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Advancements in nonenzymatic electrochemical cholesterol detection: fostering material innovation with biosensing technologies

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Cholesterol, a sterol lipid, is vital for various biological phenomena encompassing metabolism and cell functioning. Nevertheless, drastic changes in cholesterol levels will lead to severe cardiovascular disorders. The development of point-of-care technology plays a prominent role in frequent and pinpoint monitoring of cholesterol changes. The introduction of enzymatic biosensors revolutionized cholesterol detection; however, these sensors face significant challenges, including restricted stability, high expense, and sensitivity to environmental conditions. This review highlights the advancements in non-enzymatic electrochemical cholesterol biosensors, focusing on the application of novel materials, including metals and metal oxides, carbon and graphene-based materials, polymeric materials, MOFs, MXenes, photoelectrochemical materials, and advanced materials and composites, to enhance sensitivity, selectivity, and stability. Particular emphasis is placed on electrochemical techniques, material modifications, and their influence on sensing performance. For ease of comprehension and evaluation, standard statistics have been presented in a tabular format. Despite significant advancements, challenges such as miniaturization, reproducibility, and real-sample analysis persist. This review underscores the potential of nonenzymatic electrochemical biosensors to transform biosensing diagnostics and emphasizes the need for continued innovation in materials science and device integration.

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1. Introduction

Cholesterol, a dominant sterol, is most important in balancing animal cell membranes, structural integrity, and functionality. It is a key biomarker in clinical diagnostics. As a necessary biomolecule, maintaining an optimal cholesterol balance is vital for health. Elevated serum cholesterol concentrations exceeding 6.2 mM (240 mg dL^{-1}) have been strongly associated with cardiovascular disorders (CVDs), including atherosclerosis and hypertension.[1–4] CVDs are the primary cause of death globally, with nearly 17.9 million deaths yearly, as confirmed by the World Health Organization. In 2001, around 13 million deaths occurred in countries such as China and India, with projections reaching 23 million by 2030. The average total cholesterol level in healthy human serum is approximately 200 mg dL^{-1} (5.17 mM).[5,6] Given the significant role of cholesterol, precise monitoring through accessible methods is vital for diagnosing and preventing various clinical disorders, including heart diseases. Additionally, determining the cholesterol content in food is essential for recommending low-cholesterol diets and personalized lifestyle adjustments.[7]

The cholesterol level in human blood serum is vital for the diagnosis of several illnesses, such as liver problems,

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diabetes, nephrotic syndrome, hypothyroidism, and cardiovascular diseases.[8–12] Additionally, monitoring dietary cholesterol intake is essential, as excess consumption can lead to high cholesterol levels, increasing the risk of various health problems. Following a proper dietary plan of cholesterol intake can help reduce these risks and promote better health.[7,13,14] The major significance of cholesterol as a biomolecule is that monitoring its levels in daily life is essential for maintaining overall health. In addition to traditional chemical approaches, several advanced methods have been developed for cholesterol detection, including colorimetric techniques,[15,16] high-performance liquid chromatography (HPLC),[17] fluorescence assays,[18,19] enzymatic assays, microphotometry, gas chromatography, and mass spectrometry.[20–23] However, many of these techniques are associated with various drawbacks, such as high expense, complicated methodologies, and time-intensive procedures.

To handle these challenges, biosensors have appeared as a promising tool, giving exceptional sensitivity, low detection limits, and high reproducibility.[24,25] The inventiveness in the size and type of electrode used in biosensors enables their blending into miniaturized instruments, presenting an interesting opportunity for the evolution of point-of-care tools.[26–28] A biosensor is an analytical device designed to measure the concentration of a specific analyte utilizing biological elements like enzymes, antibodies, cells, or DNA. These biological interactions are subsequently transformed into an electrical signal through a transducer, enabling precise quantification.[29–31] Biosensors are majorly categorized based on the nature of the transduction used. In these devices, the coated electrode surface interacts with the analyte, prompting an electrical signal through the transducer in the form of current, potential, conductivity, or resistivity.[32] Depending on the operative mechanism, biosensors are categorized as amperometric, potentiometric, conductometric, or impedimetric sensors, respectively.[7,26,33]

The electrochemical analysis of cholesterol is crucial in the context of sensors and lab-on-a-chip devices for pharmaceutical and biomedical analysis. The research on electrochemical cholesterol biosensors commenced in the

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1990s and earlier, primarily focusing on the utilization of enzymes.[34] The material coated on the electrode surface is immobilized with certain enzymes that interact with the analyte, cholesterol, assisting it to undergo oxidation to cholest-4-en-3-one and hydrogen peroxide (H_2O_2). The process generates a measurable output signal in the form of current or potential, permitting the measurability of the analyte concentration.[35,36] A broad variety of materials has been considerably studied for applications in enzymatic, nonenzymatic, and redox-based electrochemical biosensors, where nonenzymatic biosensors are gaining significant attention due to their diverse advantages.[37–39]

Unlike enzymatic, non-enzymatic biosensors exhibit the recent trends with more advantageous properties. The enhanced capability to incorporate various metal nanoparticles and nanocomposites, along with the advanced interaction of the modified electrode and the analyte, demonstrates the dominant sensitivity, conductivity, and biocompatibility of nonenzymatic biosensors. Additionally, these attributes facilitate the fabrication of miniaturized devices, as the materials used demonstrate exceptional stability under varied environmental conditions. Polymer composites, carbon-based materials, metal nanoparticles, metal sulfides, oxides, and their nanocomposites are primary choices for nonenzymatic electrochemical cholesterol detection.[33]

This review aims to present a comprehensive and insightful analysis of recent advancements in nonenzymatic electrochemical biosensing strategies for cholesterol detection. It focuses on nonenzymatic sensing platforms enhanced with metals and metal oxides, carbon and graphene-based materials, polymeric materials, MOFs, MXenes, photoelectrochemical materials, and advanced materials and composites, emphasizing their specific interactions with cholesterol molecules. By highlighting recent innovations in nonenzymatic detection strategies, the review underscores the potential of emerging materials to drive forward the development of next-generation biosensors.

2. Cholesterol: a vital biomolecule for life

Cholesterol is essential for producing cell membranes, hormones, and vitamin D, and is also a key component for the synthesis of steroid hormones and bile acids.[40] Cholesterol is transported in the bloodstream with the

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assistance of lipoproteins, which are complexes composed of lipids and proteins. Lipoproteins are categorized into three groups; they are low-density lipoproteins (LDL), high-density lipoproteins (HDL), and triglycerides (TG).[41] LDL is referred to as "bad cholesterol" due to its association with a greater possibility of artery disease, while HDL is referred to as "good cholesterol" and serves a preventative function by minimising this risk. TG is present in a chemical form regulated by hormonal activities and acts as a main energy source, especially during fasting conditions.[42–44]

An elevated cholesterol level is vitally associated with atherosclerosis, where foam cells are observed forming on the walls of an artery.[45] Surveys conducted over the past 20 years have expressed additional health issues like chronic kidney disease (CKD)[46] and Alzheimer's disease,[47] especially among people with familial hypercholesterolemia. It is also proven that animals with greater cholesterol concentration in the liver cause non-alcoholic fatty liver disease (NAFLD), and a more severe effect can cause non-alcoholic steatohepatitis (NASH).[48–50] Even though there are many issues caused by cholesterol, these disease treatments have not been studied much. Hence, cholesterol lowering and its toxicity have gained special attention in disease and sensing applications.[51]

3. Electrochemical biosensors: bridging biology and electronics

Electrochemical sensors function by detecting analyte concentrations through measurable output signals. The process of the target analyte interacting with the electrode surface, which is coated with a specialized material, produces an electrochemical response, providing quantitative results about the analyte.[52] The recognition layer mainly contains electrode materials which has the ability to selectively bind with the analyte. Traditionally, cholesterol sensors utilized enzyme-modified electrodes, where enzymatic interactions facilitated cholesterol detection. However, recent advancements have shifted toward the use of nanocomposites or nanomaterials that undergo redox reactions, offering improved performance. The incorporation of redox mediators further enhances the sensor's sensitivity, selectivity, and stability. Additionally, the transducer converts the electrochemical interactions between the nanocomposite-modified electrode and cholesterol into electrical signals, which are subsequently processed and displayed in a user-readable format, as shown in Fig. 1.[33] Based on their electrochemical response, biosensors are categorized into four main types: potentiometric, amperometric, conductometric, and impedimetric.

3.1 Potentiometric biosensors

A biosensing device comprises a biological recognition element interfaced with an electrochemical transducer as illustrated in Fig. 2. The device operates by measuring the potential change among the reference and indicator electrodes using a high-impedance voltmeter under quasi-null current conditions. The reference electrode gives a steady potential, whereas the indicator electrode potential responds to ion interactions within the analyte solution. This change empowers the detection and quantification of particular target species. The most widely used potentiometric sensors are membrane-based ion-selective electrodes (ISEs), ion-selective field-effect transistors (ISFETs), screen-printed electrodes, chemically modified electrodes, and solid-state devices. These sensors have been considerably utilized in biosensing applications due to their sensitivity, selectivity, and rapid response properties.[53]

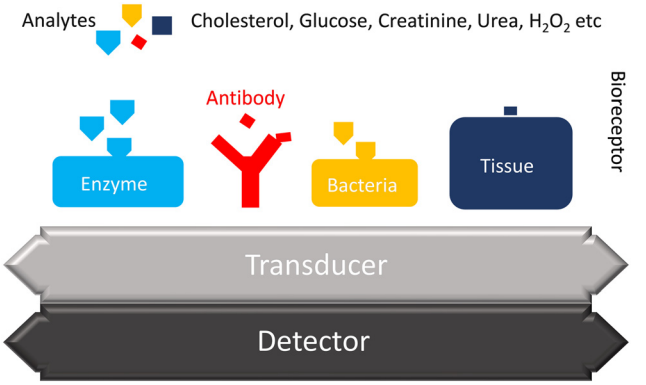

Fig. 1 A visual representation of a biosensor.

3.2 Amperometric biosensors

Amperometric biosensors are devices that produce an electrical signal based on the redox reactions of the target analyte. These sensors facilitate quantitative analysis by measuring the current produced during the oxidation or reduction of the synthesized sample upon interaction with the biological analyte. Among different biosensing applications, amperometric biosensors have confirmed significant success, heading to their commercialization due to their ability to transfer electrons competently within the system. The output signal in these sensors rises from electron exchange between the electrode and the analyte, allowing precise electrochemical detection. The fundamental working principle involves the application of a potential difference between two electrodes, inducing a redox reaction of the analyte, which is subsequently converted into a measurable electric current within the electrochemical setup[54] as shown in Fig. 3.

3.3 Conductometric biosensors

Conductometric sensors measure conductivity across a range of frequencies. Traditionally, conductometry and capacitance monitoring have utilized conductance to determine reaction rates. The conductometric technique specifically involves measuring conductance changes due to ion migration. Capacitance measure is employed during a biorecognition

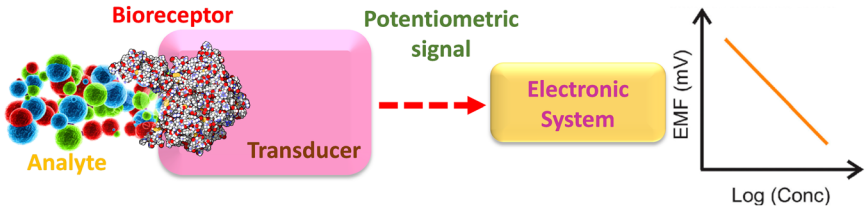

Fig. 2 Diagrammatic representation of the potentiometric biosensor.

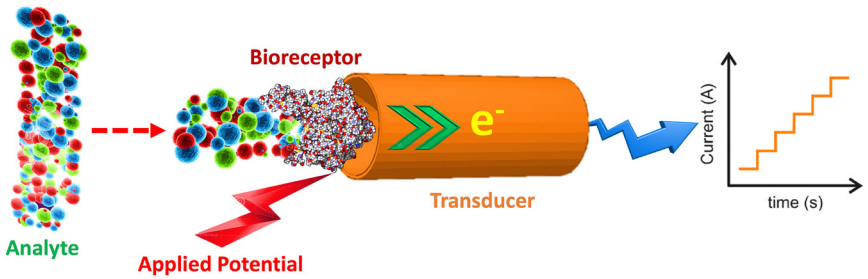

Fig. 3 Visual representation of an amperometric biosensor.

reaction that induces an alteration in the dielectric constant of the solution near a bioreceptor, thus serving as a transducer as demonstrated in Fig. 4. A prime example is the antigen–antibody reaction. If antibody molecules are immobilized within two metal electrodes of a given area, the addition and subsequent binding of an antigen to the antibody will significantly alter the dielectric constant of the medium among the electrodes, leading to a measurable shift in capacitance.[55,56]

3.4 Impedimetric biosensors

Research on impedimetric biosensors has been increasing; however, due to their time-consuming nature, they have been used particularly on enzyme-based sensors. This biosensing is operated to determine the substrate or product included in the enzymatic reaction, as demonstrated in Fig. 5. They are mainly preferred for the enzymatic approach. Multiple studies have been carried out, but the binding reaction at the surface is not sufficient to produce large changes in the signal.[57,58] Impedimetric biosensors are very beneficial for studying information based on surface properties, and new techniques, like polymer and nano, show greater potential in developing newer affinity-based impedimetric sensors. In recent years, the modification of biochips and lab-on-chip devices has attracted greater interest among young researchers.[59]

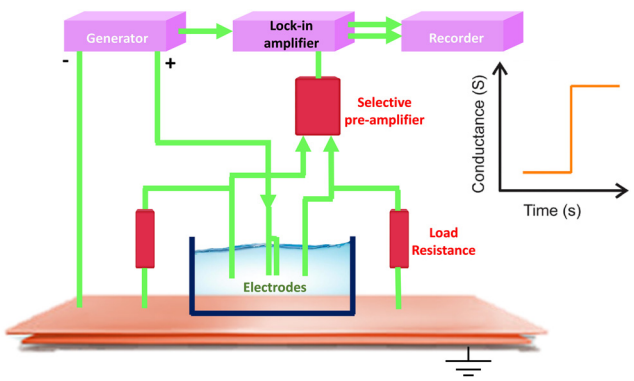

Fig. 4 Schematic representation of a conductometric biosensor.

4. Cholesterol detection technologies: sensors & mechanistic insights

The electrochemical initiation of cholesterol biosensors typically involves the incorporation of enzymes into the system, with cholesterol oxidase (ChOx) and cholesterol esterase (ChE) being the most commonly employed enzymes. These enzymes exhibit strong binding affinity toward the target biomolecules, facilitating effective cholesterol detection and yielding highly sensitive and reliable sensing performance.[33]

The use of ChE as an enzymatic catalyst initiates the hydrolysis of cholesterol esters, breaking them down into free cholesterol and fatty acids. This reaction allows the finding of the total cholesterol concentration in a sample. In contrast, ChOx promotes the oxidation of cholesterol, resulting in cholest-4-en-3-one and hydrogen peroxide (H_2O_2) as a byproduct. The latest studies on electrochemical enzymatic cholesterol biosensors are compiled in Table 1, which additionally emphasises electrode configurations, detection methodologies, nanomaterials, and the performance measurements that

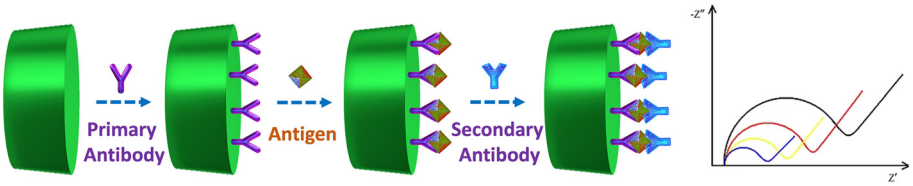

Fig. 5 Schematic representation of an impedimetric biosensor.

Table 1 Electrochemical enzymatic cholesterol biosensors based on nanomaterials

Catalyst	Working electrode	Method of detection	Electrolyte	Sensitivity ($\mu A\ mM^{-1}\ cm^{-2}$)	LOD (μM)	Linear range (mM)	Response time (s)	Ref.
Nanoporous Au/ChOx	Screen printed	CV	PBS containing 3.0 mM H_2O_2	32.68	8.36	0.05–6	3	60
$CoFe_2O_4$@MoS_2/Au/ChOx	Glassy carbon	CV	PBS, pH 7.0	194	0.09	0.005–0.1	—	61
CS/GO/MO/CHOx	Glassy carbon	CV	PBS, pH 7.0	234.63	16	0.1–20	—	62
MoS_2–AuNPs/Nafion/ChOx	Glassy carbon	CA	PBS, pH 7.0	4460	0.26	0.0005–0.048	—	63
Bi–Pt/ChOx/Nafion	Polycrystalline platinum	CA	0.1 PBS (pH = 7.2)	3.41	50	0.05–22	4	64
ZnO@ZnS/ChOx	Glassy carbon	CV	PBS (0.05 M, pH 7.0)	52.67	20	0.4–3.0	5	65
TiO_2NPs/ChOx	Pencil graphite	CV	0.1 PBS (pH = 7.3)	—	4480	3–10	2	66
AuNPs/ChOx + ChEt + GO/Ag	Screen printed	LSV	1 mol L^{-1} NaOH	32.48	0.00259	2.5×10^{-7}–0.013	—	14
rGO–nPd/ChOx + ChEt	Glassy carbon	CA	PBS, 0.1 M of pH 7.2	5120 ± 50	0.05	0.005–0.08	4	67
MWCNTs/TiO_2/Au/ChOx	FTO	DPV	0.1 M PBS, pH 7.4	50 066	0.0018	0.00005–0.08	3	68
Fe_3O_4/ChOx	ITO	EIS	50 mM PBS, pH 7.0	3320	6400	0.064–10.3	25	69
ZNT/ChOx/Nafion	Si/Ag	CA	0.1 M PBS buffer	79.40	0.0005	0.001–13.0	2	70
ZnO/ChOx	Au	CA	0. 1 M PBS buffer (pH 7.4)	23.7	0.00037	1×10^{-6}–5×10^{-4}	5	71
$NiFe_2O_4$/CuO/FeO–Chit/ChOx	ITO	DPV	PBS, pH 7.0	16.63	810	0.13–12.9	10	72
Chit–MWCNTs/Pt–Au@ZnO/ChOx	Glassy carbon	CA	PBS, pH 7.0	26.8	0.03	0.0001–0.759	6	73
MWCNTs/ChOx	Screen printed	CV	0.001 M H_2SO_4	15 310 ± 10	47	0.047–0.28	6	74
$Ti_3C_2T_x$/ChOx/Chit	Glassy carbon	DPV	1 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl	1.3266×10^8	0.00011	3.0×10^{-7}–4.5×10^{-6}	—	75
Chit–CdS/ChEt–ChOx	ITO	CV	PBS	0.384	470	0.64–12.9	—	76
GCE/ChOx/Au/MWNT	Glassy carbon	CA	PBS (pH 7.0)	—	0.5	0.002–1.4	15	77
ChOx/CS/TH/Cu_2O/GCE	Glassy carbon	DPV	PBS 100 mM, pH 7.4	70 220	0.0018	0.01–1	—	78
ChOx–PAMAM G5.0@PNE@Fe_3O_4/Nafion	Screen-printed electrode	CA	PBS (10 mM, pH 7.4)	1049.92	0.12	0.001–11.0	—	79

Abbreviations: ChOx – cholesterol oxidase, NPs – nanoparticles, CS – chitosan, GO – graphene oxide, MO – molybdenum oxide, rGO – reduced graphene oxide, ChEt – cholesterol esterase, MWCNTs – multi-walled carbon nanotubes, ZNT – zinc oxide nanotube, ITO – indium tin oxide, FTO – fluorine doped tin oxide. CV – cyclic voltammetry, DPV – differential pulse voltammetry, CA – chronoamperometry, LSV – linear sweep voltammetry, EIS – electrochemical impedance spectroscopy. TH – thionine. PNE – polynorepinephrine.

go along with them, like sensitivity, linear range, limit of detection (LOD), and response time. The redox activity of H_2O_2 works as an intrinsic redox mediator in electrochemical sensors, excluding the supportive redox mediators. In amperometric sensing, the considered current is directly proportional to the cholesterol concentration, as H_2O_2 endures oxidation at a specific applied potential, thereby facilitating the exact quantification of cholesterol levels. At this specific potential, +0.7 V *vs.* RHE describes the anodic potential required for the oxidation of H_2O_2, which is a confine of this technique. This drawback can be alleviated by introducing redox mediators into the enzymes, thereby improving the electron transfer mechanism.[33]

Redox mediators can be distinguished as enzymatic and nonenzymatic based on their electron transfer mechanism. Enzymatic redox mediators contain an enzyme and a chemical mediator, assisting indirect electron transfer between the enzyme and the electrode. In comparison, nonenzymatic redox mediators permit direct electron transfer amongst redox-active species, such as ferrocene,[6] Prussian blue,[80] methylene blue,[7] and hydroquinone—and the electrode surface.[81] These mediators act as a prominent part in boosting both the sensitivity and selectivity of biosensors, thereby improving their overall analytical performance.[6]

Although enzymatic and redox mediator biosensors demonstrate high sensitivity, rapid response time, and

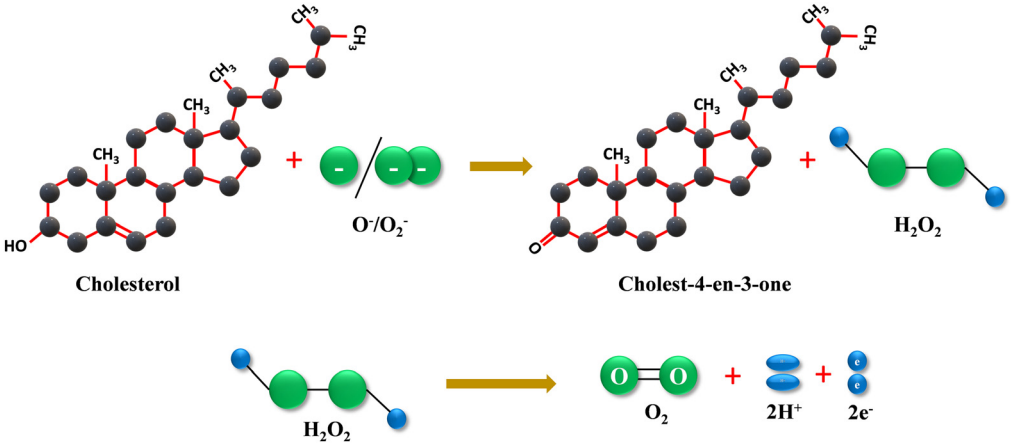

Fig. 6 The reaction mechanism underlying the application of nanomaterial-based sensing substrates for non-enzymatic cholesterol detection.

excellent selectivity, they have limitations, including high cost and susceptibility to environmental factors such as temperature and pH. To address these challenges, researchers are developing materials that are easier to synthesize, more sensitive to analytes, and more cost-effective for non-enzymatic cholesterol sensing.

Nonenzymatic biosensors utilize nanomaterials to modify stability, reproducibility, and operational simplicity. Fig. 6 illustrates the mechanism of a redox reaction happening at the transducer surface when nanomaterials interact. The elementary principle of nonenzymatic biosensors stays constant among all materials, aiming for the electrochemical oxidation of cholesterol. In this process, a conductive electrolyte facilitates the interaction between the electrode and the analyte, generating an electrical signal as the output. Various conductive electrolytes are utilized to determine cholesterol concentration based on these electrical signals. At the same time, different types of electrodes, along with modified nanomaterials and nanocomposites, are extensively explored for improved nonenzymatic cholesterol detection.[33]

5. Design and performance of non-enzymatic nanomaterial-based cholesterol sensors

The exploration of nanomaterials (NMs) has gained significant interest owing to their extensive applications in sensors, supercapacitors, water splitting, batteries, and other advanced technologies.[82–87] Among their outstanding characteristics, the greater catalytic performance, large active surface area, and tunable electrical and optical properties make NMs specifically advantageous for biosensing applications. Notably, these properties can be exactly controlled and specialised to increase the conductivity and surface area of NMs, which are essential factors in improving sensor performance.[88]

Advances in materials science have been the primary focus of creating nonenzymatic cholesterol sensors, as the electrode surface's features fundamentally govern the devices' electrocatalytic performance, sensitivity, and stability.[89,90] To present an organised viewpoint, the materials that have been published can be broadly classified into numerous classes: (a) metal and metal oxide nanoparticles, which demonstrate an extensive range of redox activity and exceptional electrical conductivity; (b) nanomaterials based on graphene and carbon, which offer variable surface properties, large surface areas, and superior charge transfer; (c) polymeric materials, which are admired for their inherent biocompatibility, processability, and structural versatility; (d) MXenes and metal–organic frameworks (MOFs), which possess fascinating electrochemical behaviours and integrate high porosity, chemical tunability, and a significant amount of active sites; (e) photoelectrochemical (PEC) biosensors which boost charge separation and sensitivity through involving light-mediated electrochemical techniques and (f) additionally, advanced materials and composites, wherein the synergistic interactions of several constituents can obtain improved sensitivity, selectivity, and operational stability. Furthermore, to simplify the evaluation of the various approaches addressed in the literature, this classification underlines the material-dependent design principles that improve the functionality of next-generation cholesterol-sensing technologies.

a. Metal and metal oxide nanoparticles

Non-enzymatic electrochemical cholesterol sensors typically use metal and metal oxide nanoparticles owing to their vast surface area, configurable electronic structures, and excellent electrocatalytic activity. As metals enhance swift electron transfer, transition metals like Ni, Cu, and Pd, in conjunction with their oxides, enable numerous active sites for the oxidation of cholesterol, increasing sensitivity while minimising detection limits. These materials are ideal choices for robust electrochemical sensing platforms

considering their chemical stability and ease of synthesis, which additionally allows the production of repeatable and reliable sensor functionality.[91]

An effective, simple, and cost-efficient approach for a non-enzymatic cholesterol biosensor based on a Cu_2O–TiO_2 nanostructure was developed. Titanium dioxide nanotubes (TNTs) were synthesised *via* titanium (Ti) foil anodization, followed by sonication for 15 minutes in different solvents and subsequent treatment with an N_2 stream. The TNTs were grown on Ti foil by a two-step anodization technique and annealed at 450 °C for 2 hours. Cu_2O decoration was done through sonication, followed by a chemical bath deposition method. Electrochemical characterization methods, like electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and chronoamperometry, were done using a three-electrode system, where pristine and Cu_2O nanoparticle-decorated TNTs acted as the working electrodes. All experiments were done in a 0.1 M phosphate buffer solution (PBS) at pH 7.0. Cholesterol stock solutions were prepared by dissolving cholesterol in 2 mL of 2-propanol, followed by dilution with 0.1 M PBS as required.

The electrode initially demonstrated a sensitivity of 1258.9 $\mu A\ mM^{-1}\ cm^{-2}$. Upon incorporating Cu_2O, a fivefold enhancement in sensitivity was observed, reaching 6034.04 $\mu A\ mM^{-1}\ cm^{-2}$, with a limit of detection (LOD) of 0.05 mM, a wide linear range of 24.4–622 μM, and a rapid response time of 3 s as demonstrated in Fig. 7. CV analysis revealed two distinct oxidation peaks, corresponding to the oxidation of Cu to Cu^{2+} and Cu^{1+}, respectively, along with a single reduction peak associated with the reduction of Cu^{2+} to Cu^{1+}. Notably, after the introduction of Cu_2O into the TNTs, a diminished reduction peak was obtained, which was ascribed to the oxygen reduction within the Cu_2O crystal structure. This phenomenon is linked to the (111) plane of Cu_2O, which exhibits a lower binding energy with adsorbed intermediates, thereby influencing the electrochemical response.

The electrochemical oxidation mechanism of cholesterol is represented in Fig. 7. The incorporation of Cu_2O into TNTs significantly enhanced the electrocatalytic activity, leading to improved cholesterol oxidation. The proposed mechanism, as depicted below, elucidates this enhancement in electrocatalytic performance in eqn (1)–(4).

$$Cu_2O + O_2 + e^- \rightarrow Cu_2O + O_2^- \quad (1)$$

$$O_2^- + H_2O \rightarrow H_2O_2 + \cdot OH \quad (2)$$

$$O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2 \quad (3)$$

$$\cdot OH + \text{Cholesterol} \rightarrow \text{Oxysterols} \quad (4)$$

Furthermore, the electrode demonstrated good stability and high sensitivity to thermal variations and pH. In addition to its favourable electrochemical performance, it exhibited a

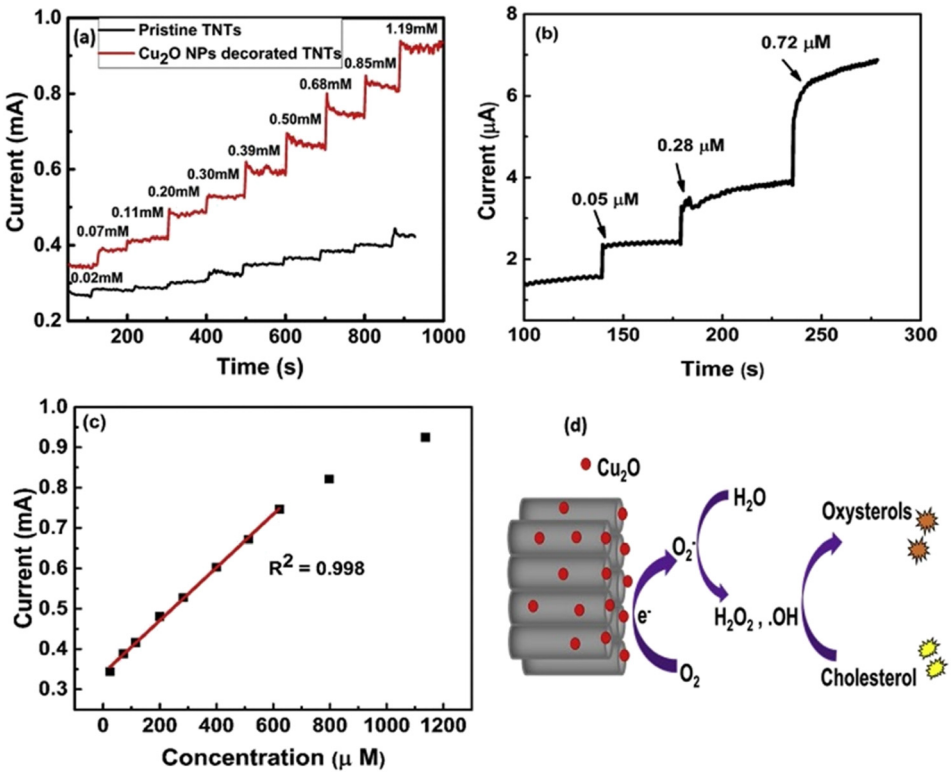

Fig. 7 (a and b) Chronoamperometric plot of the pristine TNTs and Cu_2O decorated TNTs upon addition of cholesterol; (c) linear calibration curve; (d) diagrammatic presentation of cholesterol oxidation at the electrode surface (reprinted from ref. 92 with permission from Elsevier).

lower redox potential, which effectively minimized interference from other species, highlighting its robustness for clinical applications.[92]

A reliable and stable electrocatalyst for cholesterol sensing is synthesized using mesoporous $NiCo_2S_4$ nanoflakes *via* a hydrothermal method. The resultant nanoflakes were utilized as working electrodes by coating them onto nickel foam. The mesoporous behaviour of the nanoflakes showed a greater surface area, attributed to the presence of smaller nanoparticles. The developed nanoflakes exhibited excellent electrochemical performance, achieving a high sensitivity of 8623.6 μA mM^{-1} cm^{-2}, with a linear detection range of 0.01 to 0.25 mM and a LOD of 0.01 μM. Furthermore, the sensor showed thermal stability and reproducibility for up to eight weeks. It also demonstrated a strong response in real sample analysis, underscoring its practical applicability for medical applications.[93]

A novel Ag–Cu_2O@TNTs nanostructure has been synthesised, proving excellent sensitivity for nonenzymatic cholesterol detection. The synthesis of this hybrid nanostructure involves the deposition of TiO_2 on the Ti_6Al_4V plate through anodization, followed by the decoration of these nanotubes with Cu_2O nanomaterials by a wet chemical bath deposition method. Subsequently, Ag is electrochemically deposited onto the Cu_2O@TNTs hybrid to improve the electroactive surface area, thereby increasing the rate of electron transfer. The electrochemical characteristics of the nanomaterial are examined by a three-electrode system with 0.1 M PBS as the electrolyte. CV, amperometry, and EIS studies were conducted to characterize the material's performance. The overall electrochemical reaction occurring within the system can be represented as follows in eqn (5) and (6):

$$\text{Cholesterol} + Cu_2O + Ag + O_2 \rightarrow \text{Cholesterol–quinone} + Cu + Ag^+ + H_2O \quad (5)$$

$$\text{Cholesterol–quinone} + Cu_2O + Ag + O_2 \rightarrow \text{Cholesterol-3-one} + Cu + Ag^+ + H_2O \quad (6)$$

The chronoamperometric response of the Ag–Cu_2O@TNTs electrode, with a LOD of 1.15 mM, exhibited a sensitivity of 12 140.06 μA mM^{-1} cm^{-2}, which is much more than the Cu_2O@TNTs electrode (11 437.0 μA mM^{-1} cm^{-2}), with a LOD of 0.057 mM. Furthermore, the electrode was tested using real human blood serum samples, yielding excellent results. These findings underscore its sensing performance in clinical diagnostics.[94]

Co_3O_4 nanosheets modified with varying concentrations of CdS nanoparticles (0.02, 0.026, and 0.033 mg) were synthesized using a hydrothermal approach modified with the successive ionic layer adsorption and reaction (SILAR) method. The electrochemical properties of the obtained materials were thoroughly examined through CV and amperometric analysis. The Co_3O_4@CdS-3 electrode (0.026 mg of CdS) displayed greater sensing performance for cholesterol detection, proving a broad linear detection range of 250–5000 μM in 0.1 M PBS among the three. Notably, an exceptional sensitivity of 13 564.8 μA mM^{-1} cm^{-2} was observed. Furthermore, it exhibited a remarkable LOD of 0.001 μM, along with excellent reproducibility over four weeks and high selectivity against potential interfering species. The electrode validated its steady performance in real sample analysis, showcasing its ability for practical applications in cholesterol biosensing.[95]

b. Nanomaterials based on graphene and carbon

Graphene, carbon nanotubes, and carbon spheres are a few examples of carbon-based nanomaterials which have become extremely versatile platforms for cholesterol sensing through their great surface area, strong electrical conductivity, and structural flexibility. Carbon surfaces' intrinsic mechanical flexibility and biocompatibility provide point-of-care and miniaturised sensor designs, while modification can further boost their selective interactions with cholesterol molecules, improving selectivity.[96,97]

Most recently, a SnO_2–Pd/C nanocomposite has been developed *via* a two-step process. The functional sensing electrode was constructed by hydrothermally generating SnO_2 that was subsequently decorated with Pd/C and coated on nickel foam. In comparison with pristine SnO_2–NF (546 μA mM^{-1} cm^{-2}), the resulting electrode had almost three-fold greater sensitivity of 1560 μA mM^{-1} cm^{-2}, suggesting a greatly enhanced electrocatalytic performance. In addition, the sensor exhibited a wide linear response range (200 μM–2 mM) in an alkaline medium (0.1 M KOH), a low detection limit (28 μM), and a limit of quantification of 34 μM. Notably, the SnO_2–Pd/C–NF biosensor revealed exceptional reliability by keeping 97% of its stability after 30 days. It was confirmed as well with human serum samples, underlining its potential for clinical evaluation.[98]

New findings show that iron oxide-decorated etched carbon nanotube-modified screen-printed carbon electrodes, which can be easily, straightforwardly, and affordably synthesised, can be used to generate cholesterol and high-density lipoprotein (HDL) sensors. The enzymatic HDL sensor exhibited a noteworthy sensitivity of 2136 μA mM^{-1} cm^{-2} across 0.6–1.2 mM with a detection limit of 0.2 mM, while the non-enzymatic cholesterol sensor showed a sensitivity of 0.93 μA mM^{-1} cm^{-2} over the range of 90–1080 μM and a detection limit of 64.17 μM. Their clinical utility and scientific reliability have been confirmed in human serum samples, underscoring the application of such nanostructure-based electrochemical platforms for the sensitive and selective monitoring of HDL and cholesterol, both vital biomarkers for the detection and management of cardiovascular disorders.[99]

Gelatin–Au@CD nanoconjugate films coated on ITO electrodes were employed to generate a novel enzyme-free electrochemical biosensor for determining the presence of cholesterol. With a linear range of 2–20 mM, a correlation value of 0.95, and a sensitivity of 1.36 μA mM^{-1} cm^{-2}, the

modified nanoconjugate surface demonstrated an environment that's favourable to an enhanced electrochemical reaction. Nitrogen ion irradiation was applied to boost sensitivity. At a fluence of 10^{16} ions per cm^2, transformations to the electrode surface rose to 2.99 μA mM^{-1} cm^{-2}, which is a 54% increase. This straightforward, affordable, and enzyme-free technique has tremendous potential for effective cholesterol monitoring with better stability.[100]

A NiO flower-like structure, which is further modified by the addition of graphene, has been used in a cholesterol-sensing application. Here, graphene acts as an external support to enhance the electrochemical behaviour of NiO through its external properties, such as its ambipolar nature, extraordinary energy structure, and carrier mobility. A single carbon layer on copper was deposited by chemical vapor deposition with reagents methane and oxygen at ~1000 °C and coated on a glassy carbon electrode utilizing the PMMA technique. With the help of electrolyte $Ni(NO_3)_2$ with acetate buffer, NiO was deposited on graphene flakes, and further, it was annealed in air at 270 °C. The prepared electrode was examined for electrochemical behaviour, CV and CA by using PBS and 1 M KOH as an electrolyte.

The cholesterol stock solution was prepared by the addition of isopropanol and Triton X-100 with varied concentrations of cholesterol. The mechanisms involved in this process are represented by eqn (7) and (8):

$$NiO + OH^- \leftrightarrow NiOOH + e^- \quad (7)$$

$$Ni^{3+} + \text{Cholesterol} \rightarrow Ni^{2+} + \text{Cholestenone} \quad (8)$$

This study shows the linear range from 2 mM to 40 mM with a LOD of 0.13 mM and response time of 5 seconds. It also has a high sensitivity of 40.6 mA mM^{-1} cm^{-2} and good stability.[101]

c. Polymeric materials

Polymeric materials with tunable chemical properties, structural flexibility, and biocompatibility are interesting platforms for nonenzymatic cholesterol sensing. Molecularly imprinted polymers (MIPs) offer highly selective sites for recognition that mimic the molecular structure of cholesterol, yet conductive polymers such as polyaniline, polypyrrole, and poly(methyl orange) have been extensively utilised for better charge transfer and electrode stability. When taken together, these polymer-driven techniques provide encouraging pathways leading to a future generation of biosensors that are durable, stable, sensitive, and cost-effective.[102,103]

According to a current study on polymer-based sensing, a pencil graphite electrode (PGE) modified by graphene oxide (GO) and functionalised by imprinted poly[2-(dimethylamino) ethyl methacrylate] (poly[DMAEMA]) films has been developed by electropolymerization. A sensor that exhibited a limit of detection of 0.85 mM, a limit of quantification of 2.85 mM, a linear response range of 1–6 mM, and a sensitivity of 40.52 μA mM^{-1} cm^{-2} was obtained by meticulously altering the electropolymerization parameters, that is, the template-to-monomer ratio, number of cycles, scan rate, rebinding duration, and pH. Additionally, following 10 days, the device retained exceptional selectivity against cholesterol despite potential interfering species, keeping 85.52% of its initial response, suggesting strong stability.[104]

Nanohybrids made from polyoxometalate (POM) have drawn a lot of attention owing to their multifunctionality and extensive redox activity. The polypyrrole (PPy)–$SiMo_2VW_9$ nanohybrid, which was designed for both energy storage and cholesterol monitoring, is an example. In 0.5 M H_2SO_4, the hybrid displayed battery-type charge storage performance by yielding a specific capacitance of 174.5 F g^{-1} with power and energy densities of 799.94 W kg^{-1} and 15.51 Wh kg^{-1}. The sensitivity of 7.97 mA mM cm^{-2} as a nonenzymatic cholesterol sensor, associated with its LOD of 0.06 mM and LOQ of 0.2 mM in the range 0.03–0.58 mM, underlines the potential of PPy–POM nanohybrids as multifunction materials for electrochemical energy and biosensing applications.[105]

Similarly, the selectivity of a reported MIP sensor proved three times higher for cholesterol than cholecalciferol, with a linear response in the 0.01–1 mM range with a low detection limit of 0.31 mM. The results of this approach reveal the potential of MIPs as efficient, nonenzymatic alternatives for accurate cholesterol detection, with a clinical recovery rate of 98.81%.[106]

d. MXenes and metal–organic frameworks (MOFs)

MOFs are porous, crystalline materials with an extensive surface area that offers an abundance of active sites for selective analyte interaction in sensing. MXenes are 2D transition metal carbides and nitrides with unique surface terminations and elevated conductivity, which enables strong adsorption and rapid electron transfer. These features work together to make them extremely helpful for the electrochemical detection of biomolecules like cholesterol.[107,108]

According to news reports, the Zn–Cu(terephthalic acid) metal–organic framework@graphite rod [Zn–Cu(TPA) MOF@GRP] hybrid rod acts as an affordable, freestanding working electrode for non-enzymatic cholesterol monitoring. Featuring a broad linear range of 2.5–200 μM, an unusually low detection limit of 0.0028 μM, and a high sensitivity of 333.33 μA mM^{-1} cm^{-2}, the sensor revealed diffusion-controlled and reversible redox activity. It displayed excellent reliability and reproducibility, sustaining stability for over 60 days, and remarkable selectivity against typical interfering species like glucose, urea, dopamine, ascorbic acid, hypoxanthine, and xanthine. Additionally, its practical relevance was

demonstrated for the highly accurate recognition of cholesterol in milk, showing its potential in trustworthy biosensing applications.[108]

The present investigation introduces a $Ti_3C_2T_x$ MXene nanosheet-derived, highly sensitive, enzyme-free cholesterol sensor that was developed by *in situ* etching (LiF/HCl). Considering a broad linear range (1–250 nM, $R^2 \approx 0.99$), high sensitivity (≈3.012 mF nM^{-1} cm^{-2}), and a low detection limit (0.07 nM), the MXene-adapted paper electrode proved outstanding results. In a real sample (egg yolk) evaluation, the sensor attained 93–95% recovery and showed remarkable adaptability, selectivity, repeatability, and long-term stability. These findings suggest its great promise for precise, real-time cholesterol monitoring in biological settings.[109]

There currently exists minimal study on non-enzymatic electrochemical cholesterol sensors on the basis of MOFs and MXenes. In an effort to enhance selectivity, stability, and clinical application, future research ought to focus on logically developing such materials, either alone or in hybrid designs.

e. Photoelectrochemical (PEC) biosensors

Photoelectrochemical (PEC) biosensors offer a further method of signal amplification utilising light-induced charge separation in photoactive materials to speed up electrochemical reactions.

When light activation and electrochemical detection are employed together, the sensitivity, selectivity, and detection limits for cholesterol sensing are significantly raised. PEC platforms, which rely on hybrid composites or nanostructured semiconductors, possess tunable optical and electric properties that enable low-background, quick, and highly responsive sensing, thereby acceptable for advanced non-enzymatic cholesterol detection.[110–112]

A cerium-doped zinc oxide (ZnO) nanocomposite with differing weight percentages was synthesized, adopting the wet chemical method. This study aims to develop photoelectrochemical biosensors for nonenzymatic cholesterol detection. Initially, ZnO nanoparticles were synthesized and subsequently doped with cerium at concentrations of 1, 2, and 3 wt%. Among the doped nanocomposites, ZnO doped with 3 wt% cerium displayed dominant electrochemical characteristics, as verified by CV. Furthermore, light irradiation significantly highlighted the photoelectrochemical features of the synthesized composite.

The presence of cerium resulted in the formation of one lowest unoccupied molecular orbital (LUMO), effectively shortening the band gap, which facilitated increased electron transfer and enhanced redox activity as demonstrated in the mechanism (Fig. 8). The replacement of Zn^{2+} ions by Ce^{4+} is described by eqn (9) and (10).

$$CeO_2 \xrightarrow{ZnO} Ce_{Zn}^{oo} + O_o^x + \frac{1}{2}O_2 + 2e^- \quad (9)$$

$$CZO(Ce_{Zn}^{oo}, O^{ox}) + h\gamma \rightarrow CZO(Ce_{Zn}^{oo}, O^{ox}) + e^{-*} + h^+ \quad (10)$$

Upon irradiation with light, the material exhibited an increased generation of free electrons, while cerium doping effectively decreased charge carrier recombination, thereby enhancing the photoelectrochemical characteristics. This emphasizes the significance of light exposure on boosting the material's activity.

The literature demonstrated a twofold improvement in sensitivity, attaining 2.812 mA mM^{-1} cm^{-2} under light compared to 1.37 mA mM^{-1} cm^{-2} under dark conditions through chronoamperometry as illustrated in Fig. 8. The sensor exhibited a LOD of 17 μM under light and 28 μM in the dark, with a corresponding LOQ of 54 μM (light) and 98 μM (dark) in a 0.1 M KOH solution. Moreover, the sensor revealed a linear detection range of 80 μM to 2 mM and possessed 97% stability over 60 days. Additionally, its quick sensing ability was examined through the detection of cholesterol in human serum, underscoring its ability for clinical applications.[113]

Furthermore, ZnO/C-modified FTO served as the photoanode and Fe-doped $CuBi_2O_4$ (CBFO) as the photocathode in the creation of a self-powered PEC sensor for cholesterol identification. MIPs were utilised for better selectivity. The sensor exhibited good sensitivity (1.248 μA cm^{-2}), a low detection limit (0.26 nmol L^{-1}), and an extensive linear range (1–1000 nmol L^{-1}). Its high recovery (98.6–107%) and low RSD (2.38–4.25%) in milk samples revealed its practical usability and highlighted its ability for quantitative cholesterol monitoring in food, pharmaceutical, and environmental matrices.[114]

f. Advanced materials and composites

With the objective of attaining improved sensitivity, broader detection ranges, and more rapid responses, hybrid composites—which comprise doped semiconductors and layered nanostructures—combine conductivity, catalytic activity, and structural stability. These complementary effects promote robustness and reproducibility, facilitating practical real-sample analysis applications.

A nanocomposite-based nonenzymatic cholesterol sensor by incorporating nickel oxide (NiO) with molybdenum disulfide (MoS_2), followed by electrochemical polymerization of poly (methyl orange) (MO) from an MO/phosphate-buffered saline (PBS) solution on a screen-printed carbon electrode (SPCE), was synthesised. The combination of MoS_2 enhanced the electron density in the conduction band, providing increased electron transfer and converting O_2 to superoxide (O_2^-). Additionally, the presence of H_2O led to the production of hydrogen peroxide (H_2O_2) and hydroxyl radicals, involving the oxidation of cholesterol. The sensor showed a linear detection range from 25.86 μM - 0.39 mM, with a LOD of 5.17 μM and a limit of quantification (LOQ) of 0.24 mg dL^{-1}. Furthermore, the sensor exhibited a high sensitivity of 30.63 mA mM^{-1} cm^{-2}. A real sample analysis of

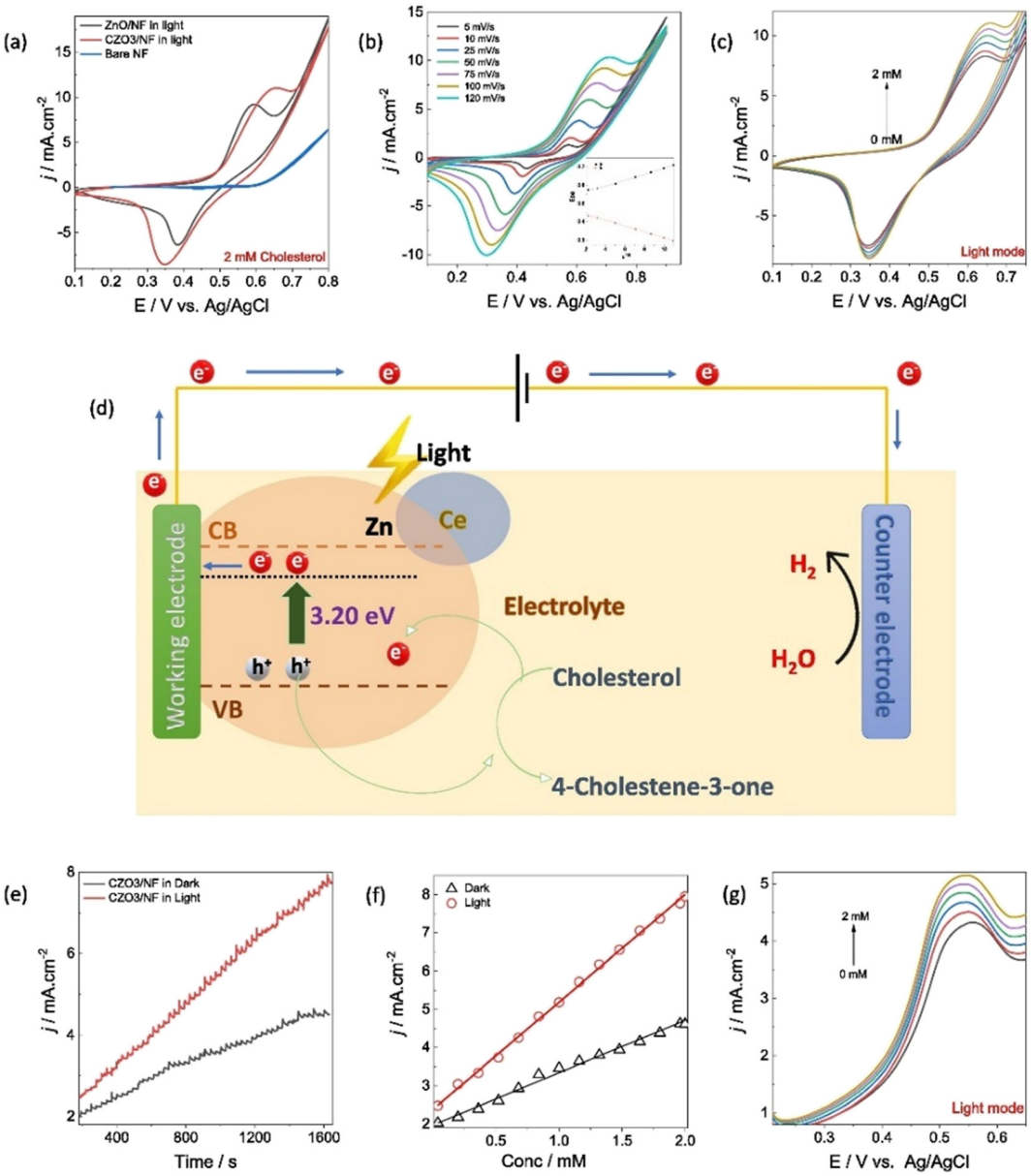

Fig. 8 The study involving (a–c) CV, (e and f) chronoamperometry, and (g) DPV, along with the proposed (d) mechanism of cholesterol oxidation in photoelectrochemical cholesterol biosensors (reprinted from ref. 113 with permission from Elsevier).

milk and yogurt was conducted using CV and DPV, confirming its practical applicability.[115]

Au nanoparticles deposited on CdS quantum dot-decorated anodic TiO_2 nanotubes (TNTs) offered an enzyme-free cholesterol sensor with a broad linear range (0.024–1.2 mM), LOD of 0.012 μM, a quick response of 1 s, and a high sensitivity (~10 790 μA mM^{-1} cm^{-2}). Practically applicable, selective, and interference-free cholesterol identification was rendered achievable by the combined effect of CdS QDs and Au NPs, significantly accelerated electron transport, and active surface area.[116]

Recently, a very sensitive non-enzymatic cholesterol identification technique has been created with a novel electrochemical platform built around a poly(ionic liquid)–cobalt polyoxometalate (PVIM–Co_5POM) composite supported on carbonaceous materials. In addition to exhibiting a broad linear dynamic range spanning 1 fM to 5 mM and distinct sensitivities across lower ranges (1 fM–200 nM) with 210 μA μM^{-1} cm^{-2} and higher (0.5 μM–5 mM) with 64 μA μM^{-1} cm^{-2} concentration regimes, the sensor revealed an ultralow detection limit of 1 fM (1×10^{-15} M) and an extremely quick reaction time of ~5 s. Additionally, the sensor was accurately tested in human serum at physiological pH while retaining good selectivity despite the presence of common interferents such as ascorbic acid, glucose, and uric acid. The extensive linear range and excellent sensitivity were sustained after it was incorporated into a flexible paper-based device, emphasising the viability

of PVIM–Co_5POM/MNC composites for effective cholesterol biosensing.[102]

Moreover, Table 2 provides a comprehensive summary of all other relevant articles.

6. Challenges and future outlook

Despite significant advancements in the past few decades, numerous kinds of scientific and technological barriers still hinder non-enzymatic cholesterol biosensors from being utilised extensively in clinical and commercial settings. Possibilities for upcoming innovations that extend beyond material research into the fields of device engineering, clinical approval, and electronic incorporation are highlighted by an in-depth examination of these drawbacks.

a. Selectivity, interference, and matrix effects

Selectivity continues to be an essential barrier, especially in complicated biological substrates where analytical accuracy is frequently compromised by electroactive interferents, including glucose, uric acid, and ascorbic acid. Whereas approaches that involve surface enhancement, molecular imprinting, and heteroatom doping have recently been attempted to boost detection for cholesterol, these have yet to demonstrate reliable and consistent outcomes throughout a variety of sample contexts. In future decades, overcoming interference-triggered constraints might call for the creation of multipurpose interfaces that integrate chemical selectivity with electronic discrimination.

b. Stability and reliability

An additional key problem concerning nanomaterial-derived electrodes is their enduring stability and reproducible properties. Functionality decline can be triggered by surface passivation and compound degradation, including structural modifications over a prolonged period. Even though embedding within a preventive matrix or the development of core–shell frameworks has boosted durability, there still exist a handful of systematic evaluations performed under physiologically relevant conditions. Consequently, adopting standardized techniques for stability testing that ensure consistency across batches and laboratories ought to be of greater importance for future research.

c. Real sample analysis and clinical translation

The conversion of laboratory findings into clinically pertinent results is greatly impeded by the relatively small amount of research that employs real biological fluids, such as serum, plasma, or whole blood. Since biological matrices are typically complex and comprise proteins, salts, and other interfering species, the accuracy and repeatability of sensors may diminish by inducing electrode fouling, nonspecific adsorption, or signal suppression. Sample pretreatment approaches include dilution, filtering, or selective separation which tend to be required to decrease these matrix effects; however, such steps add to operational complexity and hinder the viability of direct point-of-care applications. It is essential to carefully assess sensors against identified enzymatic evaluations on an appropriate sample, which comprises a range of cholesterol levels and real-world variability, for the purpose of achieving clinical translation. Furthermore, it is necessary to carry out a systematic evaluation of variables such as sensor stability, repeatability, and selectivity in physiological contexts. In addition to improve non-enzymatic cholesterol sensors' dependability, tackling such problems will make it more feasible to incorporate them into practical diagnostic platforms, especially portable and point-of-care devices, narrowing the gap between bench-scale research and clinical application.

d. Integration and monitoring advancements

System-level integration has grown progressively more vital for emerging biosensing techniques. Ongoing and personalised health management has been made feasible by the integration of cholesterol sensors with wearable components, wireless tracking systems, and microfluidic innovations. Nonetheless, it continues to prove technically challenging to incorporate flexible, affordable, and scalable system designs using high-performance nanomaterials. The rollout of these kinds of technologies could be accelerated through research efforts aimed at hybrid production procedures and user-friendly gadget design.

e. Lab-on-a-chip and point-of-care devices

Decentralising cholesterol screening entails the miniaturisation of biosensors towards point-of-care (POC) or lab-on-a-chip (LAB) platforms. Although scalable electrode generation is being made practical by advancements in screen printing, 3D printing, and laser writing, it remains challenging to achieve the right balance between expensive and disposable electrodes with excellent analytical performance. Alongside sensor optimisation, subsequent research ought to make regulatory compliance and device manufacturing capability the highest priority.

f. Data sharing and digital health integration

The introduction of biosensors into digital health networks is additionally necessary for their wider adoption. The clinical utility of cholesterol biosensing devices can be greatly enhanced through combining them with cloud-based analytics, safe data-sharing systems, and AI-powered tools for supporting decisions. Nevertheless, there is still a lack of research on subjects about safeguarding information, interoperability, and regulatory structures.

Table 2 Performance of nonenzymatic cholesterol biosensors

Catalyst	Working electrode	Method of detection	Electrolyte	Sensitivity ($\mu A\ mM^{-1}\ cm^{-2}$)	LOD (μM)	Linear range (mM)	Response time (s)	Ref.
a. Metal and metal oxide nanoparticles								
Fe_2O_3 NP-ZnO NR	FTO electrode	CA	5 mM $[Fe(CN)_6]^{3-/4-}$ and 100 mM KCl solution	642.8	~12.4	0–9	—	117
ZnO/WO_3	FTO glass substrate	DPV	0.1 M PBS solution (pH = 7.2)	176.6	0.0055	0–0.32	—	118
TiO_2 nanotubes	Ti_6Al_4V thin plate	CA	0.1 M NaOH solutions	~62.5	0.12	0–15	17	119
NiO/CuO	Glassy carbon electrode	CV	0.1 M PBS, pH 7.4	10.27	5.9	0.8–6.5	3	120
Oxidized Zn–In nanostructure	Copper substrate	CA	PBS, pH~7	81	—	0.5–9	1	121
MB/BCD/Fe_3O_4	SPCE	CA	50 mM PBS, pH 7.4	15	2.88	0.00288–0.15	—	122
Copper(I) sulfide nanoplates	Copper rod	CA	pH 7.0 acetate buffers	62.5	0.1	0.01–6.8	—	123
Au-/nPts	Au	CA	—	226.2	15	0–5	—	124
Ag-NPs/ZnO-NRs	Printed electrode	CV	20 mM PBS (pH 7.0)	135.5	184	1–9	25	125
b. Nanomaterials based on graphene and carbon								
MWCNTs- βCD	SPCE	DPV	2 mM PBS	—	0.0005	0.000001–0.003	—	126
PANI/MWCNTs/starch	Carbon paste electrode	CA	0.1 M PBS-5	800	10	0.032–5	4–6	127
Pencil lead	Pencil graphite	DPV	0.3 M lithium perchlorate ($LiClO_4$)	1455.22	—	0.63–9.38	—	128
MnO_2/GR	Pencil graphite electrode	CV, DPV	PBS, pH 7.0	6.3869×10^7	0.00042	1.2×10^{-6}–2.4×10^{-5}	—	129
APC-Fe_2O_3	Carbon paste electrode	CV, DPV	1 mM $K_4[Fe(CN)_6]/K_3[Fe(CN)_6]$ in 1 M KCl	2.4309×10^7	0.008	2.5×10^{-5}–3.0×10^{-4}	—	130
GO–MIP	Glassy carbon electrode	CV	0.1 M PBS	—	0.0001	10–0.0000001	~120	131
PANInf–GMF	ITO electrode	DPV	—	3.90	50	0.05–12	—	132
Grp/β-CD/methylene blue	Pt wire	DPV	PBS (pH 7.4)	10	1	0.001–0.1	~20	133
MIP–MWCNTs/Au NPs	Screen printed	LSV	$[Fe(CN)_6)]^{3-/4-}$ (0.05 M)	5000	0.01	0.00001–0.008	—	134
CuO–RGR/1M3OIDTFB	Carbon paste	SWV	PBS (at pH 7.4)	90.2	0.009	0.00004–0.3	—	135
PtNPs/CNTs	ITO	CA	50 mM PBS	8.7	2.8	0.005–10	—	136
CX6@rGO	Glassy carbon	DPV	2.0 mM $[Fe(CN)_6]^{3-/4-}$ redox couple (1: 1) with 0.1 M KCl	99	0.20	0.0005–0.05	—	137
β-CD/PNAANI/rGO	Glassy carbon	DPV	2.0 mM $[Fe(CN)_6]^{3-/4-}$ redox couple (1: 1) with 0.1 M KCl	—	0.5	0.001–0.05	—	138
β-CD@N-GQD/FC	Glassy carbon	DPV	PBS (pH 7.0)	—	0.08	0.0005–0.1	—	139
c. Polymeric materials								
C-pept/PLLA NM	Screen printed	EIS	PBS (pH 7.0)	2910	6.31	0.002–0.015 and 0.02–0.04	—	140
PEDOT–taurine	Screen printed	CA	1 mM $[Fe(CN)_6]^{3-/4-}$ in 0.1 M KCl solution	—	0.95	0.003–1	—	141
d. MXenes and metal–organic frameworks (MOFs)								
MXene/CTS/Cu_2O	Freestanding self electrode	CV	1 mol L^{-1} NaOH	215.71	49.8	0.0498–0.2	—	142
e. Photoelectrochemical (PEC) biosensors								
Au@CdSe@Au nanoparticles	ITO	Photoelectrochemical	—	—	0.0023	1×10^{-6}–1×10^{-5}	—	143
f. Advanced materials and composites								
Ph-SF		FLED	—	—	0.022	—	—	144
				—	0.263	1–5	—	
Cu_2O/MoS_2	Glassy carbon electrode	CA	NaOH (pH 7.0)	111 740	2.18	0.0001–0.18	10	145
PANI–HCl/ZnO NC	Conducting carbon paper	CA	PBS buffer of pH 7.5	3.8×10^8	300	0.75–1.5	—	146
Cu_2O@TNTs–Ti_6Al_4V	SiC paper	CA	0.1 M PBS (pH 7.4)	~10 981.25	~42	0.1–1.15	3	147
Au@NiO/PPy	Glassy carbon electrode	DPV	1 M KOH	7600	0.58	0.01–0.1	—	148
MB/CIT-BCD@Fe_3O_4	SPCE	Amperometry	50 mM PBS (pH 7.4)	20	3.93	0–0.1	—	149
Ru–Pi/PPY	Carbon fiber paper	DPV	$RuCl_3 \cdot xH_2O$ (0.002 M) and PBS (pH 7.0)	1.9988×10^7	0.000054	0.00016–0.02	—	150

Table 2 *(continued)*

f. Advanced materials and composites								
NiVP/Pi	Ni foam	CA	0.1 M PBS (pH = 7.4) and 0.1 M NaOH	5.51018×10^6 36 800	1×10^{-9}	1×10^{-6}–0.01; 0.1–10	—	151
PMO–BMCP	BMCP	CA	0.1 M-PBS at pH 7	226.30	0.0517	0.001–15.5	2.7	152

Abbreviations: PhSF – silk fiber functionalized phosphorene quantum dots, PPy – polypyrrole, C-pept – C-peptide, PLLA – poly-L-lactic acid, NP – nanoparticle, NR – nanorod, PANI – polyaniline, NC – nanocomposite, TNTs – TiO_2 nanotubes, MB – methylene Blue, CIT-BCD – citrate-modified β-cyclodextrin, CD – carbon dot, GR – graphite, APC – activated porous carbon, CTS – chitosan, PMO – poly methyl orange, PNAANI – poly(*N*-acetylaniline), BMCP – Cu/Ni bimetal-dispersed carbon nanofiber/polymer nanocomposite, MWCNTs – multi-walled carbon nanotubes, GO – graphene oxide, MIP – molecularly imprinted polymer, βCD – β-cyclodextrin, GMF – graphene micro-flower, Grp – graphene, PEDOT – poly(3,4-ethylenedioxythiophene), RGR – reduced graphene, 1M3OIDTFB – 1-methyl-3octylimidazoliumtetrafluoroborate, CX6 – calix[6]arene, rGO – reduced graphene oxide, GQD – graphene quantum dot, FC – ferrocene, FTO – fluorine doped tin oxide, ITO – indium tin oxide, SPCE – screen-printed carbon electrode, CV – cyclic voltammetry, DPV – differential pulse voltammetry, CA – chronoamperometry, LSV – linear sweep voltammetry, EIS – electrochemical impedance spectroscopy, SWV – square wave voltammetry, FLED – fluorescence electronic device.

g. Toxicity and biocompatibility

Lastly, it is unthinkable to put aside the matter of material safety. Transition-metal oxides, sulphides, phosphides, or carbon nanostructures have been employed in many advanced sensing technologies; yet, relatively little is understood about their long-term biocompatibility and toxicity characteristics. Clinical application will require a thorough assessment of cytotoxicity, biodegradability, and ecological impact in addition to exploring the possibility of ecologically conscious and naturally biocompatible substitutes.

h. Outlook

When taken as a whole, those challenges emphasise the significance of a comprehensive approach that extends beyond material synthesis. Non-enzymatic cholesterol biosensors' success relies on integrating rigorous clinical verification, digital health adoption, device miniaturisation, and materials innovation. In order to turn these promising laboratory-scale systems into reliable, inexpensive, readily accessible medical devices for practical applications in healthcare, interdisciplinary cooperation among chemists, materials scientists, engineers, physicians, and data scientists becomes valuable.

7. Conclusions

Nonenzymatic cholesterol biosensors happen to be an alternative to enzymatic sensors due to their high stability, low cost, and superior electrochemical performance. Sensitivity, selectivity, and LOD have been highly emphasized by evolution in nanomaterials, electrode modifications, and electrochemical processes. Nevertheless, there are several barriers that need to be determined, including electrode stability, selectivity problems, fabrication complexity, and integration into practical applications. The selection of material and the production of wearable and adaptable sensors to enrich data analysis should be the main objectives of future research. Likewise, regulatory approvals, reliable analytic methods, and validation against clinical standards are vital for marketing and regenerative development. Official permissions and clinical evidence are also mandatory to enable real-time cholesterol monitoring and commercialisation. Nonenzymatic cholesterol biosensors have tremendous potential to remodel point-of-care features and personalised treatment with nurtured interdisciplinary hard work.

Conflicts of interest

There are no conflicts to declare.

Data availability

This review article is based on previously published studies and does not include new experimental data. All sources of information are properly cited within the manuscript. Any data referenced or discussed can be accessed through the original publications as indicated in the reference list.

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