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Review

Cholesterol biosensors: A review

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ABSTRACT

Cholesterol is the most important sterol synthesized by most of the human cells majorly in the liver. It is a necessary constituent of cell membranes, it acts as a precursor for the synthesis of steroid hormones, vitamin D, and bile acids. Cholesterol is transported in plasma primarily in the form of low-density lipoproteins (LDL), the principal route for its removal from tissues to the liver is in high-density lipoproteins (HDL), followed by excretion in the bile. Cholesterol level is less than 200 mg/dL in healthy persons. 200 and 239 mg/dL is considered borderline high and 240 mg/dL and above is considered a biomarker for cardiovascular diseases, heart attack, strokes, peripheral arterial disease, type 2 diabetes and high blood pressure. Several methods are available for detection of cholesterol, among them, most are burdensome, time-consuming, require sample pre-treatment, high-cost instrumental set-up, and experienced personnel to operate. Biosensing approach overcomes these disadvantages, as these are highly specific, fast, easy, cost-effective, and highly sensitive. The review describes the various cholesterol biosensors. Cholesterol biosensors work ideally within 1 to 300 s, in pH range, 7.0–8.6, temperature 25–37 °C and cholesterol concentration range, 0.000025–700 mM, the detection limits being in the range, 0.000002–4 mM, with working potential −0.05 to 0.65 V. These biosensors measured cholesterol level in fruit juices, beverages, sera and urine samples and reused up to 200 times over a period of 15 to 50 days, while stored dry at 4 °C (Table 1). Future perspective for further improvement and commercialization of cholesterol biosensors are discussed.

1. Introduction

Cholesterol is a sterol and biosynthesized in all animal cells as it is an essential structural component of all animal cell membranes [1]. Beside this, cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acid and vitamin D [2]. Cholesterol is carried in the blood by molecules called lipoproteins. A lipoprotein is a complex consisting of lipid (fat) and protein. There are three main types of lipoproteins [3]. The three main types are:

- LDL (low density lipoprotein) – LDL is known as bad cholesterol. It acts as carrier of cholesterol from the liver to different cells of body. Its high serum level indicates higher risk of arterial diseases [4].
- HDL (high density lipoprotein) – It is referred as good cholesterol. It is reported that HDL prevents arterial disease. It transfers the cholesterol away from the cells and back to the liver. In the liver it is either broken down or expelled from the body as waste [5].
- Triglycerides (TG) – Most of the fat which we eat in food, exists in this chemical form. The source of it in our body is diet taken from outside or some part of TG also originates from others such as carbohydrates. The diet which we take, if have not consumed by body that gets converted into TG and gets stored in fat cells. Whenever we fast, that TG gets released from fat cells and used as source of energy. This process is controlled by hormones [6].

Serum cholesterol level is of major concern as it plays important role in the diagnosis and treatment of various cardiovascular diseases [7], hypothyroidism [8], nephrotic syndrome [9], diabetes [10] and liver diseases [11]. Its synthesis in body is directly regulated by the cholesterol levels present. Higher intake of it leads to a net decrease in endogenous production, whereas lower intake has the opposite effect [12]. Therefore, various methods like chromatographic [13],

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Table 1
A comparison table of various electrochemical biosensors for determination of cholesterol.

NO.	Type of electrode	Linear Range (mM)	Detection limit (mM)	Potential (V)	Sensitivity	Time (s)	pH	Precision (%)	Storage stability	References
1.	ChOx/PDMS/NiO/Pt	0.12–10.23	0.10	0.5	45 $\mu AmM^{-1}cm^{-2}$	–	–	1.25	–	[26]
2.	ChOx/CHER/AuNPs/SPCE	0.012–10.23	0.0078	−0.5	–	300	8.6	5.2	–	[28]
3.	ChOx/PBNPs/SPCE	0–15	0.2	0	2.1 $\mu AmM^{-1}cm^{-2}$	200	7.0	–	–	[29]
4.	ChOx/GO/AuNPs/SPCE	0.000025–12.93	0.000002	0.2	0.084 $\mu AmM^{-1}cm^{-2}$	120	8.6	4.95	–	[30]
5.	Apo- ChOx/PTBA/FAD/PGE	0.0008–0.0048	0.0002	0.65	0.21 $\mu A\mu M^{-1}$	2	7.4	–	35	[30]
6.	Apo- ChOx/PABA/FAD/PGE	0.0008–0.0056	0.0003	0.65	0.022 $\mu A\mu M^{-1}$	2	7.4	–	15	[30]
7.	ChOx/Poly (CBNP)/PGE	0.0025–0.0275	0.0004	−0.7	1.49 $\mu A\mu M^{-1}$	–	7.0	4.17	–	[31]
8.	ChOx/P (BImTh:Fmoc-Gly-OH)	0.0003–0.010	0.0002	–	2.1 $mAmM^{-1}cm^{-2}$	–	7.0	4.4	25	[32]
9.	ChOx/Poly (GMA-co-VFc/MNP/GCE)	0.00001–0.0001	0.000005	–	47 $\mu AmM^{-1}cm^{-2}$	1	7.0	–	18	[33]
10.	ChOx/PG	1.29–10.33	0.09	–	–	30	6.8	1.59	–	[34]
10.	ChOx/PB/GCE	8–4.5	4	−0.05	0.54 $\mu AmM^{-1}cm^{-2}$	25	7.0	6.7	50	[35]
11.	ChOx/sol–gel CHIT/MWCNT/PB/GCE	4–700	1	−0.05	1.55 $\mu AmM^{-1}cm^{-2}$	13	7.0	4.2	50	[36]
12.	ChOx/PTH/HRP/GCE	0.025–0.125	0.0063	–	0.18 $\mu A\mu M^{-1}$	–	7.0	–	–	[37]
13.	ChOx/PB sol–gel	0.001–0.08	0.00012	−0.05	0.329 $\mu A\mu M^{-1}$	< 60	6.8	–	35	[40]
14.	ChOx/Epoxyresin/Pt	1–8	0.1	0.5	0.00063 $\mu A\mu M^{-1}$	25	7.07	–	–	[42]

colorimetric [14], enzymic colorimetric [15], spectrophotometric [16] and microphotometric [17] are available for serum cholesterol determination. But these methods suffer with many disadvantages such as they are time consuming, cumbersome, labour intensive, less specific and less sensitive. Beside these general drawbacks of different conventional methods, there are specific limitations of each method such as interference of several substances with the colour reaction in colorimetric method, spots are often faint, and results are difficult to reproduce in case of thin layer chromatography.

To overcome these drawbacks the concept of biosensor was introduced [6].

Biosensor is an analytical tool that measures an analyte using a biological element (e.g. enzyme, DNA, Antibody) and converts that signal into a detectable form (Fig. 1). Transducer converts the biological signal into a measureable signal [18].

Based on transducer biosensors can be classified into following types:

1.1. Electrochemical

The basic principle for this class of biosensors is that chemical reactions between immobilized biomolecule and target analyte produce or consume ions or electrons, which affects measurable electrical properties of the solution, such an electric current or potential [19]. These are of two types: Amperometric and Potentiometric

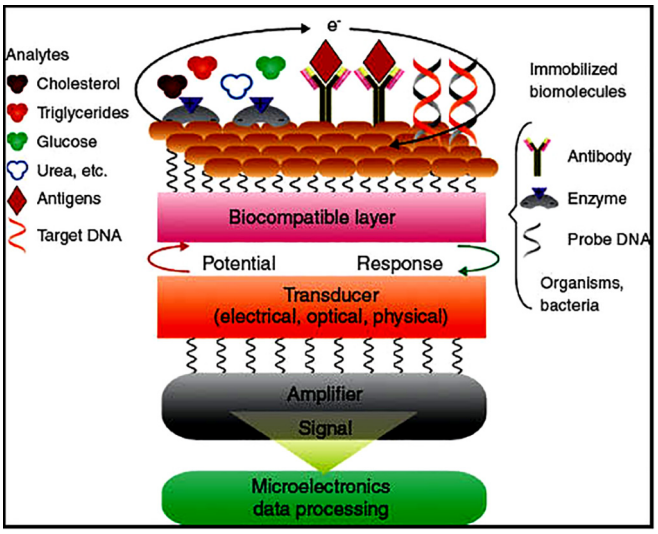

Fig. 1. Basic principle of biosensor [18].

1.1.1. Amperometric

Amperometric biosensors measures the current generated by enzyme catalyzed reactions such as electroxidation/electroreduction/hydrolysis/phosphorylation [20].

1.1.2. Potentiometric

This class of biosensors quantifies the difference in potential that is generated across an ion-selective membrane separating two solutions at virtually zero current flow [21].

1.2. Conductometric (Impedimetric)

Conductometric biosensors sense the change in conductivity or resistivity of the reaction mixture as ions or electrons are produced during the course of biochemical reaction [22].

1.3. Ion-sensitive

Biosensors based on ion-selective field-effect transistors (ISFETs) earlier considered as a category of potentiometric sensor [23].

1.4. Optical

The optical biosensors are based on fluorescence or optical diffraction. A fluorescence-based device detects the change in frequency of electromagnetic radiation emission which is generated by either absorption of radiation or by generation of an excited state lasting for a very short time [24].

1.5. Piezoelectric (mass-sensitive)

These are based on the coupling of the bioelement with a piezoelectric component like quartz-crystal coated with gold electrodes. Different materials e.g. quartz, tourmaline, lithium niobate or tantalate, oriented zinc oxide or aluminium nitride exhibit the piezoelectric effect and can be used for fabrication of piezoelectric biosensors [25].

Different types of cholesterol biosensors have been reported such as electrochemical, paper based, optical, reagentless and piezoelectric.

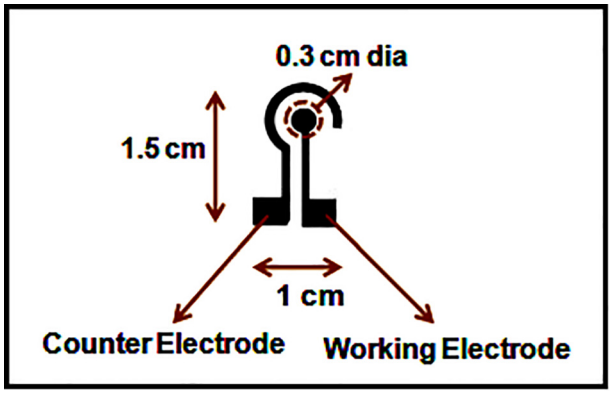

Fig. 2. PDMS/NiO electrode photomask [27].

2. Electrochemical cholesterol biosensors

2.1. Microfluidic electrochemical biosensor

Microfluidic devices displays a large number of benefits over other biosensors like less sample requirement, rapid process, highly sensitive and real time monitoring. Therefore a microfluidic biosensor based on polydimethylsiloxane (PDMS) microchannels with dimensions 300 μm (w) × 40 μm(h) × 1 cm(l) has been designed using SU8 photolithography and replica molding technique. To fabricate the biosensing chip, platinum (Pt) electrodes were designed by photolithography process. The working transducer was decorated with nickel oxide (NiO) thin film with the help of sputtering technique while bare Pt thin film worked as the counter electrode (Fig. 2). Microchannels are sealed reversibly with the chip by conformal contact process. Cholesterol oxidase (ChOx) enzyme was immobilized over the NiO thin film for specific detection of cholesterol. Highly sensitive detection of cholesterol in a wide range of concentration (0.12–10.23 mM) with a low detection limit of 0.10 mM was achieved using the fabricated microfluidic amperometric biosensor, thus demonstrating its ability for point-of-care diagnostics [26].

2.2. Screen printed carbon electrode (SPCE) based cholesterol biosensors

Biosensors based on nanomaterials represents the integration of material science, molecular engineering, chemistry and biotechnology can markedly improve the sensitivity and specificity of biomolecule detection, hold the capability of detecting or manipulating atoms and molecules, and have great potential in application such as biomolecular recognition, pathogen diagnosis and environment monitoring. Moreover, nanomaterials provide high specific surface thus already enabling the immobilization of an enhanced amount of bioreceptor units [27].

2.2.1. Gold nanoparticles (AuNPs) based SPCE

In this work, a simple and ultrasensitive cholesterol biosensor based on enzymatic silver deposition was designed by immobilizing ChOx and cholesterol esterase (CHER) onto the surface of gold nanoparticles (AuNPs) modified SPCE. By the catalytic action of CHER and CHOD, the cholesterol was hydrolyzed to generate hydrogen peroxide (H_2O_2) which can reduced the silver (Ag) ions in the solution for the deposition of metallic Ag on the surface of Au NPs modified SPE. The ultrasensitive detection of cholesterol was achieved by anodic stripping voltammetry (ASV) measurement of the enzymatically deposited Ag. The influence of relevant experimental variables was optimized. The anodic stripping peak current of Ag depended linearly on the concentration of cholesterol in the range of 5–5000 μg/mL with the regression correlation coefficient of 0.9983. A detection limit of 3.0 μg/mL was attained by 3 sigma-rule. In addition, the ultrasensitive cholesterol biosensor exhibited higher specificity, acceptable reproducibility and excellent recoveries for cholesterol detection [28].

2.2.2. Prussian blue nanoparticles based SPCE

The work describes the construction and optimization of a cholesterol biosensor based on SPCE modified with inkjet-printed Prussian blue nanoparticles (PBNPs). The deposition of PBNPs using inkjet printing led to the highly facile fabrication of sensors with excellent sensitivity and reproducibility for the measurement of H_2O_2. Further integration of the sensor with a microfabricated low volume (4 μL) sample cell allowed the measurement of cholesterol in serum with the addition of cholesterol oxidase. The biosensor exhibited a sensitivity to cholesterol of 2.1 μA/mM cm^2 (r^2 = 0.97, n = 5) and was linear in the range of 0–15 mM [29].

2.2.3. Graphene oxide (GO) and AuNPs based SPCE

The work demonstrates a simple and ultrasensitive cholesterol biosensor based on graphene oxide (GO) and AuNPs co-mediated enzymatic silver deposition was designed by immobilizing ChOx, CHER and GO onto the surface of AuNPs modified SPCE. Under the synergistic effect of CHER, ChOx and GO, the cholesterol was hydrolyzed to generate H_2O_2, which can reduce the silver (Ag) ions in the solution to metallic Ag which deposited on the surface of AuNPs modified SPE. The ultrasensitive detection of cholesterol was achieved by anodic stripping voltammetry measurement of the enzymatically deposited Ag. Under optimal conditions, the anodic stripping peak current of Ag increased with the increasing cholesterol concentration in the range from 0.01 μg/mL to 5000 μg/mL with a limit of detection of 0.001 μg/mL (S/N = 3). In addition, the ultrasensitive cholesterol biosensor exhibited higher specificity, acceptable reproducibility and excellent recoveries for cholesterol detection [30].

2.3. Pencil graphite electrode (PGE) based cholesterol biosensors

2.3.1. Boronic acid modified PGE based cholesterol biosensor

An amperometric cholesterol biosensor was fabricated by electropolymerization method of conducting polymers on PGE after which apo-ChOx was immobilized onto a flavin adenine dinucleotide (FAD) monolayer by reconstitution method. In this study two well-known and widely used materials in the field of biosensors thiophene-3-boronic acid and 3-aminophenyl boronic acid were electropolymerized onto PGE and modified with cholesterol oxidase. Electrochemical performance of modified electrodes and their properties were compared. Based on the results, thiophene-3-boronic acid based electrode shows sensitivity 10 times higher response than that of one based on 3-aminophenyl boronic acid [31].

2.3.2. (Z)-4-(4-(9H-carbazol-9-yl) benzylidene)-2-(4-nitrophenyl) oxazol-5(4H)-one CBNP PGE

A simple and robust cholesterol biosensor was designed by immobilizing ChOx onto a conducting polymer modified PGE (Fig. 3). For this purpose, monomer, (Z)-4-(4-(9H-carbazol-9-yl) benzylidene)-2-(4-nitrophenyl) oxazol-5(4H)-one (CBNP), was synthesized and electrochemically polymerized on an electrode to achieve an effective immobilization platform for enzyme immobilization. After electropolymerization of the monomer CBNP, electrochemical and spectroelectrochemical properties were investigated. Through the presence of nitro group on the polymer backbone hydrogen-bonding between enzyme molecules and polymer was achieved. Moreover, strong π–π stacking between aromatic moities in the polymer and aromatic residues of the enzyme enables a sensitive and reliable biosensor by conserving the crucial structure of biological molecules during the enzymatic reaction. The efficient interaction of the enzyme with the polymer coated surface brings easy and long-life detection of the substrate, cholesterol. After successful immobilization of ChOx with the help of glutaraldehyde as the crosslinking agent, amperometric

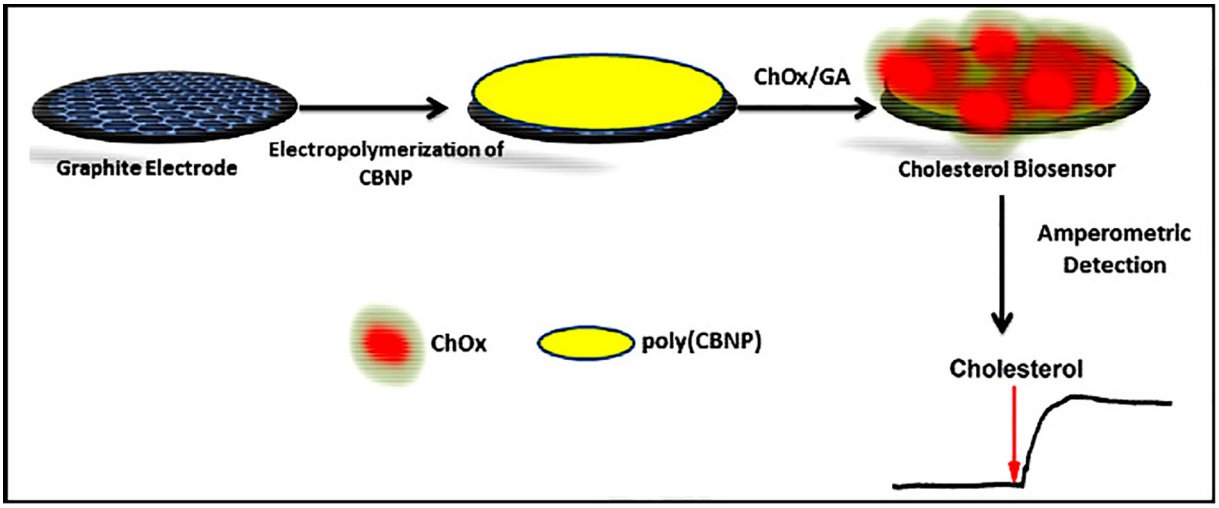

Fig. 3. Preparation of CBNP based cholesterol biosensor [31].

biosensor responses were recorded at −0.7 V vs Ag wire in phosphate buffer (pH 7.0). (37.3 μM), I_{max} (3.92 μA), LOD (0.4063 μM) and sensitivity (1.49 μA μM^{-1} cm^{-2}) values were determined. Finally, the prepared biosensor was successfully applied for determination of cholesterol content in real blood samples [32].

2.3.3. Conducting polymer modified PGE

2-Heptyl-4,7-di(thiophen-2-yl)-1H-benzo[d]imidazole) (BImTh) was synthesized. Electrochemical copolymerization of this monomer with 2-(((9H-fluoren-9-yl)methoxy)carbonylamino)acetic acid (Fmoc-Gly-OH) was achieved on a PGE and used as a matrix for amperometric cholesterol biosensing studies. In order to prepare a new cholesterol biosensor, ChOx was covalently immobilized onto the copolymer coated PGE. Cholesterol was used as the substrate and the decrease in oxygen level as a result of enzymatic reaction was monitored at −0.7 V vs Ag reference electrode in a phosphate buffer (50 mM, pH 7.0). Kinetic parameters, storage stabilities and surface characteristics were investigated. K_M^{app}, I_{max}, LOD and sensitivity were calculated as 6.25 μM, 9.69 μA, 0.17 μM and 2.47 mA/mM cm^2, respectively. This biosensor was applied to the determination of total cholesterol in serum samples [33].

2.3.4. PG electrode based biosensor

Serum cholesterol was determined by an amperometric device based on immobilization of cholesterol oxidase onto pencil graphite rod. It required low potential to generate electrons from H_2O_2, which does not allow ionization of serum substances. The optimum response was shown at pH 6.8 & 25 °C within 30 s. Biosensor exhibited linearity in range from 1.29×10^{-3} to 10.33×10^{-3} M with detection limit of 0.09×10^{-3} M. Mean analytical recovery of added cholesterol (100 mg/dl and 200 mg/dl) in serum was 85.0% & 90.0% respectively. Within batch and between batch coefficients of variations were 1.59% & 4.15% respectively. A good correlation (r = 0.99) was obtained between serum cholesterol values by standard enzymic colorimetric method and the present method. No interference by metabolites was observed in the method. The enzyme electrode was reused 200 times over a period of 25 days, when stored at 4 °C [34].

2.4. Glassy carbon electrode (GCE) based cholesterol biosensors

2.4.1. Magnetic nanoparticles (MNPs) modified GCE

A highly sensitive cholesterol biosensor was fabricated using poly (GMA-co-VFc)/MNP and cholesterol oxidase. The enzyme was immobilized on to GCE surface using poly (GMA-co-VFc) redox polymer containing super paramagnetic iron oxide nanoparticles (MNPs), which greatly enhanced electron transport between electrode and enzyme, giving a biosensor very good sensitivity, 47 μA mM–1, linear range of 0.01–0.12 μM, a detection limit of 0.005 μM and incredible response time of 1 s. The biosensor retained 70% of its original response after 18 days, when stored in 0.1 M PBS (pH 7.0) at 4° C, and it losses 18% of its initial activity in 20 continues assays [35].

2.4.2. Sol-gel chitosan/silica and multiwalled carbon nanotubes (MWCNTs) based GCE

A new type of amperometric cholesterol biosensor based on sol-gel chitosan/silica and multiwalled carbon nanotubes (MWCNTs) organic-inorganic hybrid composite material was developed. The hybrid composite film was used to immobilize ChOx on the surface of Prussian blue-modified glass carbon electrode. Effects of some experimental variables such as enzyme loading, concentration of Triton X-100, pH, temperature, and applied potential on the current response of the biosensor were investigated. Analytical characteristics and dynamic parameters of the biosensors with and without MWCNTs in the hybrid film were compared, and the results show that analytical performance of the biosensor can be improved greatly after introduction of the MWCNTs. Response time, sensitivity, linear range, limit of detection (S/N = 3), and apparent Michaelis-Menten constant Km are 25 s, 0.54 μA mM^{-1}, 8.0×10^{-6} to 4.5×10^{-6} M, 4.0×10^{-6} M, and 0.41 mM for the biosensor without MWCNTs and 13 s, 1.55 54 μA mM^{-1}, 4.0×10^{-6} to 7.0×10^{-4}, 1.0×10^{-6} M, and 0.24 mM for the biosensor with MWCNTs, respectively. The activation energy of the enzyme-catalyzed reaction was measured to be 42.6 kJ mol^{-1}. This method has been used to determine the free cholesterol concentration in real human blood samples [36].

2.4.3. Poly(thionine)-modified GCE

A simple and cheap cholesterol biosensor was designed by immobilizing ChOx and horseradish peroxidase (HRP) onto a poly(thionine) (PTH) modified GCE (Fig. 4A). Being mediated by hydroquinone (HQ), the immobilized HRP exhibited excellent electrocatalytic activity in reducing H_2O_2, which was produced from cholesterol by the enzymatic reaction of ChOx (Fig. 4B). The linear detection range for cholesterol was 25–125 μM, with a detection limit (S/N = 3) and a sensitivity of 6.3 μM and 0.18 μA/cm^2/μM, respectively, under optimal conditions. The highly reproducible and sensitive GCE/PTH/ChOx/HRP sensor exhibited an interference-free signal for cholesterol detection with excellent recoveries for real sample analysis [37].

2.4.4. Graphene/ionic liquid-modified GCE

ChOx and catalase (CAT) were co-immobilized on a graphene/ionic

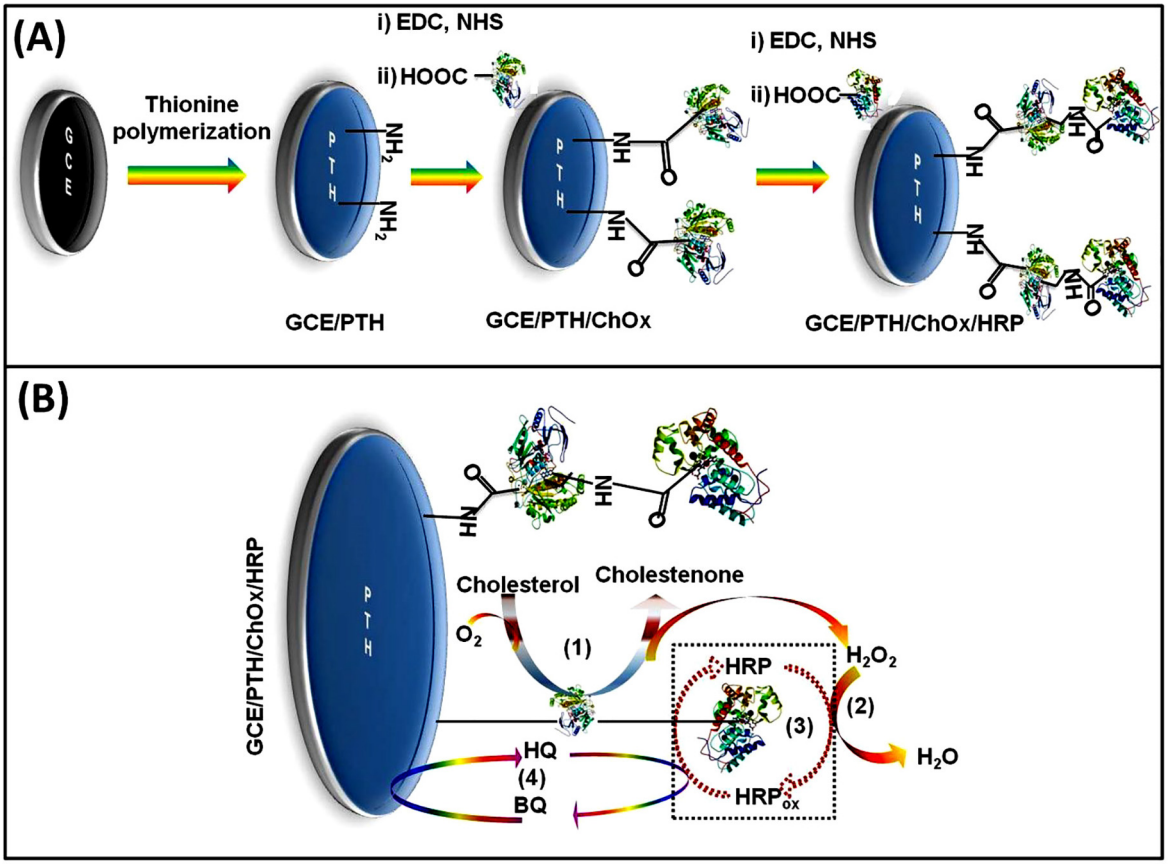

Fig. 4. (A) Preparation of polythione modified GCE (B) Chemical reactions involving in preparation of polythione modified GCE [36].

liquid-modified GCE (GR–IL/GCE) to develop a highly sensitive amperometric cholesterol biosensor. The H_2O_2 generated during the enzymatic reaction of ChOx with cholesterol could be reduced electrocatalytically by immobilized CAT to obtain a sensitive amperometric response to cholesterol. The direct electron transfer between enzymes and electrode surface was investigated by cyclic voltammetry. Both enzymes showed well-defined redox peaks with quasi-reversible behaviors. An excellent sensitivity of 4.163 mA mM^{-1} cm^{-2}, a response time less than 6 s, and a linear range of 0.25–215 μM ($R^2 > 0.99$) have been observed for cholesterol determination using the proposed biosensor. The apparent Michaelis–Menten constant () was calculated to be 2.32 mM. The bienzymatic cholesterol biosensor showed good reproducibility (RSDs < 5%) with minimal interference from the coexisting electroactive compounds such as ascorbic acid and uric acid. The CAT/ChOx/GR–IL/GCE showed excellent analytical performance for the determination of free cholesterol in human serum samples [38].

2.4.5. Hemoglobin-encapsulated chitosan-modified GCE

A cholesterol biosensor based on direct electron transfer of a hemoglobin-encapsulated chitosan-modified GCE has been developed for highly sensitive and selective analysis of serum samples. Modified by films containing hemoglobin and cholesterol oxidase, the electrode was prepared by encapsulation of enzyme in chitosan matrix. The H_2O_2 produced by the catalytic oxidation of cholesterol by cholesterol oxidase was reduced electrocatalytically by immobilized hemoglobin and used to obtain a sensitive amperometric response to cholesterol. The linear response of cholesterol concentrations ranged from 1.00×10^{-5} to 6.00×10^{-4} mol/L, with a correlation coefficient of 0.9969 and estimated detection limit of cholesterol of 9.5 μmol/L at a signal/noise ratio of 3. The cholesterol biosensor can efficiently exclude interference by the commonly coexisting ascorbic acid, uric acid, dopamine, and epinephrine. The sensitivity to the change in the concentration of cholesterol as the slope of the calibration curve was 0.596 A/M. The relative standard deviation was under 4.0% (n = 5) for the determination of real samples. The biosensor is satisfactory in the determination of human serum samples [39].

2.4.6. Silicic sol-gel matrix at a Prussian blue modified GCE

Prussian Blue-modified GCE was employed for fabrication of a cholesterol biosensor by immobilization of cholesterol oxidase (ChOx) in a layer of silicic sol-gel matrix. It detected hydrogen peroxide produced by ChOx at 0.05 V. Biosensor possessed half-lifetime of about 35 days. Cholesterol can be determined in the concentration range of 1×10^{-6} to 8×10^{-5} M with a detection limit of 1.2×10^{-7} M. Compounds like ascorbic acid and uric acid do not interfere with the determination [40].

2.5. Platinum electrode (Pt) based cholesterol biosensors

2.5.1. Polypyrrole film modified Pt based cholesterol biosensors

The preparation of a cholesterol amperometric biosensor using a platinized Pt electrode as a support for the electropolymerization of a polypyrrole film, in which ChOx and ferrocene monocarboxylic acid (electron-transfer mediator) were co-entrapped, is described (Fig. 5). All the biosensor preparation steps (platinization and electropolymerization) and the cholesterol determination take place in the same flow system. The presence of the mediator enhances the sensitivity and selectivity of the platinized biosensor without modifying the dynamic parameters of the response, and the platinized layer improves the operational lifetime of the mediated sensor. The sensitivity obtained was 88.51 nA mM^{-1} and the limit of detection was 12.4 μM of cholesterol. The analytical properties of the biosensor for the flow-injection determination of cholesterol were studied and compared with those of other simpler amperometric biosensor configurations [41].

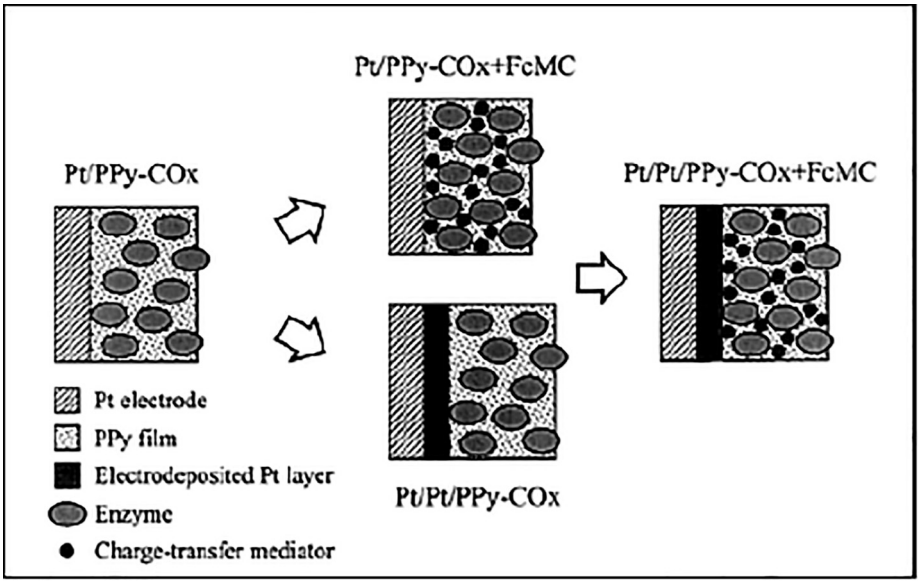

Fig. 5. Fabrication of polypyrrole film modified platinum electrode [40].

2.5.2. Epoxy resin maembrane bound onto Pt electrode based cholesterol biosensor

Amperometric method was described for estimation of free cholesterol using epoxy resin membrane bound cholesterol oxidase. It worked efficiently within 25 s at +0.5 V and pH 7.0 7 45 °C and showed linearity with cholesterol concentration in the range 1–8 mM. Biosensor exhibited sensitivity of 0.63 μA/mM having detection limit of 0.1 mM. The half life of the biosensor was 6 months, when stored in reaction buffer at 4 °C [42].

2.6. Gold electrode (AuE) based cholesterol biosensor

2.6.1. Bismuth subcarbonate ($Bi_2O_2CO_3$) nanoplates based AuE

This study reports the development of a highly sensitive and selective amperometric cholesterol biosensor based on the bismuth subcarbonate ($Bi_2O_2CO_3$) nanoplates synthesized by the facile hydrothermal process at low temperature. The detail characterization of as-synthesized material in terms of their morphological, structural and compositional properties revealed that the nanoplates are synthesized in large quantity with well-crystallinity. The fabricated biosensors exhibit a very high and reproducible sensitivity of 139.5 $\mu A m M^{-1} cm^{-2}$, wide linear range from 0.05 mM to 7.4 mM, fast response time of ~4 s and low detection limit of 10 μM (S/N = 3) for cholesterol sensing. The anti-interference ability, reproducibility, and long-term stability were also assessed. To the best of our knowledge; this is the first report which demonstrates the use of $Bi_2O_2CO_3$ nanostructures for the fabrication of highly sensitive and selective cholesterol biosensor [43].

2.6.2. PANI modified Au electrode

The polyaniline (PANI)/Au nanocomposite was successfully fabricated through a seed-mediated strategy. And the hybrid nanostructure evaluated as a new material for cholesterol biosensor was demonstrated. UV–vis spectra proved the production of conductive PANI and the increase of the particle size of the Au nano-seeds. Scanning electron microscopic measurements displayed that the synthesized PANI/Au exhibited a spherical structure with dimensions of about 300 nm in diameter. Energy dispersive X-ray spectrogram demonstrated the ingredient of the composite. Cyclic voltammetry and electrochemical impedance spectroscopy investigation of the PANI/Au modified electrode indicated the good conductivity of the composite. Direct electron transfer of ChOx was obtained in pH 7.0 phosphate buffer solution when ChOx was further immobilized on the PANI/Au modified electrode. This result showed that the PANI/Au nanocomposite was a good candidate for the development of the cholesterol biosensor. The biosensor displayed a response time of 3 s. Some common interferents like glucose and ascorbic acid did not cause interference due to the use of a low operating potential [44].

2.7. ITO electrode based cholesterol biosensor

Sophisticated molecular architectures can be produced with the layer-by-layer (LbL) method, which may combine distinct materials on the same film (Fig. 6). In this study, cholesterol amperometric biosensor was designed from LbL films containing hemoglobin (Hb) and cholesterol oxidase in addition to the polyelectrolytes poly(allylamine hydrochloride) (PAH) and poly(ethylene imine) (PEI). Following an optimization procedure, an LbL film deposited onto ITO substrates, with the architecture $ITO(PEI/Hb)_5(PEI/COx)_{10}$, yielded a sensitivity of 93.4 $\mu A\ \mu mol\ L^{-1}\ cm^{-2}$ for cholesterol incorporated into phospholipid liposomes, comparable to state-of-the-art biosensors. Hb acted as efficient electron mediator and did not suffer interference from phospholipids. Significantly, cholesterol could also be detected in real samples from chicken egg yolk, with no effects from potential interferents, including phospholipids. Taken together these results demonstrate the possible fabrication of low cost, easy-to-use cholesterol amperometric biosensors, whose sensitivity can be enhanced by further optimizing the molecular architectures of the LbL films [45].

2.8. Paper based cholesterol biosensor

A novel nanocomposite of graphene (G), polyvinylpyrrolidone (PVP) and polyaniline (PANI) has been successfully prepared and used for the modification of paper-based biosensors via electrospraying (Fig. 7). The droplet-like nanostructures of G/PVP/PANI-modified electrodes are obtained with an average size of 160 ± 1.02 nm. Interestingly, the presence of small amount of PVP (2 $mg\ mL^{-1}$) in the nanocomposites can substantially improve the dispersibility of G and increase the electrochemical conductivity of electrodes, leading to enhanced sensitivity of the biosensor. The well-defined cyclic voltammogram of standard ferri/ferrocyanide is achieved on a G/PVP/PANI-modified electrode with a 3-fold increase in the current signal compared to an unmodified electrode. This modified electrode also exhibits excellent electrocatalytic activity towards the oxidation of H_2O_2. Furthermore, ChOx is attached to G/PVP/PANI-modified electrode for the

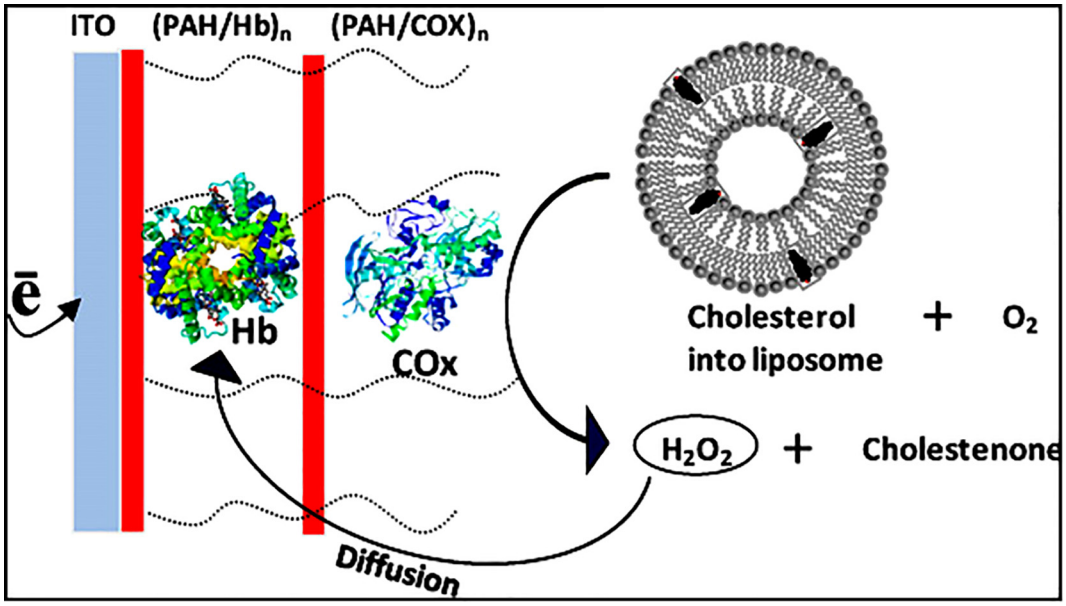

Fig. 6. Mechanisms involved in detection of cholesterol using Hb as mediator of electrons for detection of H_2O_2 [44].

amperometric determination of cholesterol. Under optimum conditions, a linear range of 50 μM to 10 mM is achieved and the limit of detection is found to be 1 μM for cholesterol. Finally, the proposed system can be applied for the determination of cholesterol in a complex biological fluid (i.e. human serum) [46].

2.9. Cu/Ni-dispersed CNF-based electrode

A novel Cu/Ni bimetal-dispersed carbon nanofiber/polymer nanocomposite (BMCP)-based electrode was developed in this study for the measurements of cholesterol (Fig. 8). Dendritic nanofibers of poly methyl orange (PMO), which served as the recognition element for cholesterol, were successfully grown over BMCP, using electro-polymerization. Tested using various electrochemical techniques including chronoamperometry and differential pulse voltammetry, the prepared PMO-BMCP biosensor showed a high sensitivity ($226.30\ \mu A\ mM^{-1}\ cm^{-2}$) and low detection limit ($0.002\ mg\ dL^{-1}$) over 0.04–600 $mg\ dL^{-1}$ concentration range, with remarkable linearity ($R^2 = 0.999$), which were either higher or comparable to most of the cholesterol sensors discussed in literature. The biosensor exhibited good reproducibility and long-time (~8 months) stability of the electrochemical activity. The presence of interfering bioactive agents, namely, glucose, uric acid, creatinine, urea and p-acetamido phenol had negligible effect on the measurement of cholesterol. The proposed material and method described in this study can be used to develop similar non-enzymatic electrochemical biosensors for other bioactive molecules in blood, such as glucose and creatinine [47].

3. Liquid crystal based cholesterol biosensor

A cholesterol biosensor was fabricated by coating 4-cyano-4′-pentylbiphenyl (5CB) in a transmission electron microscope (TEM) grid with poly(acrylicacid-b-4-cyno-4′-undecyl acrylate) (PAA-b-LCP), followed by co-immobilization of ChOx and HRP on the PAA brushes. This TEM grid sensor was tested for stability and sensitivity of the immobilized enzymes to cholesterol. Cholesterol was detected by a planar-to-homeotropic orientational change of 5CB at a limit of 0.8 mM. The high stability, ease of preparation, and specific detection of cholesterol in a complex mixture, could provide a new and convenient method for screening cholesterol levels in a sample using a polarized optical microscope [48].

4. Photoelectrochemical cholesterol biosensor

Herein, a novel electrochemical–photoelectrochemical (PEC) dual-mode cholesterol biosensor was fabricated based on graphene (G) sheets interconnected-graphene embedded titanium nanowires (TiO_2(G)-NWs) 3D nanostacks (designated as G/Ti(G) 3DNS) by exploiting the beneficial characteristics of G and TiO_2-NWs to achieve good selectivity and high sensitivity for cholesterol detection (Fig. 9). The G/Ti(G) 3DNS was fabricated by the reaction between functionalized G and TiO_2(G)-NWs. ChOx was subsequently immobilized in to G/

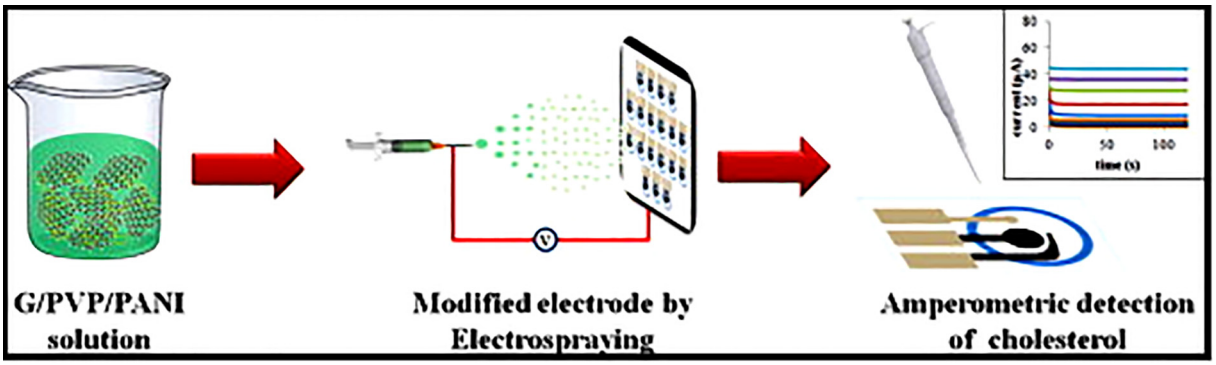

Fig. 7. Fabrication of paper based electrode for cholesterol estimation [45].

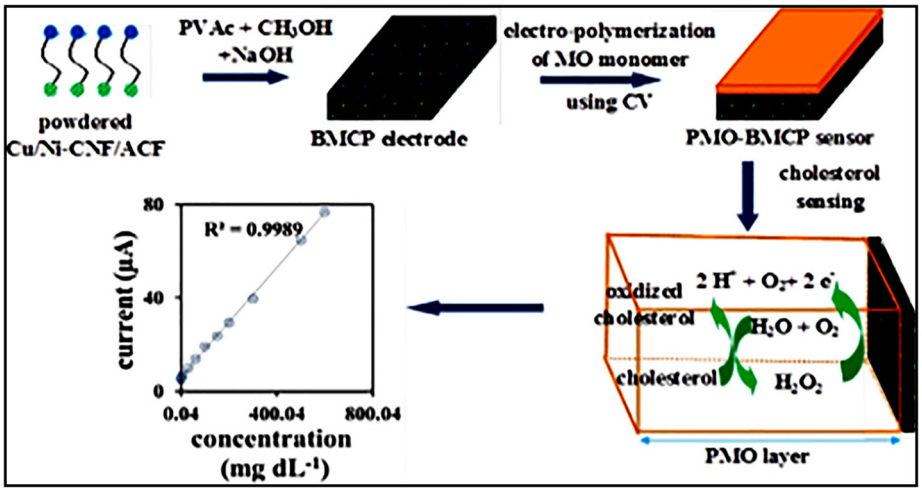

Fig. 8. Cu/Ni bimetal-dispersed carbon nanofiber/polymer nanocomposite (BMCP)-based electrode preparation [46].

Ti(G) 3DNS using chitosan (CS) as the binder and the dual mode G/Ti (G) 3DNS/CS/ChOx biosensor was fabricated. The electro-optical properties of the G/Ti(G) 3DNS/CS/ChOx bioelectrode were characterized by cyclic voltammetry and UV–vis diffuse reflection spectroscopy. The cyclic voltammetry of immobilized ChOx showed a pair of well-defined redox peaks indicating direct electron transfer (DET) of ChOx. The amperometric reduction peak current (at −0.05 V) linearly increased with increase in cholesterol concentration. The G/Ti(G) 3DNS/CS/ChOx bioelectrode was selective to cholesterol with a remarkable sensitivity (3.82 μA/cm^2 mM) and a lower detection limit (6 μM). Also, G/Ti(G) 3DNS/CS/ChOx functioned as photoelectrode and exhibited selective detection of cholesterol under a low bias voltage and light irradiation. Kinetic parameters, reproducibility, repeatability, storage stability and effect of temperature and pH were evaluated [49].

5. Electrochemiluminescent (ECL) cholesterol biosensor

5.1. Au/hollowed-TiO_2 nano composite based ECL biosensor

This study describes a novel approach to promote the analytical performance of electrochemiluminescent (ECL) biosensor of cholesterol by a nanocomposite (AuNPs/ion liquid/hollowed TiO_2 nano-shell) prefunctionalized indium tin oxide glass to load the cholesterol oxidase. Here the enzymatically produced hydrogen peroxide greatly intensified the ECL of luminol under the catalysis of the nanocomposite. The quantification of cholesterol was directly accomplished by calibrating the ECL sensing output. The formation and properties of the nanocomposite and the functionalized electrode have been confirmed by electrochemistry, spectroscopy and electron-microscopy. The prepared biosensor exhibits a linear regression ranged from 8.33×10^{-9} M to 4.17×10^{-7} M with an ultralow detection limit (LOD) of 6.30×10^{-9} M. It also possesses the excellent specificity for cholesterol from potential interferents, the favorable repeatability/stability for use or storage, the good reproducibility and adequate recovery for real sample test. In addition, for the detection of clinical serum sample, it is well consistent with the datum from medical cholesterol kit, indicating its reliability. It is in promising of the application for the cholesterol detection in those clinical specimens of teeny amount of sample and limited concentration [50].

5.2. Hemin-functionalized graphene based ECL biosensor

A novel ECL enzyme biosensor for the ultrasensitive detection of cholesterol was designed based on a bi-pseudoenzymatic reaction to generate a coreactant of peroxydisulfate for signal amplification. In this work, hemin-functionalized graphene (hemin-GR) was synthesized and used to immobilize ChOx to construct an ECL biosensor for cholesterol. When cholesterol was added to the detection solution, ChOx catalyzed the oxidation of cholesterol to generate H_2O_2, which could be further catalyzed by hemin to produce O_2 as the coreactant in the peroxydisulfate system for signal amplification. The linear range for cholesterol detection was 3.3–1500 nM, with a lower detection limit of 1.0 nM (signal to noise ratio = 3). Therefore, the detection limit and sensitivity of the biosensor were improved. This novel strategy offers advantages of simplicity, improved sensitivity, good selectivity, and repeatability, and therefore, holds promise for use in sensitive bioassays for clinical determination of cholesterol levels [51].

5.3. MoS_2 quantum dots (MoS_2 QDs) and graphene quantum dots (GQDs)

Developing a novel non-enzyme mimetic in biosensors is of great significance. Here, a synergetic peroxidase-like activity was disclosed for mixed MoS_2 QDs and GQDs. The high catalytic effect of this mixture was studied on the ECL system. It was observed that the simultaneous presence of MoS_2 QDs and GQDs had a powerful enhancing effect on the chemiluminescence (CL) emission of rhodamine B (RB)-H_2O_2 reaction. MoS_2 QDs and GQDs mixture (prepared with a ratio of 3:2) showed a superior catalytic activity when compared to each of the constituents. A linear relationship was acquired between the CL emission intensity and H_2O_2 concentration in the range of 1.5–460 nmol L^{-1}. On the other hand, since the enzymatic oxidation of cholesterol leads to the production of H_2O_2; the offered CL system was examined to detect cholesterol after its oxidation by ChOx enzyme. Herein, a further improvement was achieved by MoS_2 nanosheets. The MoS_2 nanosheets increased the performance of ChOx in cholesterol oxidation process. The obtained results confirmed a highly selective and sensitive determination of cholesterol concentration in a linear dynamic range of 0.08–300 μmol L^{-1}, with a detection limit (3S) of 35 nmol L^{-1}. The developed method was successfully applied for the detection of cholesterol level in human serum samples [52].

5.4. AuNPs based ECL biosensor

A novel cholesterol biosensor was prepared based on AuNPs-catalyzed luminol electrogenerated ECL. Firstly, l-cysteine-reduced graphene oxide composites were modified on the surface of a GCE. Then, AuNPs were self-assembled on it. Subsequently, cholesterol oxidase (ChOx) was adsorbed on the surface of AuNPs to construct a cholesterol biosensor. The stepwise fabrication processes were characterized with cyclic voltammetry and atomic force microscopy. The ECL behaviors of

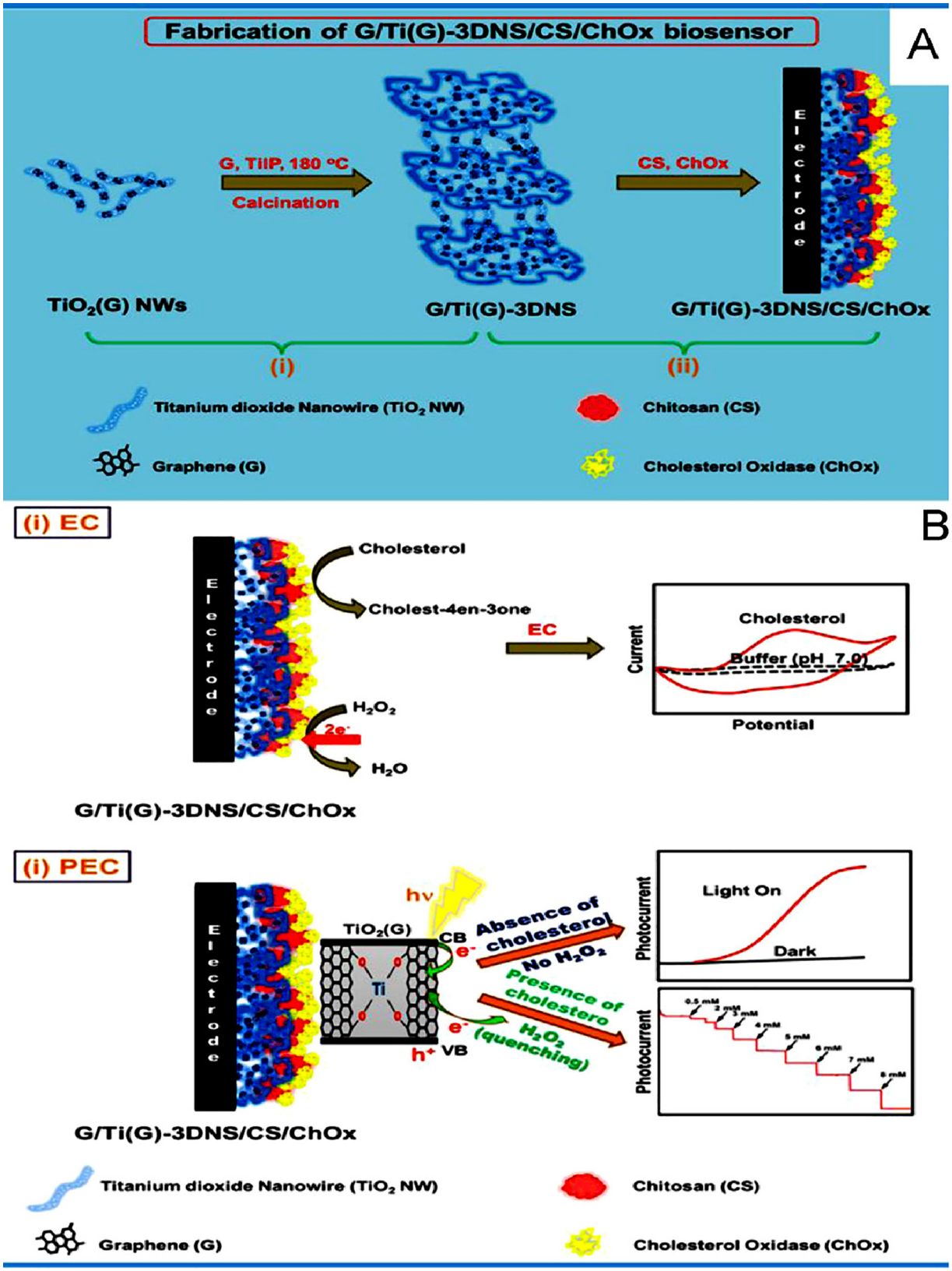

Fig. 9. Fabrication of photoelectrochemical biosensor [48].

the biosensor were also investigated. It was found that AuNPs not only provided larger surface area for higher ChOx loading but also formed the nano-structured interface on the electrode surface to improve the analytical performance of the ECL biosensor for cholesterol. Besides, based on the efficient catalytic ability of AuNPs to luminol ECL, the response of the biosensor to cholesterol was linear range from 3.3 μM to 1.0 mM with a detection limit of 1.1 μM (S/N = 3). In addition, the prepared ECL biosensor exhibited satisfying reproducibility, stability

and selectivity. Taking into account the advantages of ECL, we confidently expect that ECL would have potential applications in biotechnology and clinical diagnosis [53].

6. Electrospun polyaniline nanofibers based piezoelectric cholesterol biosensor

An amperometric cholesterol biosensor was fabricated using electrospun polyaniline nanofibers. Polyaniline was dissolved in chloroform with camphorsulfonic acid, and polystyrene was added in this solution. Using this mixed solution, nanofibers were formed and collected by electrospinning. Then ChOx was immobilized onto these fibers using an electrostatic layer-by-layer adsorption technique. Poly(diallyldimethylammonium chloride) was used as the counter ion source. The level of adsorption was examined and evidence of layer-by-layer adsorption was investigated using a quartz crystal microbalance (QCM) technique. A cholesterol biosensor was fabricated from these nanofibers as a working electrode, and it was used to measure the cholesterol concentration [54].

7. Graphene nanosheet based BioFET biosensor

The intensive bio-effects on graphene surface is an attention-grabbing field which determine the capability of performing rapid detection of biomolecules with high accuracy. In electrical transport measurements, the Dirac point position is gradually shifted towards negative gate voltage as cholesterol concentration increases which reveal the clear influence of cholesterol molecules on the graphene. These graphene surface modifications induce n-type doping and the charge carrier mobility is increased from $\sim 2000\,cm^2\,V^{-1}\,s^{-1}$ to $\sim 3900\,cm^2\,V^{-1}\,s^{-1}$ by increasing the cholesterol concentration. The detection of cholesterol molecules is further investigated by Raman spectroscopy, FTIR and AFM characterizations. The results indicate significant impact of cholesterol-graphene interaction on the performance of graphene devices. Furthermore, sensing approach can be quantitatively deployed for commercial use of portable graphene-based cholesterol sensing devices for biomedical applications [55].

8. Reagentless cholesterol biosensor

An efficient reagentless biosensor for determination of free cholesterol and total cholesterol has been realized using a ZnO–CuO composite matrix grown onto ITO coated corning glass substrates by pulsed laser deposition (PLD) technique. The inclusion of CuO in ZnO matrix successfully introduces redox property and provides enhanced electron communication features. The optimized ZnO–CuO composite matrix has been used for development of ChOx/(ZnO–CuO)/ITO/glass and (ChEt–ChOx)/(ZnO–CuO)/ITO/glass bioelectrodes used for selective detection of free and total cholesterol respectively in the range of 0.12–12.93 mM and 0.5–12 mM without using any external mediator. The fabricated biosensors exhibit high sensitivity of about $680\,\mu A\,mM^{-1}\,cm^{-2}$ and $760\,\mu A\,mM^{-1}\,cm^{-2}$ towards free cholesterol and total cholesterol respectively with response time of 5 s, along with high shelf life. The results are encouraging and show the promising application of the ZnO–CuO composite matrix for the realization of reagentless integrated implantable biosensor [56].

9. Optical cholesterol biosensors

9.1. Silicone-entrapped tris (4,7-diphenyl-I,10-phenanthroline) ruthenium (II) complex based cholesterol biosensors

An optical fiber biosensor for free cholesterol monitoring in serum samples is described. The luminescence, tris (4,7-diphenyl-I,10-phenanthroline) ruthenium (II) complex entrapped-Silicone, is used as an optical transducer and is sensitive to oxygen fluctuation. Of free cholesterol in serum the active range of the sensing film is measured to be 0.15–3.0 mM. This sensor was found to be stable at the 7.0 pH. Optimum temperature measured was 303 K. The detection limit of cholesterol was found to be 0.15 mM [57].

9.2. Arylamine glass beads based cholesterol biosensor

The spectrophotometric method was used for estimation of free cholesterol content, in which the enzymatic reaction showed an increase in the color intensity as the concentration of analyte increased by way of oxidation of 4-aminoantipyrine (4-AAP), a dye. The maximum activity was found to be 1.7148 g/l, at pH 7.5 and 40 °C [58].

9.3. Octadecylsilica (ODS) particles based cholesterol biosensor

For cholesterol determination an optical oxygen transducer was used, in which the sensing depends on the ruthenium(II) compound fluorescence quenching through oxygen. The results signifies that quenching of ruthenium(II) complex decreased by the increased utilization of oxygen, is a result of increases in fluorescence when there is an increase in cholesterol concentration. The diagnostic working range of cholesterol biosensor for the aqueous micelle was from 0.05 to 8.0 mM cholesterol and for the organic phase was from 0.07 to 18.0 mM cholesterol, at pH range 5.5–8.5 and 40 °C the response time was found to be 4–12 min [59].

9.4. Cellulose acetate membrane based cholesterol biosensor

Wang et al. have utilized the of $Ru(Phen)_3Br_2$ fluorescence properties was oxygen reliant for making of cholesterol biosensor based on the fiber-optic fluorescent. The optimum conditions of immobilized COD, were: (i) 2.5% glutaraldehyde (ii) 8 mg/100 mL concentration of COD (iii) of immobilization time was 4 h at 25 °C (iv) optimum pH was 7.2 [60].

9.5. Hydrogel based optic biosensor

The study described a temperature-triggered fiber optic biosensor for sequential detection of cholesterol and glucose based on poly(**N**-isopropylacrylamide)-co-acrylamide)(P(NIPAAm-co-AAm))-magnetic immobilized glucose oxidase(GOD) complex (PMIGC) and magnetic immobilized ChOx (Fig. 10). At 38 °C, the sensor will selectively detect cholesterol concentration with the detection range of 25–250 mg/dL since P(NIPAAm-co-AAm) is known to shrink above its lower critical solution temperature (LCST) of 36 °C, and will separate GOD from analytes so that PMIGC has no catalysis effect on glucose. When the temperature was switched to 25 °C (below LCST), PMIGC could catalyze the oxidation of glucose since GOD will be exposed to analytes due to the swell of P(NIPAAm-co-AAm). This sensor can be used for the detection of glucose with the detection range of 50–700 mg/dL. The optimal detection conditions for cholesterol were achieved with pH 7.0, 40 °C and 10 mg ChOx (in 75 mg carrier), and those for glucose were achieved with pH6.5, 35 °C and 12 mg GOD (in 90 mg carrier). The biosensor is shown to have outstanding repeatability, selectivity and yield satisfactory detection results on practical samples [61].

10. Conclusion and future perspective

The cholesterol biosensors are considered as an important device in clinical analysis of a large number of diseases such as cardiovascular diseases. Therefore, there is a need to develop most effective and precise device for effective monitoring of the cholesterol. The already available devices are not portable and also fail to real time monitoring of cholesterol. Moreover, they can't be used at home by patients. To keep in mind these drawbacks, there is a need to develop miniaturized cholesterol biosensor with low cost. Therefore, the upcoming research

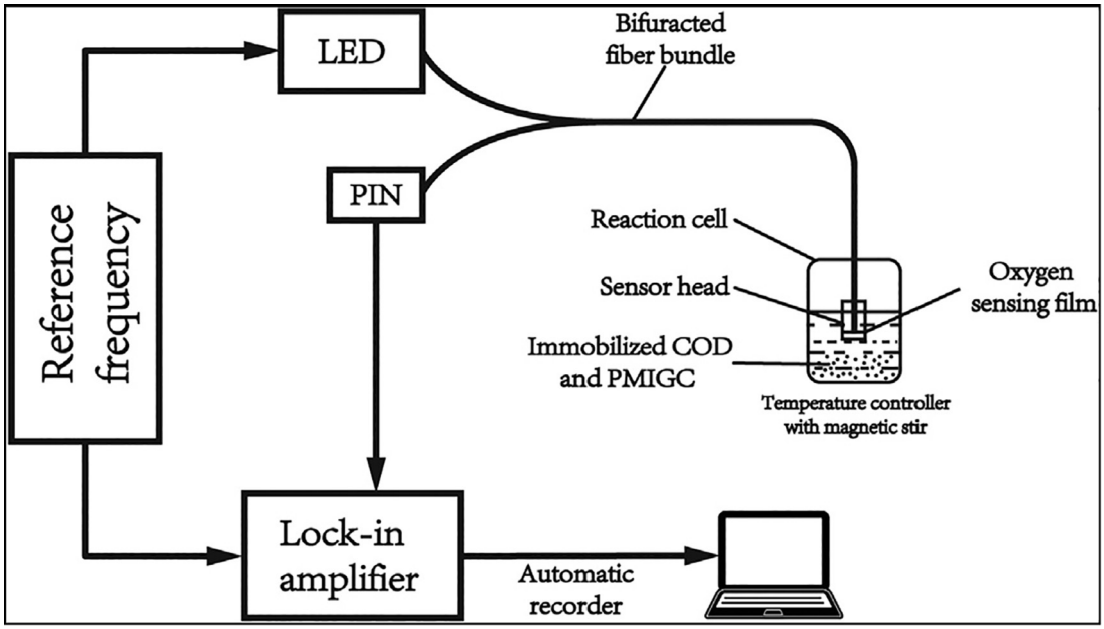

Fig. 10. Reagentless detection system for cholesterol biosensor [60].

in cholesterol biosensors would focus on fully automatic lab on chip devices so that they can be easily used by the patients at home or his/her bedside at hospitals. Labs on chip devices represents various advantageous features like rapid response, low sample intake and cost effectiveness. Additionally, the enzyme nanoparticles based cholesterol biosensor could be designed to make the fabrication process simpler and effective.

Acknowledgement

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