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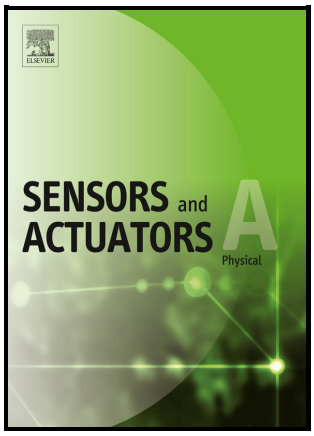

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Biosensors to detect low-density lipoprotein and oxidized low-density lipoprotein in cardiovascular disease

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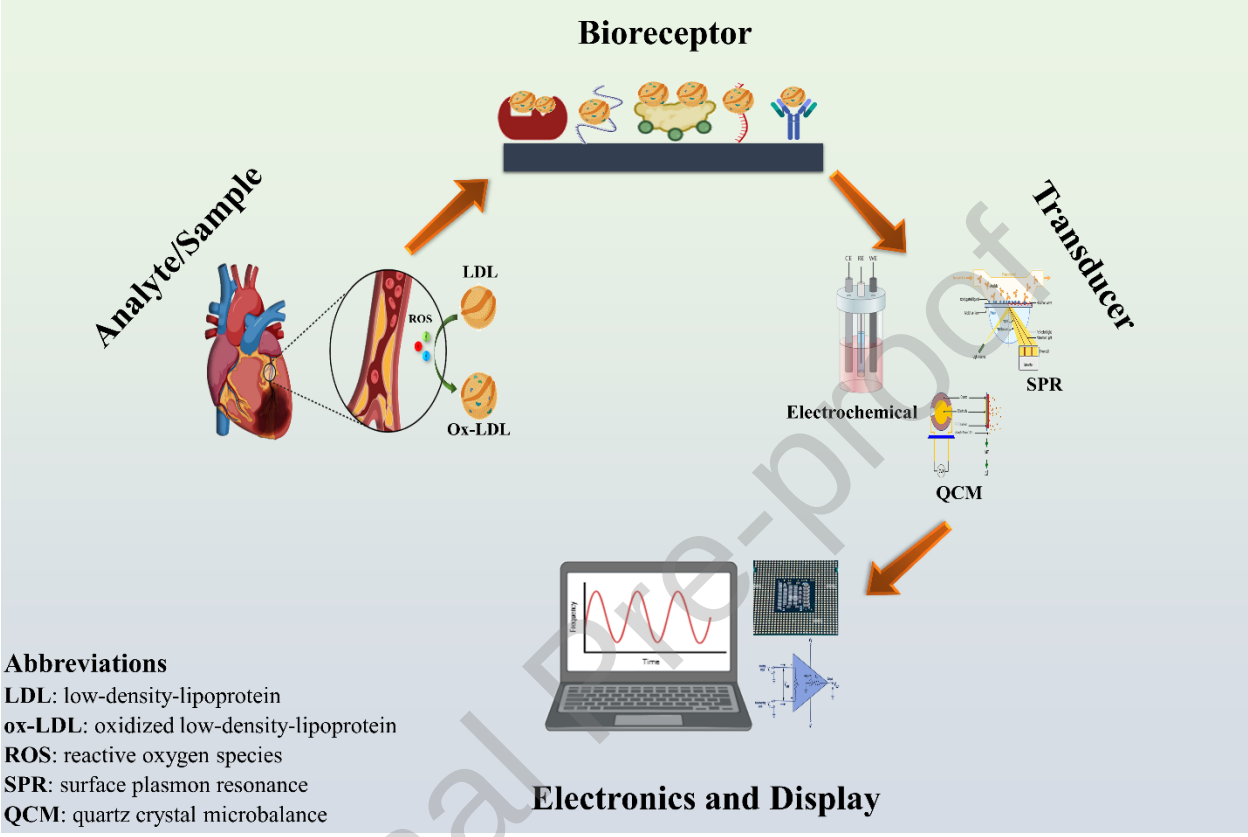
Abstract

Cardiovascular disease (CVD) is the main reason for death worldwide, with atherosclerotic disease being recognized as the most prevalent underlying cause. Various studies have linked low-density lipoproteins (LDLs) and oxidized LDLs (ox-LDLs) to atherosclerosis. Early detection of LDL and ox-LDL using biosensors can support early intervention to prevent CVD or improve management. Studies on LDL/ox-LDL biosensors focus on decreasing detection time and developing new detection methods. Techniques such as quartz crystal microbalance, surface plasmon resonance, and electrochemical sensors are powerful tools that enable early and accurate detection. As a result, this technology has the potential to change how cardiovascular disorders are detected and treated completely. The objective of this review is to provide an overview of the role of ox-LDL in atherosclerosis development and the different LDL/ox-LDL biosensor types in the context of their linear range, detection capability, and possible medical applications.

***Keywords**:* Cardiovascular disease; LDL; ox-LDL; detection; biosensors; atherosclerosis; lipids.

Graphical abstract:



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1. Introduction

Cardiovascular disease (CVD) is the most common cause of mortality and disability, claiming 17.9 million lives annually [1]. A detailed understanding of the pathophysiology of CVD is essential to inform interventional strategies that can help mitigate its impact. Epidemiological studies show low-density lipoprotein (LDL), a cholesterol carrier with a size of 20–25 nanometers (nm), to be among the main causes of atherosclerosis [2, 3].

Atherosclerosis is a condition of the arteries that can present as coronary heart disease (CHD), leading to cardiac ischemia and cerebrovascular disease. Atherosclerosis is an intricate, ongoing,

and inflammatory process primarily arising in the tunica intima (subendothelial area) of middle- to large-size arteries where blood currents or bifurcations occur [4-6]. The presence of risk factors such as male sex, family history of ischaemic heart disease, lipid-rich diet, hypertension, diabetes, and smoking accelerate atherosclerosis [7].

Research identifies ox-LDL, decreased endothelial function, and oxidative damage as contributing factors to atherosclerotic plaque development. Oxidized LDL damages endothelial cells and in turn, macrophages and monocytes migrate to the area. Foam cells are created and these aid in the progression of atherosclerosis [8-11]. Alongside ox-LDL, increasing blood LDL levels have been linked to an increased likelihood of atherosclerosis [12].

Risk assessment of CVD and monitoring of established disease requires measurement of ox-LDL and LDL in the blood. These may be detected using a range of methods, including traditional biochemical tests and cutting-edge tools like mass spectrometry and immunoassays [13, 14]. Moreover, the Friedewald equation is a prevalent technique used for quantifying LDL-cholesterol using levels of high-density lipoprotein cholesterol (HDL-C), triglycerides, and total cholesterol [15, 16]. Despite its convenience, this approach has some limitations. For example, in people with elevated triglyceride levels or atypical lipid profiles, or in those at high or very high risk fo CVD, it might not be accurate [17, 18]. Immunoassays that use antibodies with an affinity for LDL or ox-LDL are another widely used technique for distinguishing and measuring LDL and ox-LDL. The enzyme-linked immunosorbent assay (ELISA) is one of the most popular immunoassay methods for quantifying LDL [19, 20]. Another technique is Mass spectrometry (MS). This technology allows for the accurate molecular identification of cellular lipids, including neutral lipids like cholesteryl esters and triacylglycerols [21, 22] and lipoproteins [23, 24]. Liquid chromatography/tandem mass spectrometry (LC-MS/MS) is used in targeted lipidomics and can

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also measure specific lipid compounds such as LDL [25, 26]. However, these technologies are expensive and can be time-consuming [26].

Consequently, finding cost-effective and simplistic methods to measure LDL and ox-LDL is crucial. In recent years, various novel techniques such as sensors and nanotechnology-based biosensors have emerged for detecting LDL and ox-LDL. Biosensors are particularly advantageous over diagnostic tools like ELISA. This is due to their ability to detect LDL and ox-LDL with high selectivity, sensitivity, ease, rapidity, low cost, and transportability [27-29]. This review provides an overview of the roles of LDL and ox-LDL in the development of atherosclerosis and introduces the concept of biosensors, the different types, targeted LDL and ox-LDL on various biosensor platforms, and their application in the assessment of cardiovascular risk. Finally, like other review articles in the field of heart disease biomarker detection [30, 31], it highlights important clinical parameters such as detection range, limit of detection (LOD), and bioassay design.

2. The relationship between ox-LDL and atherosclerosis development

Normally, LDL in plasma contains triglycerides and cholesterol esters surrounded by free cholesterol, phospholipids, and an apolipoprotein B (ApoB) layer. This layer helps transport hydrophobic cholesterol through the bloodstream [32]. When plasma LDL levels increase, atherosclerosis occurs [33]. Under pathological circumstances, LDL containing ApoB in the plasma may infiltrate the subendothelial intima of blood vessels via compromised endothelial cells. Reactive oxygen species (ROS) can cause lipoprotein oxidation and formation of ox-LDL which leads to a sequence of events resulting in plaque formation and possible thrombosis, as shown in **Figure 1**. In these circumstances, LDL gets changed into ox-LDL [34, 35]. When ox-LDL stays in the subendothelium, it attracts monocytes to the tunica intima where they become macrophages.

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Oxidized-LDL is engulfed by macrophages because it is a powerful binding agent for scavenging receptors (CD36, SR-BI, and SR-AI/II) on macrophages [36]. The innate immune system recognizes and detects oxidized specific epitopes (OSEs) present on ox-LDL after they undergo oxidation. This recognition facilitates the uptake of ox-LDL by macrophages. Macrophages absorb ox-LDL via their scavenging receptors, and ox-LDL buildup causes macrophages to have a foamy look, referred to as "foam cells." This eventually leads to the formation of atherosclerotic plaque lesions and subsequent thrombosis with possible myocardial infarction or stroke in the event of rupture, **Figure 1** [37, 38].

For the cell to remain in balance and avoid oxidative stress, redox homeostasis is crucial. When redox homeostasis is imbalanced, free radicals such as ROS and reactive nitrosative species (RNS) are produced [39, 40]. Internal and external variables can imbalance the redox system and increase ROS formation, including ionizing radiation (IR), ultraviolet exposure (UV), environmental contaminants (pesticides such as deltamethrin and paraqual), air pollutants (such as particulate matter (PM)), smoking, enzymes (like xanthine oxidase, NADPH oxidase, and nitric oxide synthase), radiation, physical forces (oscillatory shear) and heavy metals (such as copper, cobalt, arsenic, chromium, etc.) [41]. Other biomolecules such as proteins, nucleic acids, and lipids are also susceptible to damage, and this can lead to disease or cell death [32, 40, 42, 43].

3. The biosensors design process

Biosensors are low-cost, quick, and highly accurate alternative methods to LC-MS/MS, ELISA, and MS for the detection of LDL and ox-LDL.

A biosensor assesses biological/chemical responses by producing signals corresponding to an analyte concentration. Three functional components make up every biosensor. The initial component of the biosensor is the biological element, which identifies the analyte and provides a

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response signal. The second, and most critical, part of any biosensor device is the transducer, which converts the signal produced into a response that can be detected. The biosensor's third component, the detector, analyses and amplifies the signals before showing them on an electronic display system, **Figure 2**. Biosensors are used in a variety of applications such as drug discovery, monitoring of diseases and disease-causing microorganisms, and identification of pollutants in addition to detection of disease indicators in bodily fluids like urine, blood, sweat, and saliva [44, 45].

To improve the signal-to-noise ratio of a biosensor in order to reduce the sample quantities required, nanoparticles can be employed and the biosensor can be reduced to the micro- or nano-scale. When the biosensor is scaled down to the nanoscale, the ratio of the surface to the volume of the sensing region increases, and the size of the diagnosis electrode and biomarker of the target approach are analogous. This reduces nonspecific bonding and increases bonding effectiveness towards the target. Consequently, the bioreceptors can function as activated transducers in the system for sensing, enabling single-molecule identification. Further, the requirement for lower specimen quantities reduces assay costs [45-47].

Low-density lipoprotein/ox-LDL sensors are a group of bioreceptors that demonstrate marked potential for biomedical diagnosis owing to their stability and selectivity in replies. These sensors can assess plasma LDL/ox-LDL levels to assess the risk of atherosclerosis [48, 49]. New approaches for detecting LDL/ox-LDL using biosensors are being explored. Low-density lipoprotien/ox-LDL diagnosis transducers can be classified into three types: (1) piezoelectric; (2) electrochemical and (3) optical. In continuation, the basic axioms of various transducers, such as quartz crystal microbalance (QCM), electrochemical and surface plasmon resonance (SPR), and

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studies on these will be discussed in relation to their significance in qualitative and quantitative LDL/ox-LDL analyses.

3.1 Quartz crystal microbalance-based LDL/ox-LDL biosensors

The piezoelectric QCM biosensor is based on a mechanical transducer that works on mass diagnosis principles. It is highly sensitive and capable of diagnosing the target analyte in its area, which causes the resonance frequency to change. Piezoelectric-based biosensor technology has several advantageous features including compact size, high speed, high throughput, and excellent specificity and sensitivity [50]. Piezoelectric lipoprotein immunosensors have been used to trap and detect ligands on particles of lipoprotein [51].

Chunta et al. developed new types of synthetic polymers called molecularly imprinted polymers (MIPs) which mimic specific receptors found in living organisms [52]. These were created to assist in the rapid measurement of LDL in biological samples. To find the best combination of building blocks for these polymers, different ratios of three monomers (acrylic acid (AA), methacrylic acid (MAA), and vinyl pyrrolidone (NVP) were tested. The most effective combination was found to be MAA and NVP in a 3:2 ratio by weight, which allowed for a linear response to LDL cholesterol levels between 4 and 400 $mg.dL^{-1}$.

The offered biosensor could detect the LOD for LDL-C to 4 $mg.dL^{-1}$ and the limit of quantification (LOQ) to 13 $mg.dL^{-1}$. Very-low-density lipoprotein (VLDL) leads to 1 to 3% of the LDL signal, HDL to 4 to 6%, and human serum albumin (HSA) to 0 to 2%. With a precision of 95–96% and a confidence margin of 95%, the LDL-MIP sensor offers high analytical accuracy. The sensor answers were highly correlated to the results obtained from the method of standard with $R^2 = 0.97$, demonstrating the application of the sensor to real-world specimens analyzed using

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LDL-MIP. This indicates that the method could be used to effectively determine LDL levels in human serum samples.

In another study, Chunta et al. [53] developed a type of sensor that employs a molecularly imprinted polymer (MIP) to detect LDL and high-density lipoprotein (HDL). The researchers used a common mixture of vinyl pyrrolidone (NVP) and methacrylic acid (MAA) in ratios of 2:3 and 3:2 to create MIPs for each lipoprotein class. The MIPs were deposited onto a three-electrode QCM that served as selective elements for HDL and LDL, respectively. The QCM was equipped with three gold electrodes and coupled with non-imprinted polymer (NIP), LDL-MIP, and HDL-MIP, resulting in a three-channel sensor array. The sensor array response to lipoprotein binding was measured by the shift in resonance frequency of the QCM.

Frequency changes were observed with concentrations of LDL solutions ranging from 200 to 400 $mg.dL^{-1}$. The LDL-MIP-coated electrode experienced a greater negative frequency shift (Δf: 1400-4016 Hz) compared to the HDL-MIP coated electrode (Δf : 0 to 318 Hz) and the NIP coated electrode (Δf: 86 to 933 Hz). The responses of HDL-MIP and NIP were slightly reduced because of the non-specific binding of LDL to the polymer film. This also illustrates HDL-MIP to LDL cross-reactivity, which is displayed as LDL to HDL-MIP effectiveness relative to the LDL-MIP signal. The LDL component in the HDL-MIP signal ranges from 9 to 19%. Additionally, 0 to 3% of the LDL-MIP reaction is produced when LDL-MIP and HDL cross-react. All of the LDL that has adhered to the sensor array can be removed by the washing procedure. Although Chunta and his colleagues couldn't improve LDL diagnosis over the previous study [52], this sensor has the advantage of reusability, which was not evaluated in the previous study.

Very low-density lipoprotein particles are critical in moving endogenous triglycerides (TGs) produced in the liver into the bloodstream. They account for approximately 55% of the

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overall TG concentration in each particle. A clinical study suggests that VLDL, like LDL, has a direct role in the advancement of atherosclerosis and cardiovascular risk [54, 55]. In the absence of a specialized specimen preparation procedure, the VLDL cholesterol content (VLDL-C) cannot accurately be determined by the enzyme immunoassay technique in clinical laboratory analysis as it can for other lipoproteins. The concentration of VLDL is calculated by examining the endogenous TGs bound in its particles and converting them to the VLDL-C estimate (TGs/5). Collection of TGs should be done after a 12-hour fasting period when exogenous TGs bound to chylomicrons (CMs) have been removed from the circulation [56]. In order to directly detect VLDL, a QCM-based sensor was developed that used biomimetic sensor components as a MIP. The sensor of VLDL-MIP demonstrated remarkable sensitivity with a linear range of 2.5 $mg.dL^{-1}$ to 100 $mg.dL^{-1}$ and a LOD of 1.5 $mg.dL^{-1}$ for VLDL-C When VLDL-MIP sensor was used for VLDL measurement at values ranging from 38 to 71 $mg.dL^{-1}$, 96 to 103% of recoveries were obtained [57], **Figure 3A**. The sensor had high repeatability and reproducibility, ranging from 1.63 to 4.74% and 4.25 to 9.04%, respectively. The sensor showed limited cross-reactivity with other lipoproteins, with 6 to 7 % of LDL, 2 to 4% of HDL, and 1% of CMs contrasted to the VLDL, **Figure 3B**.

Biosensor data for 12 hour fasting serum and non-fasting serum corresponded well with VLDL estimations calculated using TGs/5, with R^2 values of 0.997 and 0.993, respectively. This innovative sensor provides a novel technique for assessing VLDL directly from non-fasted serum without the need for any further sample preparation.

Another study was conducted to directly detect ox-LDL in serum using a MIP thin film on a QCM [58]. A schematic outlining the preparation of ox-LDL-MIP QCM sensors is provided in **Figure 3C**. The MIP sensors that were developed showed minimal response to other lipoproteins

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like LDL and HDL and no response to VLDL and HSA. The detection range of the sensor for ox-LDL was found to be 86-5600 $\mu g.dL^{-1}$, and the LOD and LOQ (quantification) were 86 and 287 $\mu g.dL^{-1}$, respectively, which allowed for the detection of ox-LDL in both normal (420 to 2100 $\mu g.dL^{-1}$) and pathological ranges. It demonstrated a recovery ranging from 92 to 107% and 1 to 8% coefficient variation precision when detecting ox-LDL. This was compared to an ox-LDL ELISA test kit. The sensor had similar characteristics with a 0.98 correlation value, but the response time of the ELISA was slower (210 minutes vs. 10 minutes) Therefore, ox-LDL-MIP QCM sensors are efficient tools to detect this sensitive biomarker.

3.2 Surface plasmon resonance-based LDL/ox-LDL biosensors

Surface plasmon resonance (SPR) biosensing is a technique that can detect bonding processes between molecules in real-time without the need for labeling [59, 60]. The method operates by detecting variations in the refractive index caused by the interaction between target and probe molecules. It has many applications in fields such as food specimen evaluation, pharmaceutical engineering, and antigen-antibody description [59, 61-63]. When compared to other techniques that require labeled molecules, SPR provides benefits such as dependable equipment, reusable sensor chips, and adaptability [63, 64]. Additionally, SPR can be used with a wide range of various nanoparticle areas as well as nanoparticles of noble metals to create SPR-based sensors [65].

Matharu et alproposed a sensor for detecting LDL using SPR technology [66]. Biotinylated heparin (B-HEP) was fixed onto a self-assembled 4-amino thiophenol (ATP) monolayer. The linking of B-HEP on the ATP layer was accomplished via the biotin-avidin binding, as outlined in **Figure 4A**. The B-HEP was immobilized on the surface of the self-assembled monolayer (SAM), which was modified with avidin. Subsequently, the nonspecific sites were blocked using bovine serum albumin (BSA). The resulting electrode was subjected to varying LDL concentrations.

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Figure 4A depicts the electrode preparation. The modified electrodes were evaluated using several techniques such as SPR, cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS), and contact angle (CA) evaluations. The CA illustrations of 4-amino thiophenol [ATP]/Au, B-HEP/biotinylated heparin and avidin [AVI]/ATP/gold [Au] and LDL/B-HEP/AVI/ATP/Au different electrodes are presented in **Figure 4B**. The contact angle value decreased from 76.98° for ATP/Au (**Figure 4B** (a)) to 44.16° for B-HEP/AVI/Au (**Figure 4B** (b)), indicating the linking of B-HEP. This decrease is attributed to numerous polar groups in B-HEP that make the level more hydrophilic, thus reducing the CA value. After linking LDL, the CA amount further decreased to 16.86° (**Figure 4B** (c)). This decrease is due to the high number of amino and carboxylic groups in LDL, contributing to increased hydrophilicity, resulting in a further decrease in the CA. The CA change confirms the stabilization of LDL on the B-HEP/AVI/ATP/Au electrode. Utilizing the SPR method, the interaction between stabilized B-HEP and LDL was studied. The modified electrode was able to detect LDL in the linear range of 20–100 $mg.dL^{-1}$ with 513.3 m°/μM sensitivity.

In another investigation, a fiber-optic sensor was created using SPR to detect LDL [67]. A gold layer was applied to a section of the optical fiber core and then ATP and anti apolipoprotein B (AAB) were added to the surface. The sensor was able to detect LDL concentrations in sample solutions ranging from 0 to 190 $mg.dL^{-1}$, with a sensitivity of 0.184 nm/mg/dL. The answer time of the sensor was approximately two minutes. Verma et al's proposed sensor was able to improve the linear range and sensitivity reported in the previous study [66]. This biosensor has the potential to measure LDL levels in human blood.

3.3 Electrochemical-based LDL/ox-LDL biosensors

Electrochemical-based biosensors are extensively researched and employed, relying on the electrochemical characteristics of the analyte and transducer for their operation. They have

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appreciable selectivity, sensitivity, and detection capabilities. An electrochemical interaction between the bioreceptor and target in the transducer area results in measurable, electrochemical signals that consist of impedance, voltage, capacitance, and current [68, 69]. Electrochemical-based biosensors can be classified based on the transmission concept, which includes impedimetric, voltammetric, conductometric, potentiometric, and amperometric [45, 68, 69].

Electrochemical biosensors have been created to detect LDL using nanomaterials and electrochemical ways. One method is CV, a type of analysis that can study the electroactivity of substances and offer data about the kinetics of electron transfer. According to the alteration at cathodic and anodic peaks, CV may be utilized to track biomolecular interactions and investigate the connection between the probe and LDL/ox-LDL [70, 71]. Another electrochemical technique used in these biosensors is EIS, which requires only low-amplitude disturbances from the steady state and provides data about the interface [70, 72, 73]. Overall, this method is used to make immunosensors with excellent sensitivity to LDL/ox-LDL [74]. As a result, more research based on LDL/ox-LDL biosensors has been devoted to reducing identification time and developing novel methods of identifying LDL/ox-LDL.

For example, Jie et al developed a new type of electrochemiluminescence (ECL) biosensor to detect LDL [75]. This was achieved by using self-assembly techniques and amplification of the signal with gold nanoparticles. Construction of the ECL biosensor and binding of LDL is illustrated in **Figure 5A**, which includes several steps such as the self-assembly of cysteamine on a gold electrode, conjugating a gold nanoparticle layer to cysteamine, covalently attaching carboxyl group functionalized CdS nanocrystal (CdS NC) to the cysteine, cross-linking apoB-100 (a ligand of the LDL receptor) on the CdS NC, blocking the non-specific binding surfaces of CdS NC with BSA, and finally, specific binding of LDL against apoB-100.

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The modified electrode was used as a biosensor to detect LDL. The concentration was determined by measuring the reduction in ECL intensity that occurred due to the bonding of LDL with apoB-100. The intensity of the ECL biosensor decreased in a linear fashion as the concentration of LDL increased, with the ability to detect LDL concentrations in the linear range of 0.025 to 16 ng. mL^{-1} and a LOD of 0.006 $ng.mL^{-1}$. The result of the ECL output generated by the biosensor over the course of ten cycles of continuous potential scanning showed that the signals were stable, meaning that the biosensor was well-suited for ECL detection.

The CdS NC used in the biosensor exhibited great ECL intensity and biocompatibility, and even provided more bonding surfaces for apoB-100 which is beneficial for detecting low concentrations of LDL. Additionally, the gold nanoparticle amplification technique was employed to improve the sensitivity of protein ECL analysis. Consequently, the biosensor displayed good reproducibility, sensitivity, stability, and a prompt response.

Another study proposed a label-free electrochemically based immunosensor to detect LDL [76]. They used a P-type semiconducting nickel oxide (NiO) slim layer as a matrix for covalently immobilizing the apo-B-100 antibody, which was achieved using EDC-NHS chemistry. The NiO slim layer was deposited using the radio frequency (RF) sputtering technique, and the process is illustrated in **Figure 5B**. In immunosenor development, low-density lipoprotein interacts with the AAB antibody. After preparation, the immunoelectron was analyzed utilizing EIS, differential pulse voltammetry (DPV), and CV. The impedance answer of the immunosensor displayed a great sensitivity of 12 kΩ/μM over a broad linear range of 0.018 to 0.5 μM, with a LOD of 0.015 μM. The immunosensor also exhibited excellent efficiency specifications and a long lifetime of 18 weeks, demonstrating the superior ability of the NiO matrix to quantify LDL accurately on a commercial scale.

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In other research the use of a slim layer of NiO used as a platform to create a biosensor for detecting total and free cholesterol and LDL without the need for any additional reagents was investigated [77]. Researchers took advantage of the redox properties of the NiO matrix to improve electron transfer between the enzyme and the electrode and remove potentially toxic mediators from the solution. The offered biosensor demonstrated great sensitivity, with a range of 63 and 27 $\mu A/mM/cm^2$ for total and free cholesterol, respectively, over a linear range of 1 to 12 and 0.12 to 10.23 mM. The biosensor's 0.12 $mA/M/cm^2$ sensitivity allowed assessment of LDL levels over a broad span of values, from 0.018 to 0.5 M. These findings show great promise for creating a biosensor to measure cholesterol levels in blood samples.

Rodriguez-Silva et al have developed an electrochemical biosensor with a supercapacitor to detect LDL [78]. Their approach involves the utilization of polyethyleneimine (PEI)-functionalized graphene oxide (GO) to develop an immunosensor for measuring LDL, which is analyzed based on supercapacitance. Polyethyleneimine is physically adsorbed onto the GO surface, which is then combined with a polymeric layer made of chitosan, a natural polymer. The presence of $-NH_2$ (amine) groups in both PEI and chitosan allows for covalent binding between the amine terminal and the -COOH (carboxylic) terminal of the AABantibody (**Figure 6A**). By studying the change in capacitance as a subordinate of LDL concentration, the BSA/AAB/chitosan immunosensor showed a consistent linear range of LDL concentrations between 10 and 120 $mg.dL^{-1}$, with a LOD as low as 10 $mg.dL^{-1}$ and a detection time of fewer than two seconds. Rodriguez-Silva was able to advance work by Kaur et al's [76, 77] by achieving a faster detection time.

This biosensor could potentially have important clinical applications for the detection and monitoring of LDL. Researchers also investigated solutions containing HDL in the linear range of

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10 to 100 mg.dL^{-1} using a similar method as for LDL. The results, shown in **Figure 6B**, indicate that the special capacitance of the platform does not consistently and proportionally respond to HDL concentrations. However, the capacitance values for LDL overlap 65–90 μF.cm^{-2}. At concentrations of HDL ranging from 20 to 40 mg.dL^{-1} and 85 to 95 mg.dL^{-1}, the capacitance of the immune electrode is similar when exposed to HDL. The capacitance of the immune electrode in the HDL concentration range of 40 to 60 mg.dL^{-1} is lower compared to the capacitance of LDL function, which enables dissociation among non-specific and specific proteins. The technique is stable, specific, and sensitive for biosensing applications, making it suitable for the quantification of other essential biomolecules in serum like cancer-related proteins and significant biomarkers. The method offers a novel approach and builds the foundation for the development of a point-of-care system that can deliver faster, more precise, and more cost-effective clinical tests in the future.

In another investigation, Assaifan et al demonstrated a simple LDL-cholesterol detection technique employing non-faradic impedimetric biosensing [79]. Unlike existing electrochemical biosensors, disclosed biosensors do not need reference electrodes or redox probes during diagnosis. A non-faradaic impedimetric biosensor for the determination of LDL-cholesterol was developed in this study. The biosensor comprised of interdigitated gold electrodes with chemical linkers, glutaraldehyde, and cysteamine (**Figure 7A**). The surface was coated with antibodies and blocked with BSA to improve biosensor selectivity and stability. The biosensors responded well to various LDL concentration infusions and held up well to samples of the blank. The LOD and sensitivity of the biosensor were 120 pg. mL^{-1} and 70 nF/log(ng.mL^{-1}) at a linear range of 500 pg.mL^{-1} to 500 ng.mL^{-1}, respectively, which is considerably lower than the suggested LDL blood level. This platform can potentially monitor people in critical care facilities or be used for continued monitoringThis enables clinicians to detect variations in blood levels and ascertain the

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efficacy of therapies. The method can be applied to other chronic illnesses that necessitate real time tracking.

Cabral-Miranda et al presented a rapid and efficient technique to detect ox-LDL, a novel immunosensor that uses three monoclonal antibodies (mAbs) vs. ox-LDL [26]. The immunosensor was designed by self-assembling cysteamine (Cyst) on a reusable screen-printed gold electrode surface. After reacting with NHS and ethyl(dimethyl aminopropyl) carbodiimide (EDAC), three mAbs were incubated in Au-Cys. Bovine serum albumin was further stabilized to guarantee that no other compounds but ox-LDL could bond to the working electrode (**Figure 7B**). This was accomplished by simulating human plasma containing ox-LDL using a standard ox-LDL solution made in serum. The linear range was from 0.5 to 18 g/mL with a LOD of 0.22 mg/mL.

This effectively assessed the ox-LDL percentage in human serum without previous dilution or chemical therapy, and a readout was obtained in less than 20 minutes. Use of multiple monoclonal antibodies on biosensing technology appears to be an effective strategy for producing a specific response to a complex multianalyte target, corresponding to the amount of ox-LDL in atherosclerotic disease. In conclusion, the developed immunosensory is quick, simple, portable, and inexpensive, with considerable potential to enhance atherosclerotic disease detection with point of care diagnosis.

In another study by Billah et al, EIS was used to observe the sensor character design and diagnosis of two cardiovascular pathologist analytes, cardiac troponin I (cTnI) and solubilized lectin-like oxidized LDL receptor-I (sLOX-1), on transfers of mixed self-assembled monolayers (mSAM) onto gold electrodes [80], **Figure 8A**. Because of its specificity and long-lasting persistence, cTnI is used as a gold standard biomarker in acute myocardial infarction (AMI) detection. The LOD for the sLOX-1 and cTnI immunosensor was a minimum of 10 to13 M for

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each target in the linear range 10^{-13} to 10^{-7} M. Levels of sLOX-1 in the blood can be used as an indicator of atherosclerotic plaque instability or rupture before an AMI occurs, allowing for earlier identification of ACS (acute coronary syndrome).

Fang et al developed an electrochemical sensor that utilizes micromotor technology to detect ox-LDL in blood [81]. This new approach simplifies the processing of blood samples. As shown in **Figure 8B**, the sensor, called Mg–Fe_3O_4@PB (Prussian blue) @ Antibody of ox-LDL @ BSA, uses Mg microspheres to move, with the help of H_2, to capture ox-LDL in whole blood. Captured Ox-LDL is collected on the surface of a magnetic glassy carbon electrode MGCE using a self-made hopper apparatus. The ox-LDL concentration is then measured through chronoamperometry using an electrochemical method. The use of micromotor technology in the offered sensor led to efficient detection of ox-LDL in whole blood, with a detection range of 0.01 to 10 μg.mL^{-1} and a LOD of 0.98×10^{-3} μg.mL-1. Compared to other studies, this new sensor provides a fast, reliable, and sensitive method for ox-LDL detection in whole blood [26, 80].

In the future, it is likely these biosensors will be used in conjunction with conventional methods for the detection of LDL/ox-LDL in whole blood samples. **Table 1** presents a comparison between different types of biosensors utilized for the detection and measurement of LDL/ox-LDL.

4. Future perspective and conclusion

Biosensors for biomarker detection are a growing trend in clinical laboratory research. Different methods, such as electrochemical, SPR, and QCM can offer insight into the relationship between LDL/ox-LDL, atherosclerotic plaque development, and acute cardiovascular events. Biosensors in terms of user-friendliness, portability, flexibility, exceptional sensitivity, extensive diagnostic capabilities, and cost-effectiveness can help to inform an individual's cardiovascular risk profile, which is essential for early CVD detection and treatment. Nevertheless, some concerns need to be

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addressed prior to achieving complete development, such as a restricted lifespan and the assessment of various markers, such as LDL, ox-LDL, HDL, VLDL, triglycerides, and total cholesterol, to accurately detect CVD. Also, further research is required to elucidate the effects of temperature, pH, time, and concentration on the functional response of biomolecular systems. This review highlights the significant potential of biosensors in CVD management. Additionally, point-of-care testing may be conducted in non-laboratory settings. The immediate evaluation of samples at the testing site avoids time-consuming specimen processing in testing labs.

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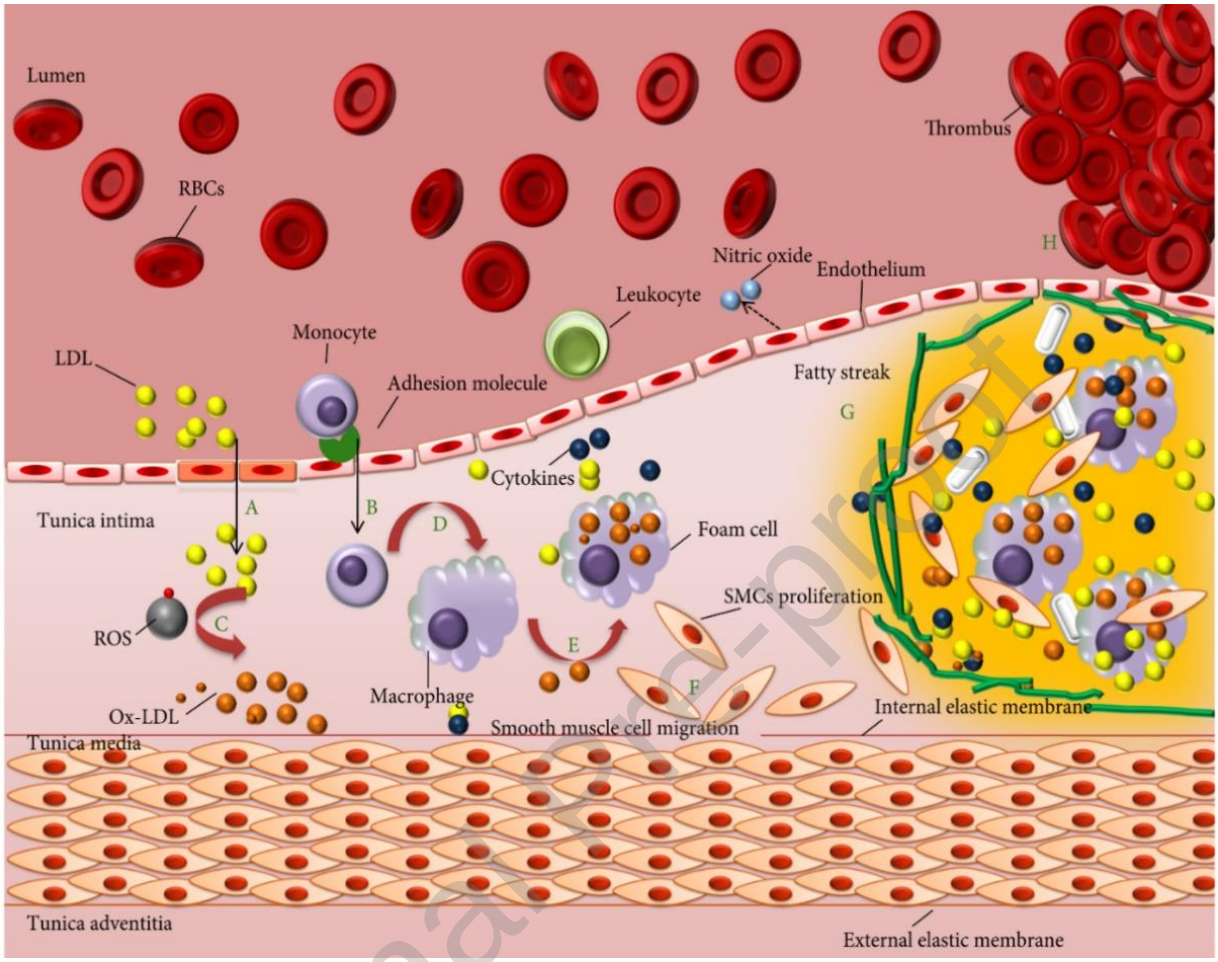

Figure 1. A schematic to depict the process of low-density lipoprotein oxidation and atherosclerosis development, taken from [3]. **(A)** LDL within the blood crosses the damaged endothelium, **(B)** Damaged endothelial cells express adhesion molecules that attract monocytes, **(C)** Monocytes migrate into the intima and produce free radicals that oxidize LDL, **(D)** Ox-LDL attracts additional monocytesand immune cells to the area , **(E)** Ox-LDL particles are engulfed by macrophages and become foam cells, **(F)** Foam cells die and release their contents which are engulfed by different macrophages, a large lesion develops **(G)** Progressive expansion of the lesion which develops into a plaque, accumulating collagen, smooth muscle cells (the tunica media), foam cells and calcium salts. The plaque remains below the endothelium until the endothelial tissue above it becomes damaged, **(H)** The damaged endothelium is less capable of producing blood coagulation inhibitors, making it more susceptible to penetration into the blood artery lumen. The clot attached to the vessel wall can cause myocardial infarction or stroke. **(Abbreviations** : LDL, low-density lipoprotein; ox-LDL, oxidized low-density lipoprotein; RBC, red blood cell; ROS, reactive oxygen species; SMC, smooth muscle cell.).

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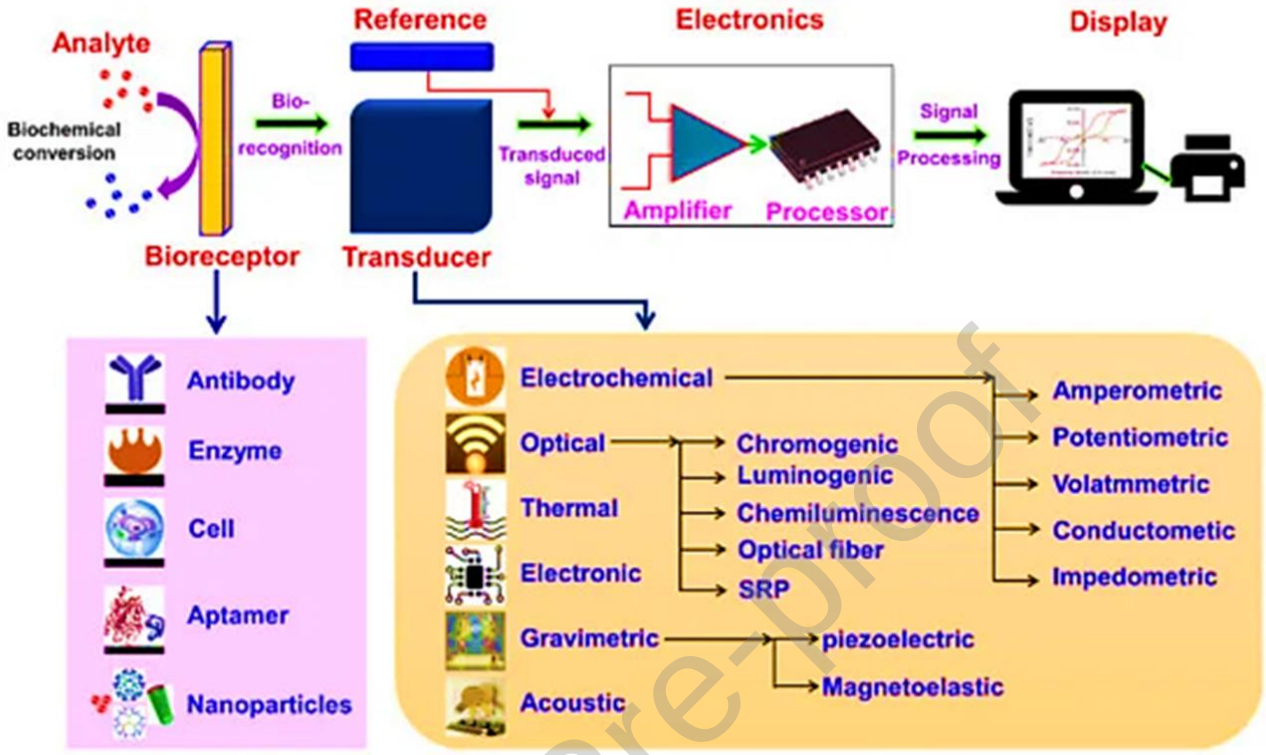

Figure 2. A schematic representation of a biosensor, including a bioreceptor and transducer (and the different types that are available), electrical devices (processors and amplifiers) and display (such as a computer/printer), taken from [45].

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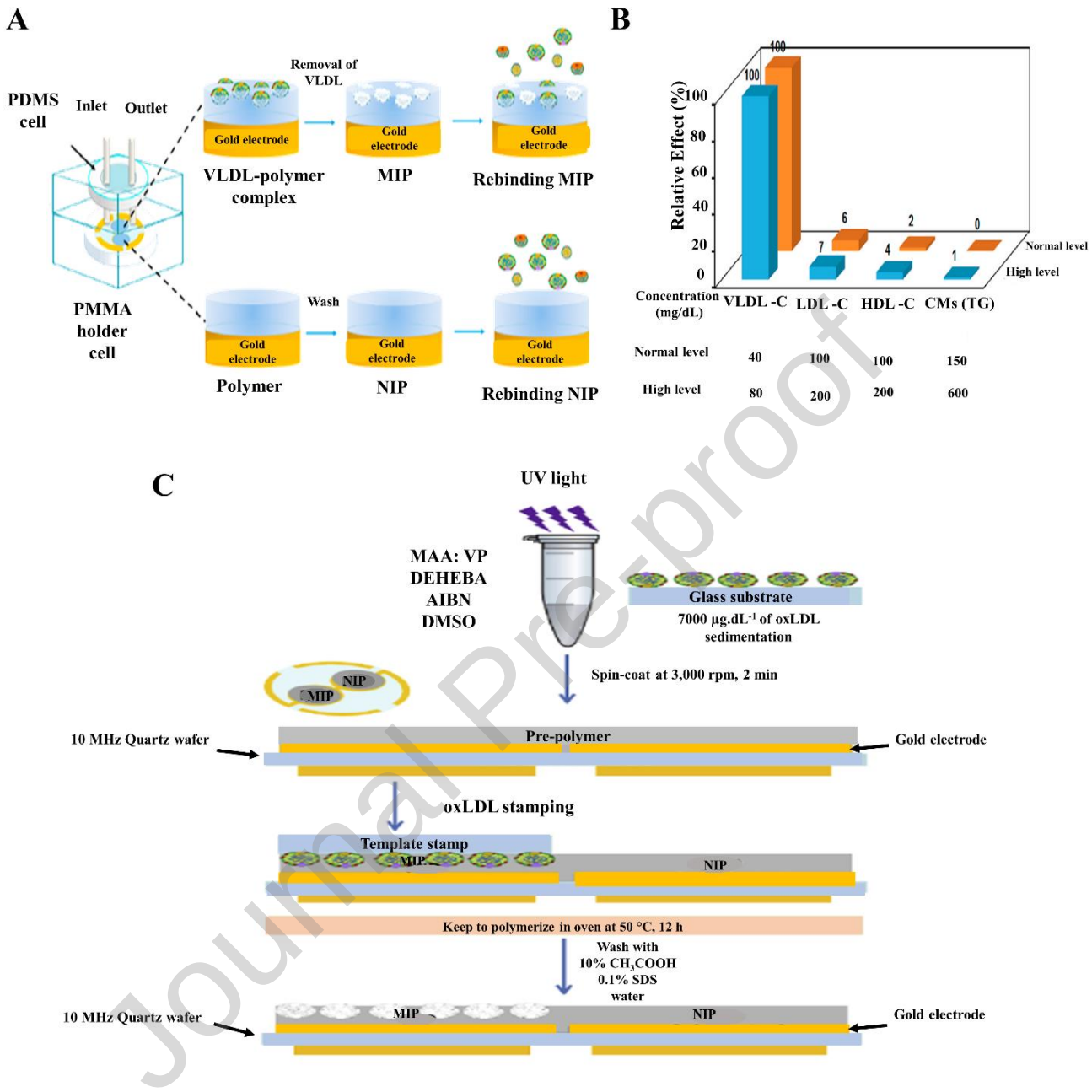

Figure 3. (A) Schematic of the VLDL-MIP QCM in the PMMA flow cell and preparation of MIP and NIP on the two-electrode QCM, taken from [57]; **(B)** VLDL-MIP sensor selectivity, taken from [57]; **(C)** illustration of preparing the ox-LDL/MIP QCM sensor [58] (**Abbreviations**: AIBN, 2,2′-azobis-(isobutyronitrile); CM, chylomicron; DEHEBA, *N*, *N*′-(1,2-dihydroxyethylene) bis(acrylamide); DMSO, dimethyl sulfoxide; HDL-C, high density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MAA, methacrylic acid; MIP, molecularly imprinted polymer; NIP, non-imprinted polymer; oxLDL, oxidized LDL; PDMS, polydimethylsiloxane; PMMA, polymethylmethacrylate; TG, triglyceride; UV, ultraviolet; VLDL, very low-density lipoprotein.).

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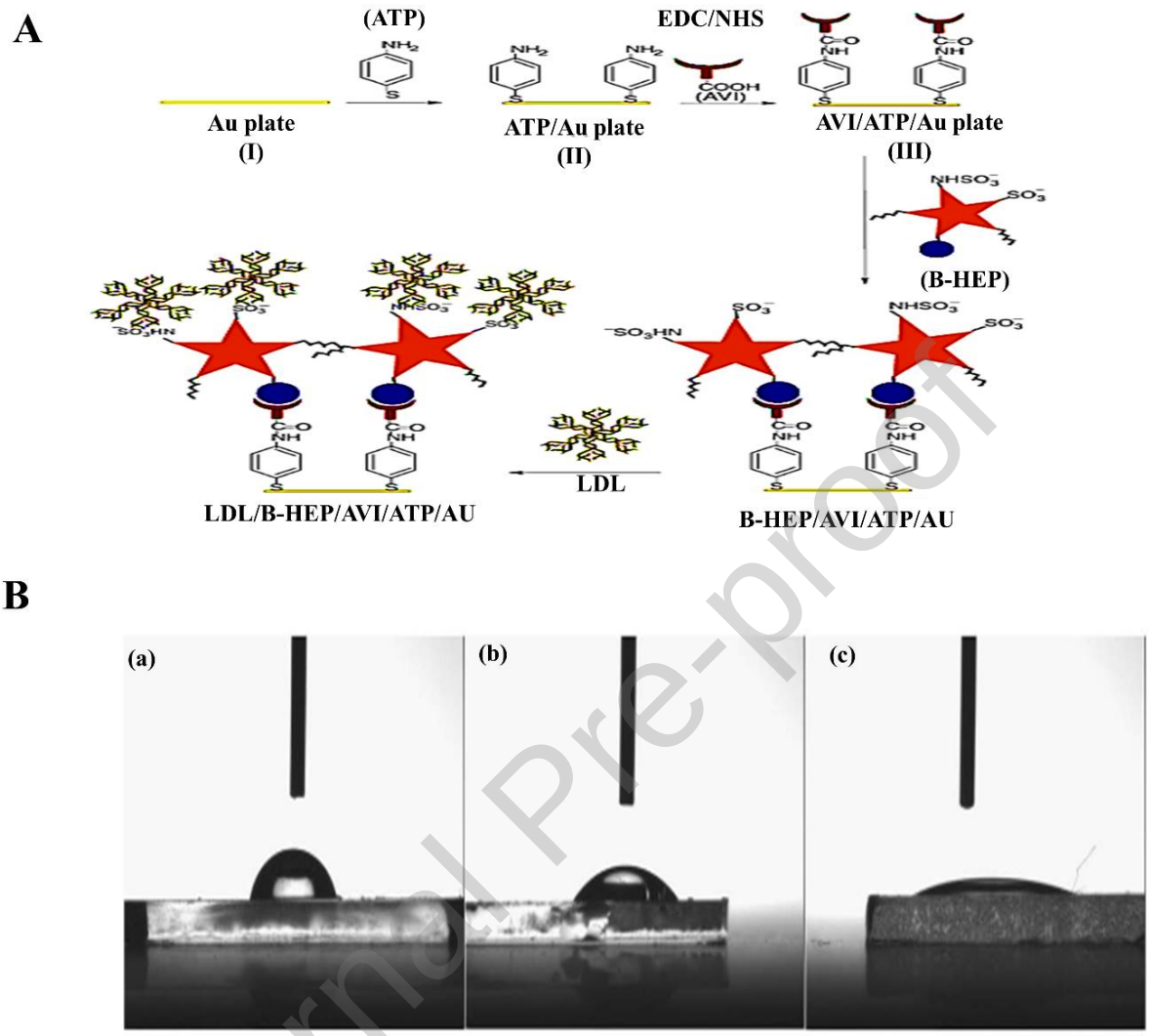

Figure 4. (A) Diagram the process of attaching LDL on the B-HEP/AVI/ATP/Au electrode, taken from [66]; **(B)** The CA evaluation of the ATP/Au, B-HEP/AVI/ATP/Au, and LDL/B-HEP/AVI/ATP/Au different electrodes, taken from [66] (**Abbreviations**: ATP, 4-amino thiophenol; Au, symbol for gold; AVI, Biotinylated heparin and avidin; B-HEP, biotinylated heparin; EDC, N-ethyl-N-(3-dimethyl amino propyl carbodiimide); LDL, low-density lipoprotein; NHS, N-hydroxy succinimide).

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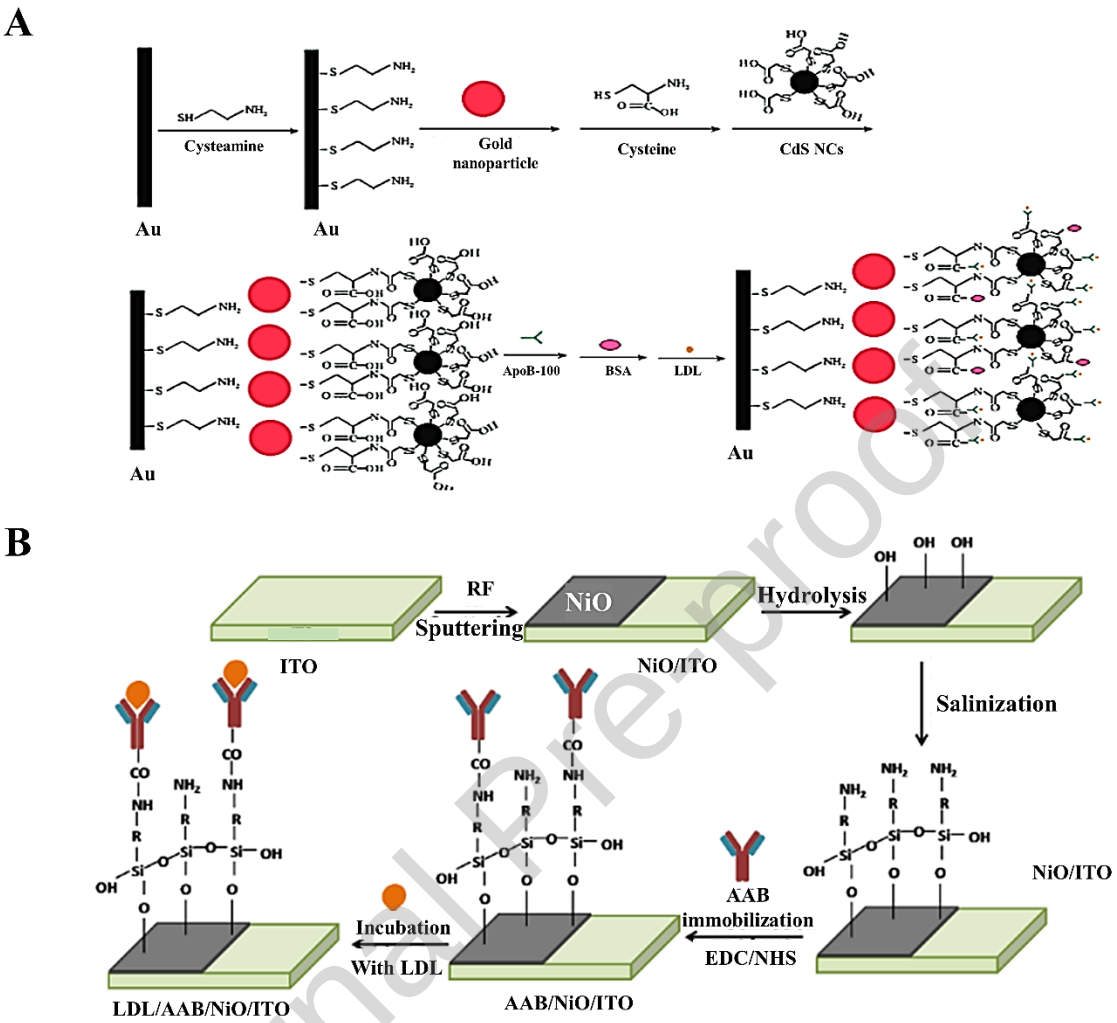

Figure 5. (A) Biosensor fabricationand binding of LDL, taken from [75]; (**B)** The process of preparing the immunoelectron and the interaction between LDL and the AAB antibody, taken from [76] (**Abbreviations**: AAB, anti-apolipoprotein B; apoB-100; apolipoprotein B-100; Au, symbol for gold; BSA, bovine serum albumin; CdS NC, carboxyl group functionalized CdS nanocrystal; ECL, electrochemiluminescence; EDC, N-ethyl-N-(3-dimethyl amino propyl carbodiimide); NHS, N-hydroxysuccinimide; LDL, low-density lipoprotein; NiO, nickel oxide; RF, radio frequency).

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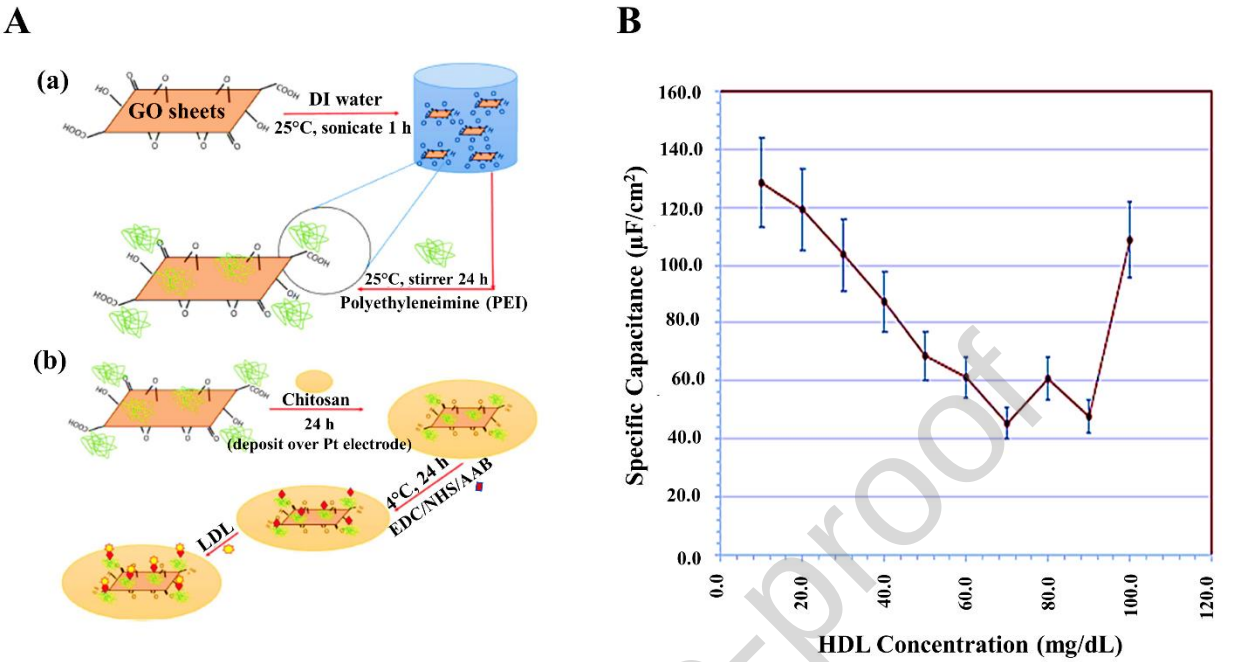

Figure 6. (A) (a) Adsorption of PEI onto GO sheets, (b) Preparation of an immuno-functionalized layer using the GO/PEI nanocomposite, and the interaction between LDL and the AAB , taken from [78]; **(B)** AAB/CS/PEI-GO biosensor detection of HDL at different concentration, taken from [78] (**Abbreviations** : AAB, anti apolipoprotein B; Au, symbol for gold; EDC, N-ethyl-N-(3-dimethyl amino propyl carbodiimide); GO, graphene oxide; HDL, high density lipoprotein; LDL, low-density lipoprotein; NHS, N-hydroxysuccinimide; PEI, polyethyleneimine).

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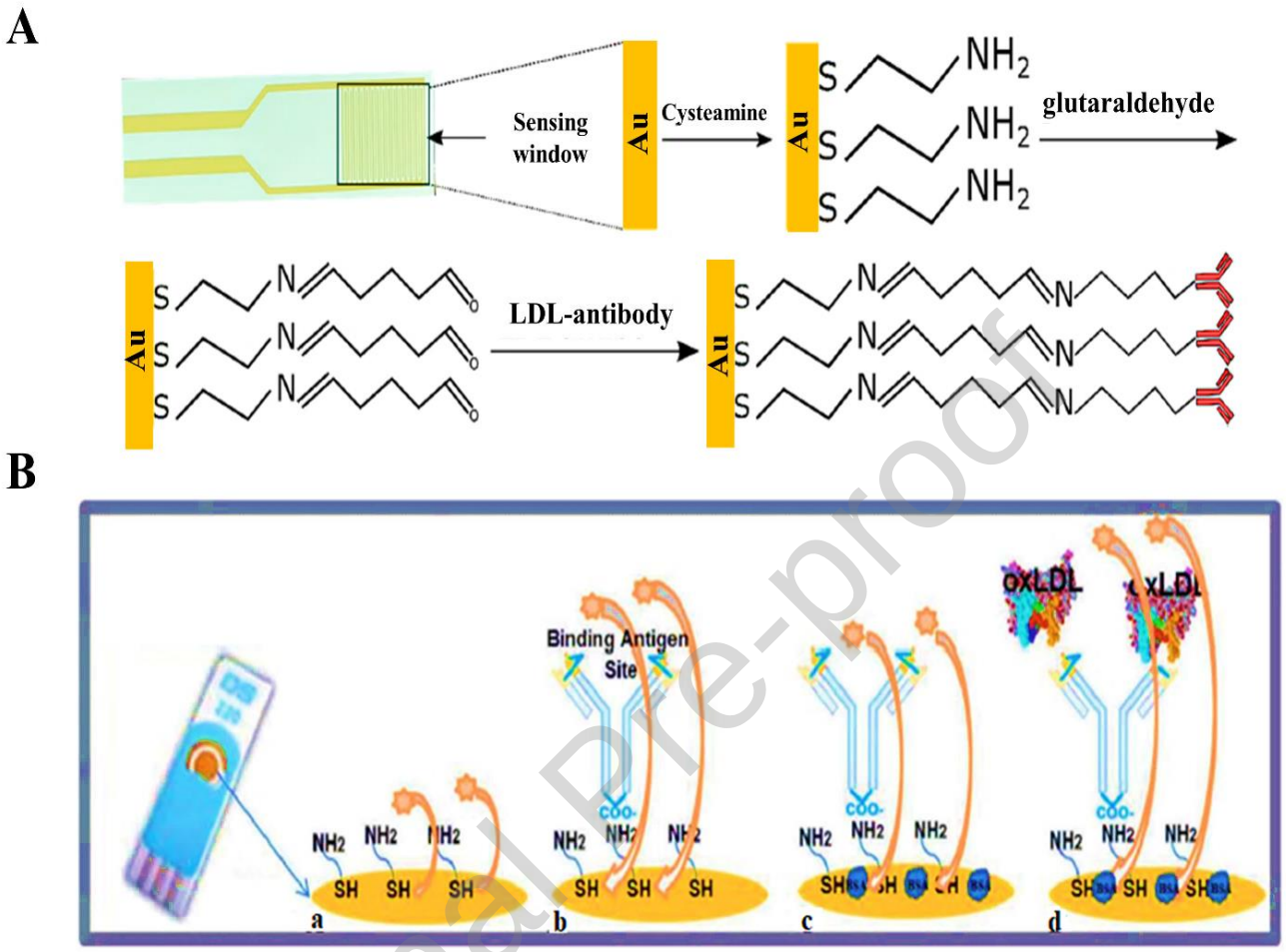

Figure 7. (A) Interdigitated gold electrodes biofunctionalizing with LDL-antibodies taken from [79]; **(B)** Immunosensor platform:(a) an Au electrode containing self-assembled Cysts; (b) antibody stabilization; (c) BSA bonding; and (d) a modified electrode incubated with ox-LDL, taken from [26] (**Abbreviations**: Au, symbol for gold; LDL, low-density lipoprotein; Ox-LDL, oxidized low-density lipoprotein).

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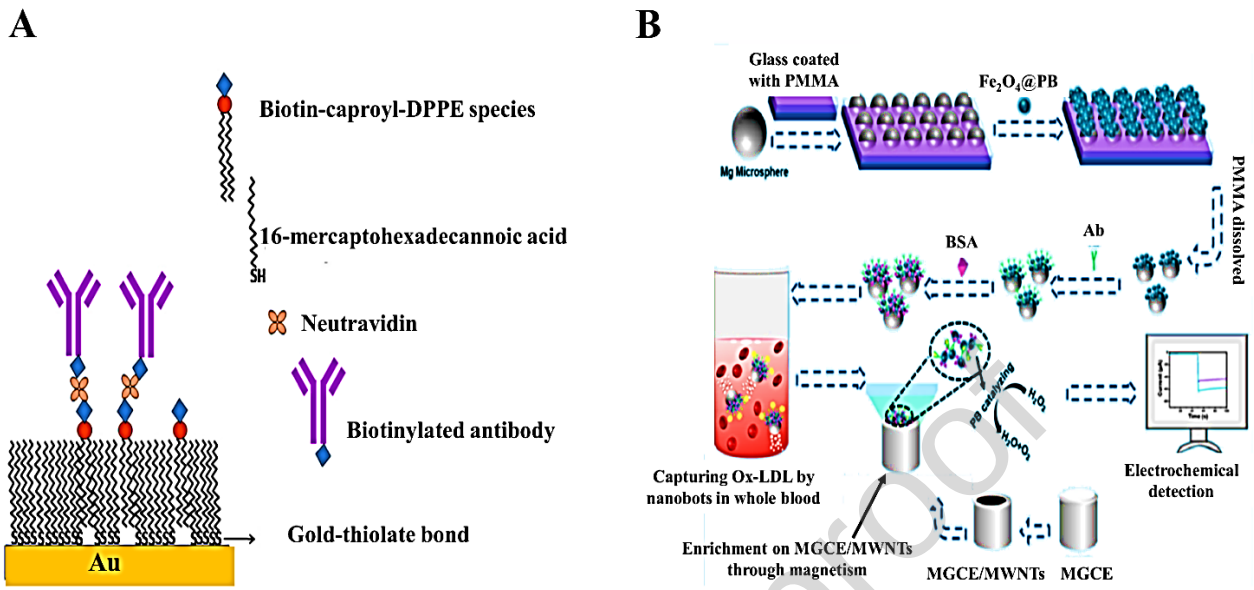

Figure 8. (A) mSAM-based immunosensor for detection of sLOX-1 and cTnI, taken from [80]; **(B)** Design and application of an electrochemical sensor for detecting ox-LDL, taken from [81] (**Abbreviations**: Ab, antibody; Au, symbol for gold; BSA, bovine serum albumin; MGCE, magnetic glassy carbon electrode; MWNT, multi-walled carbon nanotubes; Ox-LDL, oxidized low-density lipoprotein; PB, prussian blue; PMMA, polymethyl methacrylate).

Table.1. Comparison between different types of LDL/ox-LDL biosensors.

Biosensor Classification	**Detection**	**Platform**	**Method**	**Linear range/ Limit of Detection (LOD)**	**References**
QCM	LDL	MIP	QCM	Linear range:200-400 $mg.dl^{-1}$	[53]
		MIP	QCM	Linear range: 4-400 $mg.dL^{-1}$ LOD: 4 $mg.dL^{-1}$	[52]
		MIP	QCM	linear range: 2.5 -100 $mg.dL^{-1}$ LOD: 1.5 $mg.dL^{-1}$	[57]

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SPR	ox-LDL	MIP	QCM	Linear range: 86-5600 $\mu g.dL^{-1}$ LOD: 86 $\mu g.dL^{-1}$	[58]
	LDL	Au/ATP/AVI/B-HEP	SPR	Linear range: 20-100 $mg.dL^{-1}$	[66]
		Au/4-ATP/AAB	SPR	Linear range:0-190 $mg.dL^{-1}$	[67]
Electrochemical		GC/Au NPs/AgCl@PANI/apoB-100	Electrochemical biosensor/ EIS	Linear range : 0-33.5 $pg.mL^{-1}$ LOD: 0.34 $pg.mL^{-1}$	[74]
		Au/Cys/AuNPs/Cds/ apoB-100	Electrochemiluminescence biosensor	Linear range: 0.025- 16 $ng.mL^{-1}$ LOD:0.006 $ng.mL^{-1}$	[75]
		ITO/NiO/AAB	Electrochemical immunosensor/ EIS, DPV, and CV	Linear range: 0.018-0.5 µM LOD:0.015 µM	[76]
		ITO/NiO/AAB	Electrochemical immunosensor/ EIS, DPV, and CV	Free cholesterol: Linear range:0.12-10.23 mM Total cholesterol: Linear	[77]

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			range: 1-12 mM	
	GO-PEI/CS/AAB	Electrochemical immunosenor/ capacitance	Linear range: 10-120 $mg.dL^{-1}$ LOD: 10 $mg.dL^{-1}$	[78]
	Au electrode	Electrochemical biosensor/ EIS	Linear range:500 pg. mL^{-1}-500 ng. mL^{-1}	[79]
			LOD :70 nF/log(ng. mL-1)	
	Au/CysCds/AAB	SPR, Electrochemical immunoensor / EIS	Linear range:0-190 $mg.dL^{-1}$ LOD : 16.03 $mg.dL^{-1}$	[82]
	PVF/Au NPs-Mab-BSA	Electrochemical immunosensor/ EIS	Linear range: 3.5– 175 $\mu g.mL^{-1}$	[83]
	Au/4-ATP/AbM/BSA/Au/ Apt	Electrochemical immunosensor/ SWV	Linear range: 0.01- 1.0 $ng.mL^{-1}$ LOD : 0.31 $ng.mL^{-1}$	[84]
	Au/Apt	Electrochemical Aptasensor/ SWV	Linear range: 0.01- 1.0 $ng.mL^{-1}$ LOD:0.25 $ng.mL^{-1}$	[84]
	Fe_3O_4@SiO_2/LDL/MOF-Fc@Apt	Electrochemical Aptasensor/SWV	Linear range:1 ng/mL-100 $\mu g.mL^{-1}$	[85]

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	Au/ β-CD	Electrochemical biosensor/ EIS	LOD: 0.3 $ng.mL^{-1}$ Linear range : 2.5-20 $\mu g.mL^{-1}$	[86]
	ITO/rGO-NH2/AAB/BSA	Electrochemical immunoensor/ EIS	Linear range : 50-1200 $mg.mL^{-1}$ LOD : 0.5 $mg.L^{-1}$	[87]
	ITO/CNT-CH/AAB	Electrochemical immunoensor/ EIS	Linear range : 0-120 $mg.dL^{-1}$ LOD : 12.5 $mg.dL^{-1}$	[88]
	Au/Cyst/ mAbs/BSA	Electrochemical immunosensor/SWV	Linear range : 0.5-18 $g.mL^{-1}$ LOD : 0.22 $mg.mL^{-1}$	[26]
ox-LDL	Au electrode	Electrochemical immunosensors/ EIS	linear range:10^{-13}- 10^{-7} M LOD: 10^{-13} M	[80]
	Mg–Fe_3O_4@PB@ Ab @BSA	Electrochemical sensor/ chronoamperometry	Linear range: 0.01-10 $\mu g.mL^{-1}$ LOD : 0.98×10^{-3} $\mu g.mL^{-1}$	[81]

Abbreviations : AAB, anti apolipoprotein B; Ab, antibody; apoB-100; apolipoprotein B-100; ATP, 4-amino thiophenol; AVI, Biotinylated heparin and avidin; B-HEP, biotinylated heparin; BSA, bovine serum albumin; CV, cyclic voltammetry; Cyst, cysteamine; DPV, differential pulse voltammetry; EIS, electrochemical impedance spectroscopy; GO, graphene oxide; LDL, low-

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density lipoprotein; LOD, limit of detection; mAb, monoclonal antibody; MIP, molecularly imprinted polymer; NiO, nickel oxide; ox-LDL, oxidized low-density lipoprotein; PEI, polyethyleneimine; PB, Prussian blue; SPR, surface plasmon resonance; SWV, square wave voltammetry; QCM, quartz crystal microbalance.

Declaration of Competing Interest

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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