized by electrostatic tethering of Au NPs with redox ionic liquid which can retain the bioactivity of HRP during H_2O_2 sensing. The bioconjugates formed offers enhanced electron kinetics, selectivity and sensitivity [35]. Meanwhile, an Au electrode was modified with thiol and CNT for HRP immobilization that gives far better sensitivity, linearity and LOD than HRP/Au NPs electrode. CNT formed a 3D network on electrode surface that ensure more homogenous distribution of HRP [36]. Jiang et al. developed a multi-walled carbon nanotubes (MWCNTs) and metal organic framework [NH_2-MIL-53(Fe)] (MOF) modified HRP based H_2O_2 biosensor that is effective as a UV-vis method. The synergy between MWCNTs and MOF enhances the current response by reducing the electron transfer resistance and distance between redox centre and surface [37]. Feizabadi et al. improved the stability of HRP based H_2O_2 biosensor by deposited it onto a GABA-COOH functionalized MWCNTs modified GC electrode that retains 90% of its current response after ten weeks [30]. Gutierrez et al. developed a HRP based H_2O_2 biosensor by immobilizing HRP on avidin functionalized MWCNTs. Avidin/MWCNTs forms a building blocks that offer robust loading of biotinylated horseradish peroxidase and enhance the sensing performances [38].

Table 1. Detailed analytical performance of nanomaterial modified HRP based H_2O_2 biosensors.

Biosensor	Sensitivity ($\mu A\ mM^{-1}\ cm^{-2}$)	Linear Range (µM)	Detection Limit (µM)	90% Stability (Days)	Ref.
HRP@PGH/GNSs		1 - 340	0.27	35	[39]
Au/SAM/HRP/CNT	0.34	0.3 - 120	0.022	21	[36]
Au NPs/MSP-NAS-SPE		0.0001 - 10^4	0.0001	30	[34]
HRP/MB/chitosan/MoS_2/graphite microfiber		0.1 - 90	0.03	60	[40]
HRP-NpAc-IL/MWCNT/GC	55.98	10 - 270	2.7	30	[41]
CMF/Go/HRP@MS		0.1–235	0.01	14	[42]
PVA/Go/Au@Cu nanoflower	332.68	0.1 - 100	0.018	20	[43]
MWCNT-COOH-GABA/HRP/GC		0.2 - 281	0.13	21	[30]
b-HRP/MWCNT-avidin/GC	137	1 - 14	0.024		[38]
NH_2-MIL-53(Fe)/HRP/MWCNTs/GC	6149.2; 155.74	0.1- 1; 1 - 600	0.028	1	[37]
HRP/TH/ERGo/GC	6.98	0.039–0.78	0.02	14	[44]
HRP/PDA-MNPs/L-Arg/Tb/GC		0.5-30	0.23	3	[45]
HEPNP/rGo/Au		0.01–100	0.01		[46]
HRP/Au_2Ni_1/GC		0.1 -1000	0.0212	35	[32]
HRP-CHO AIL/AuNPs/GC	63.4; 51.1	20 – 720; 720 - 2770	3.7	30	[35]
HRP/LDH-CMC	22.04	20 – 6000	12.4	14	[47]

A H_2O_2 sensing bi-composite electrode was developed by introducing photo-cross-linkable PGA-HEMA and graphene nanosheets (GNSs) with HRP. The wrinkle features of GNSs increase the surface area, offer more active sites

and accelerate the electron transfer [39]. A H_2O_2 biosensor was fabricated by coating of HRP@ mesoporous silica (HRP@MS) composite on a Go modified screen printed (SP) electrode. Go was verticalized by applying a magnetic field which increased the surface area and charge transfer capacity as well as enzyme composite loading capacity by 18.74% [42]. Pandey et al. used thionine modified rGo for HRP immobilization which could detect H_2O_2 with a linearity and detection limit of 0.03–0.35 mM and 4.65 µM, respectively [44]. Another group introduced cysteamine capped rGo for immobilizing HRP encapsulated protein nanoparticles (HEPNP) for H_2O_2 detection. The cysteamine capped rGo modified biosensor gave much wider linearity and low detection limit compared to bare HRP and above indicated thionine/ rGo/ HRP electrode [46]. Zhang et al. used peroxidase mimicking MoS_2 nanosheets/graphite microfiber hybrid for HRP decoration. The catalytic nature, high surface area and active binding site of the hybrid offer H_2O_2 sensing in harsh condition with increased sensitivity, selectivity and stability [40]. Here magnetic nano-particle was employed with poly (L-arginine/toluidine blue) to immobilize HRP that can retain the catalytic activity of HRP during H_2O_2 detection in human plasma [45]. Alim et al. used multiporous material, SnO_2 NFs that can hold HRP molecules on their pours structure and shortening the distance between the active sites of HRP enzyme and the electrode. During sensing, the modified electrode displayed higher sensitivity, selectivity, linearity upto 120 µM and LOD of 0.5 µM [48].

Although HRP unable to catalyze glucose, but it can catalyze H_2O_2 that was produced during electrooxidation of glucose using Gox. Baek et al. prepared a hybrid structure for glucose sensing by packing Gox and HRP in a Cu nanoflower instead of an open layer and coating it on AuNPs doped GO NFs surface [43]. Alim et al. employed polymerized multiporous SnO_2 nanofiber to immobilize Gox and HRP for electrochemical detection of glucose that displayed a linear range from 5 to 100 µM with a detection limit of 1.8 µM. SnO_2 NFs together with highly conductive PANI and HRP effectively helps Gox to catalyse glucose [17]. Table 1 describe the detailed detection results of HRP based biosensors.

Nanomaterials in glucose oxidase based biosensor

GOx is a direct electrochemistry exhibiting enzyme that uses molecular oxygen as the main electron acceptor to catalyse the oxidation of glucose. It often exhibits slow electron kinetics when only GOx is used for sensing due to the deep embedding of GOx redox active site [49]. Therefore, metals, metal oxides, and carbon-based nanomaterials effectively participate with other supporting materials in single or combined form to accelerate the electron dynamics of GOx during sensing. Gold with polymer is a good strategy to immobilize GOx enzyme because it can systematically accelerate the sensitivity and current response of glucose biosensors. Based on these, researchers decorated Au electrodes with polynorepinephrine (PNE) functionalized Au NPs to ensure efficient immobilization of the GOx enzyme. The biosensor displayed LOD and sensitivity of 1.34 µM and 35.4 $mAmM^{-1}cm^{-2}$ during glucose sensing, respectively [50]. Zhang et al. reported an Au NPs modified Au/OPPy/GOx electrode for glucose sensing [51]. Meanwhile, polytyramine (Pty) functionalised Au NPs was employed to coat GOx on a prussian blue-modified screen-printed carbon (SPC) electrode. The high surface area containing Pty/Au NPs allow more compact GOx enzyme loading that increased sensitivity and LOD 2.4 and 3.2 times compared to GOx/Pty/PB/SPCE, respectively [52]. A low background noise exhibiting GOx based glucose biosensor was fabricated by assembling Au nanorods (Au NRs) and ionic liquid modified graphene sheets (GS-IL) using thiolated silica sol–gel on GC electrode [53].

A multicomposite materials (MCM) with property like high surface area, electron transport facilitator, stability enhancer, and conductive have been synthesized by using poly (diallyl-dimethylammonium chloride), Au NPs, nickel ferrite, CNTs and chitosan. This MCM was then electropolymerized to immobilize GOx on the GC electrode for glucose sensing [54]. Buk et al. developed a GOx/Au NPs/ carbon quantum dots/gold micro disk array electrode for glucose sensing by employing the rapid electron transfer acceleration behaviour of Au NPs and carbon quantum dots [55]. Besides, Lu et al. enhances the sensitivity of an Gox based glucose biosensor using a Pt NPs decorated polyimide-laser-engraved porous graphene (LEPG). The hierarchical porous structure of LEPG helps in redox probe diffusion to the electrode surface and Pt NPs addition increase the electroactive area [56]. Instead of LEPG, the Pt NPs was modified by poly(Azure A) to immobilize GOx for glucose sensing. This Gox/Pt NPs/ Poly(Azure A) based electrode provide suitable immobilization conditions that ultimately prevents interference activity and offers glucose detection at lower potential [57]. A molecularly imprinted structure was prepared by laser engraving of graphene on a polyimide tape which was further employed for glucose sensing by immobilizing GOx [58]. Dinesh et al. exposed GOx to high surface area containing pristine MWCNTs for glucose biosensor fabrication that demonstrated excellent sensitivity and LOD of 45 µM during glucose sensing [59]. Soleh et al. used graphene oxide to construct a Gox modified biosensor that can detect glucose, uric acid and dopamine simultaneously. Compared to pristine MWCNTs/GOx, Go/GOx biosensor exhibited 8 times lower detection limit [60]. Maity et al. employed three different nanomaterials to fabricate GOx based glucose biosensor. They synthesized a complex architecture using AuNP, rGo and MWCNTs which ensures high electron transfer kinetics during sensing. The detailed fabrication is attached in Figure 1. With all this materials, the biosensor displayed sensitivity of 246 µ Acm^{-2} mM^{-1}, 30 days stability, linearity of 1–10 mM and LOD of 64 µM [61].

Kafi et al. reported a mediator based enzymatic glucose biosensor by depositing GOx on a multiporous SnO_2 NFs modified GC electrode. The porous SnO_2 NFs entrap the enzyme and ensure GOx dispersion and its bioactivity [62]. Meanwhile, MOS_2 was used with chitosan and gelatine to modify GOx on graphite electrode. This MOS_2 excites the direct electron transfer and the fabricated electrode displayed wide linearity upto 0.8 mM and LOD of 3.18 µM towards glucose [63]. Asrami et al. reported a ZnO coated GOx/nano-ZnO/PVA/FTO electrode for glucose sensing in human serum [64]. A combination of metal and metal oxide nanomaterials was employed to ensure the accurate orientation of

Gox during glucose sensing. The biosensor was fabricated by coating of Au electrode using $NiO/Ni(OH)_2$ and subsequent immobilization of the GOx [65].

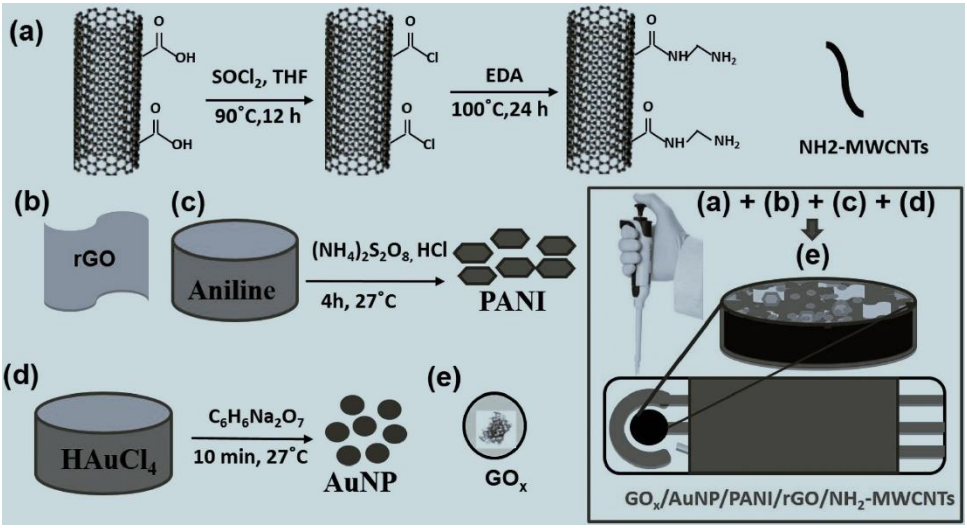

Figure 1. Schematic details of GOx/AuNP/PANI/ rGO/NH_2-MWCNTs modified SPC electrode fabrication [61].

Yoon et al. modified a polymer electrode by double sputter coating of gold and single spin coating of MOS_2 for glucose sensing. GOx deposited on Au/MOS_2 displayed higher current response and accelerated electron transfer kinetics than that of conventional gold-coated silicon electrode [66]. Rassas et al. employed Au NPs with chitosan/kappa-carrageenan to synthesize a polyelectrolyte complex (PEC) to encapsulate GOx for glucose sensing. The inclusion of Au NPs on PEC/AuNPs/GOx electrode displayed three times higher sensitivity than PEC/GOD electrode [67]. Another glucose biosensor was fabricated by immobilizing Gox on Au NPs decorated oxidized cellulose nanocrystal that demonstrated good analytical performances such as linearity of 0.1–1.6 mM, LOD of 40 µM and good recovery in human serum [68]. The detailed detection results of GOx based biosensors presented in Table 2.

Table 2. Detailed analytical performance of nanomaterial modified GOx based H_2O_2 biosensors.

Biosensor	Sensitivity (µA mM^{-1} cm^{-2})	Linear Range (µM)	Detection Limit (µM)	90% Stability (Days)	Ref.
Au/OPPy/AuNPs/GOx/Nafion	8.09	Upto 2600	40	14	[51]
PEC/AuNPs/GOx/Au	283.9	10 - 7000	5	21	[67]
GOx-TA-AuNPs-CNC/GC		100 - 1600	40	15	[68]
GOx/GC/MWCNT/Fe3O4/PDA	43.15	Upto 8000	431	10	[58]
Au-NiO/$Ni(OH)_2$-GOx	1.95	Upto 30000	1540		[65]
NF/GluOx/IL/mPEG/fMWCNTs		20 - 950	0.2	14	[69]
GOx/AuNP/PANI/rGO/NH_2-MWCNTs	246	1000 - 10000	63	60	[61]
GOx/Pt-LEPG	241.82	10 - 31500	0.3	14	[56]
GOx/CNT-Go/PAA-Fc		100 - 10000	200	6	[70]
PANI-AuNPs(6 nm)-GOx/GOx Ppy-AuNPs(6 nm)-GOx/GOx	65.4 55.4	100 16500	70 71	22 17	[71]
GOx/PoPD/MCM/GC	853.07	0.5 – 10; 10-15000	0.35		[54]
PMWCNT/Gox/GC	6600	200 - 5800	45	30	[59]
GC/Au NFs/GS-IL-Au NRs/GOx		1 - 764	0.38	40	[53]
GOx-PtNPs-PAA-aSPC	42.7	20 - 2300	**7.6**	7	[57]
Chi/GOx/PB-G/GC		15 - 1500	5.7		[60]
GOx/SnO2–NF/CH/PB/GC		500 - 5000	50	30	[62]
CQDs/AuNPs-GOx	626.06	160 - 4320	13.6	30	[55]
GOx/AuNPs/Pty/PB/SPC	33.4	0.1 - 1000	1	21	[52]
GOx/Au/MoS2/gold nanofilm		0.1 - 0.5	0.1		[66]
Nafion/GOx/ERGo-PLL/GC	2578.9	5 - 9000	2		[72]
PNE/GOx/AuNPs@PNE/Au	35.4	0.3 - 3430	1.34	7	[50]
GOx/nano-ZnO/PVA/FTO	41	200 - 20000	2	60	[73]

Nanomaterials in Hemoglobin (HB) based biosensor

Hemoglobin is a peroxidase like activity exhibiting redox protein that can detect H_2O_2 via direct electrochemistry. However, the denaturation and slow electron kinetics affect the detection efficiency and stability [74]. Until now, various methods have been explored to accelerate the electron kinetics. Nanomaterials to immobilize hemoglobin has been shown to be effective due to their large surface area, good bio-compatibility and conductivity. A H_2O_2 biosensor have been reported by immobilizing Hb on a cysteamine (Cys) and Au NPs modified SC electrode. Electrochemically deposited Au NPs increased the electro active surface area (0.07 cm^2) where cathodic and anodic peak currents were found to be 2.8 and 1.6 times larger than Nafion-Hb-SPCE, respectively (Figure 2). The developed biosensor had a linearity over 3 μM to 240 μM and LOD of 0.6 μM [75]. Xu et al. added manganese carbonate ($MnCO_3$) microspheres with Au NPs to immobilize Hb on electrode. Hb on $MnCO_3$–Au microspheres supports exhibited better electron transfer facility where electron transfer rate constant was 2.63 s^{-1}, better than Cys/Au NPs (0.785 s^{-1}) [76]. Another Hb based H_2O_2 biosensor was developed using PAMAM encapsulated Au NPs modified GC electrode. Au NPs conducted an electrical wiring mechanism to escalates the conductivity and surface area that results in increased sensitivity, selectivity and stability [77].

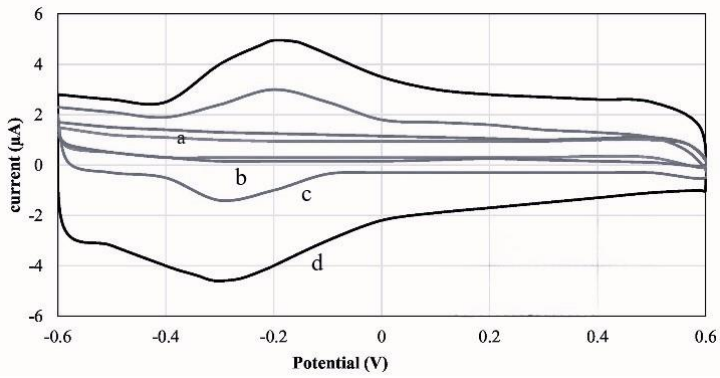

Figure 2. CV of (a) AuNP-SPC, (b) bare-SPC, (c) NF-Hb-SPC, (d) NF-Hb-Cys-AuNPs-SPC [75].

Kafi et al. construct a carbonyl functionalized SWCNTs-Hb crosslinked network for H_2O_2 sensing. Hb has created an effective networks with CNTs that helps to achieve direct electrical communication [78]. The same group employed carbon nanotube (CNT) with multiporous SnO_2 NFs to fabricate a Hb based H_2O_2 biosensor. The addition of CNTs with SnO_2 NFs offer higher surface area and increase enzyme loading. Compared to SnO_2 NFs/Hb biosensor, SnO_2-NF/CNTs/Hb biosensor displayed 33.33 times lower detection limit [79]. Here a H_2O_2 biosensor has been developed through electrospinning of Hb contained suspension. A suspension of Hb, gelatin, and MWCNTs was employed to fabricate electrospun Hb/gelatin-MWCNTs/GC microbelts electrode [80]. Same group reported an electrospun core-shell MWCNTs/gelatin-Hb nanobelts for H_2O_2 sensing. Compared to electrospun microbelts electrode, electrospun nanobelts gave lower detection limit, real sample recovery and reproducibility. Munir et al. constructed a Hb based mediator free H_2O_2 biosensor where they employed hollow BiOBr/rGo hybrids to ensure favourable microenvironment and electron transfer kinetics acceleration [81].

Table 3. Detailed analytical performance of nanomaterial modified Hb based H_2O_2 biosensors.

Biosensor	Sensitivity ($\mu AmM^{-1}cm^{-2}$)	Linear Range (μM)	Detection Limit (μM)	90% Stability (Days)	Ref.
Hb/MWCNTs-gelatin microbelts		5 - 80	0.0467	28	[82]
SnO_2-NF/CNTs/Hb/Ch/GC		1 - 140	0.03	35	[79]
Hb/3.0G PAMAM-AuNPs/GC	35.07	20 - 950.22	6.1	30	[77]
Nafion/HMHNFs/GC	68.945	0.02 – 3.6	0.007	60	[83]
Hb/NIBA-IL/MWCNT/GC	111	10 - 6300	3.2	30	[84]
core-shell MWCNTs/gelatin-Hb nanobelts		5 - 55	0.0293		[82]
NF-Hb-Cys-AuNPs-SPC	0.918	3 - 240	0.6	30	[75]
Hb/GO/Dy_2O_3/GC		5 - 300	2	5	[5]
GC/Hb/SnO_2/CHIT		5 - 150	1	35	[85]
Au/SWCNT/HB/Ch	0.3	10 - 120	3		[78]
Nafion/Hb/H-BiOBr/rGO/GC	395.8	0.1 - 420	0.02	60	[81]
Hb-MCFs@rGO/GC	0.25	250 - 8000	0.0636	15	[86]
Hb/Chit/MGC	227	5–250; 0.01–0.1	0.003	7	[87]

A nanomaterial TiO_2 was covered by CdS for Hb immobilization. During H_2O_2 sensing, The combination of Hb with CdS formed an intermediate species that exhibited an electron transfer rate of 0.45 s^{-1} [88]. Another group employed rGo

with CdS for Hb immobilization [89]. Compared to the TiO_2 supported CdS/Hb, the rGo supported CdS/Hb electrode exhibited very good detection limit. Alim et al. employed multiporous (MP) SnO_2 NFs with Hb to develop highly sensitive H_2O_2 sensor. The multiporous SnO_2 NFs allow high-density proteins/enzyme loading and escalate direct electron transfer. The developed sensor has excellent sensitivity, selectivity, linearity and stability [85]. Yuan et al. used a magnetic electrode for Hb coating. During sensing, the paramagnetic nature of Hb was utilized to oriented Hb using an external magnetic field and it gives 16% current increment during H_2O_2 sensing [87]. The interaction between Hb and $Mn_3(PO_4)_2$ unfold Hb polypeptide chains which results in opulent electroactive centers in the hydrophobic cavity. A H_2O_2 biosensor was developed based on this composite that offers higher sensitivity, electron transfer rate and excellent detection matrix [83]. The Hb based biosensors detection related results are demonstrated in Table 3.

Nanomaterials in Cytochrome C (Cyt c) based biosensor

Cytochrome C (Cyt C) is an electrontransfer protein that acts as a catalyst in many redox reactions. Although it is a less commonly used due to slow electron kinetics, a number of H_2O_2 biosensor using nanomaterial supported Cyt C have been reported recently [90]. More specifically, gold and carbon nanotube-based nanomaterials are highly functional for Cyt c-based biosensor. A Cyt C immobilizing biointerface was prepared by depositing Au NPs on graphene aerogel (GA). The GA-Au NPs formed a highly conductive 3D network that exposed the redox centre of Cyt C nearly to the electrode surface, thus enhance the electron transfer rate and sensitivity [90]. Manickam et al. integrated β-cyclodextrin-chitosan (CS-g-β-CD) hydrogel with Au nanocubes to facilitate the direct communication during H_2O_2 sensing. This hybrid matrix exhibited improved biocompatibility (in RAW 264.7 and N2a cell lines), conductivity, sensitivity and a low detection limit of 15 nM [91].

The conductivity and electron transfer rate acceleration property of MWCNTs was employed for Cyt C immobilization. Aghamiri et al. immobilized Cyt C on a polyaniline/polypyrrole/carboxylated MWCNTs modified GC electrode for H_2O_2 sensing. The cMWCNTs functionalized with polymers offers higher electrical conductivity and mechanical strength, thus together acts as a charge promoter to facilitate direct electron transfer [92]. Meanwhile, Murphy et al. used phosphonium based carboxyl functionalized ionic liquid (TPP-HA[TFSI]) modified MWCNTs to immobilize Cyt C for H_2O_2 sensing. The reported biosensor exhibited excellent linearity, sensitivity and detection limit which was 20 to 892 μM, 0.14 $\mu A \mu M^{-1} cm^{-2}$ and 6.2 μM, respectively [93]. Lee et al. decorated Cyt C on a graphene field-effect transistor (FET) for detecting H_2O_2. FET contained a single graphene layer which exhibited a hole-transport behaviour during detection that offer excellent detection characteristics. The modified electrode displayed short response time of one second, wide linear range of 100 fM-100 pM and detection limit of 100 fM [94]. A group employed metal, metal oxide and CNTs to synthesize a composite for Cyt c. The composite was prepared by functionalizing Au NPs using a magnetic N-doped carbon nanotubes (NCNTs@Fe_3O_4) that can entrap Cyt C in their structure. The good conductivity of NCNTs oriented the Cyt C and Au NPs ensures well dispersion, microenvironment and surface area for electron transfer [72].

CONCLUSION AND FUTURE RECOMMENDATIONS

Redox enzyme and protein-based nanomaterials modified biosensors are more sensitive and functional biosensor to detect H_2O_2 and glucose than traditional techniques. It prevents enzyme denaturation and accelerates the electron transfer rate. Despite their high catalytic efficiency, low stability and the need for multiple materials for redox protein and enzyme immobilization is still a factor that is reducing its usefulness and its future market potential. Besides, issues like dual or triad sensors, biocatalysts with less support materials, and more practical applications will have to be solved.

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