Anal. Bioanal. Electrochem., Vol. 16, No. 8, 2024, 786-808
https://www.doi.org/10.22034/abec.2024.715435

Analytical & Bioanalytical Electrochemistry

2024 by CEE
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Review

A Review: Nanomaterial-Based Bio-Interfaces for Cardiac Biomarker Myoglobin Electrochemical Biosensing

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Received: 2 June 2024 / Received in revised form: 18 August 2024 / Accepted: 20 August 2024 / Published online: 31 August 2024

Abstract- Detecting cardiovascular disease [CVD] at its earliest stage is vital to effectively manage patients and improve their chances of full recuperation. Currently, identifying CVD during its initial development has become a significant concern for global health. The remarkable attributes of nanoscale electrochemical biosensors make them highly appropriate for detecting CVD biomarkers in bodily fluids even in extremely low quantities. The unique advantages of biosensors that use electrochemistry, which include increased sensitivity and stability through electrode surface nanostructuring, as well as bioreceptors with heightened affinity and selectivity, have prompted exploration of new electrochemical biosensing techniques as viable alternatives to traditional methods for clinical diagnosis of cardiovascular disease. In this review, current information on electrochemical biosensing techniques and signal amplification tactics employing nanomaterials for detecting myoglobin, a biomarker for cardiovascular disease, is presented. Future research efforts must prioritize the discovery of new, specific biomarkers for cardiovascular disease. Additionally, it is crucial to develop real-time analysis techniques for these biomarkers, as well as point-of-care validation assays that have excellent selectivity and sensitivity.

Keywords- Cardiovascular biomarker; Electrochemical biosensor; Nanomaterials; Biointerface; Myoglobin

1. INTRODUCTION

Cardiovascular diseases account for 30% of adult deaths in the 30−70 year age group, which is greater than the combined mortality rate from all types of cancer [1,2]. The ability to diagnose cardiac pathology is therefore of utmost concern to clinicians. Acute myocardial infarction (AMI) is a common cardiovascular emergency [3]. With the improvement of living standards, the number of patients with cardiovascular disease (CVD) has also increased rapidly. Epidemiological surveys show that AMI has become the main cause of death and disability in Western countries. The World Health Organization (WHO) predicts that by 2020, Acute AMI will surpass all other causes of death and become the leading cause of mortality worldwide. [4]. AMI is a severe cardiovascular condition resulting from the acute and prolonged reduction of blood flow and oxygen supply to the coronary artery. The disease exhibits a rapid progression, resulting in significant rates of morbidity and mortality [5]. A number of cardiac biomarkers have attracted attention because they can act as markers for cardiovascular events [6]. Serum cardiac markers, particularly myoglobin, have a crucial role in clinical diagnosis as they serve as indicators of myocardial damage when their levels are elevated [7].

2. MYOGLOBIN BIOMARKER

With a molecular weight of 16.8 kDa, myoglobin is a small protein that is mainly used to bind, store, and transport oxygen. An investigation conducted on mice lacking myoglobin indicated that myoglobin has a significant function in the transportation of oxygen to mitochondria [8]. All mammals have it in their skeletal and cardiac muscles. It is known that the normal range of myoglobin concentration in blood is 6–85 ng/mL [9]. Following muscle cell injury, which occurs during AMI, myoglobin is released into the bloodstream, reaching concentrations of 70-200 ng/mL. This level serves as the diagnostic threshold for myoglobin [10]. This protein's low molecular weight and quick release into the bloodstream after a heart attack make it a popular cardiac biomarker for AMI diagnosis [11,12]. The first and oldest biomarker for AMI is myoglobin. Because of its 5.2-minute elimination half-life, its blood concentration increases within an hour of the heart attack, peaks between 4 and 12 hours later, and then recovers to normal within 24 hours [9,13]. Using immunohistochemistry, Examined Horike and coworker the degree of myoglobin staining in end myocardial biopsy samples from patients who had myocarditis. They discovered a relationship between myoglobin staining and both the severity of the disease and the length of acute myocarditis [14]. Myoglobin is present in skeletal muscles, however it is not exclusively associated with cardiac conditions. On the other hand, its negative predictive value is high [15]. However, myocarditis typically presents as a systemic disease that can potentially affect the skeletal muscle [16,17], it’s possible that

more severe episodes of acute myocarditis are connected to the skeletal muscle's release of myoglobin, which could result in higher blood levels of this marker. Therefore, in order to identify AMI, it is essential to have a sensitive and accurate way to find myoglobin in the bloodstream early on [12,18-20]. Numerous techniques have been documented in scientific literature for the identification of myoglobin, such as surface plasmon resonance [21,22], liquid chromatography [23,24], immunoenzymatically assay [25,26], mass spectrometry [27,28], and fluorescence [29,30]. However, even with their improved selectivity for myoglobin quantification, these techniques are extremely expensive and require a great deal of sample preparation and processing. These restrictions could make it more difficult to diagnose AMI patients since timely and reliable clinical judgments are essential to the illness's management [12,31].

Myoglobin levels must be measured accurately in order to analyze and detect AMI early on and lessen the severity and progression of heart attacks and other cardiovascular illnesses [32,33]. Consequently, myoglobin is regarded as one of the earliest indicators for detecting and confirming AMI [33]. Myoglobin can be found in saliva, which can be utilized to diagnose AMI [34-36], It serves as a marker for muscular and renal disorders in urine, but not for cardiac disorders [37-39].

Biosensors are advantageous instruments for medical diagnosis due to their compact dimensions, rapid response time, and economical nature [40-42]. An analytical tool called a biosensor is made to find particular chemical compounds in samples [43]. The two most beneficial types of biosensors for medical diagnostics are optical biosensors and electrochemical biosensors [44,45]. A biosensor that converts chemical data into an electrical signal is called an electrochemical sensor, which can be utilized for analysis. Electrochemical biosensors exhibit a strong attraction for detecting medical diagnostic analytes. The mentioned items include cancer antigens, markers for heart attacks, medications, hormones, allergies, and antibodies [46].

3. METHODS OF ELECTROCHEMICAL DETECTION

Because electrochemical biosensors transform biological events into electrical signals, they provide a compelling way to analyze the composition of biological samples [47-50]. The reaction under research generates a detectable current that called amperometric, a charge accumulation or measurable potential that is potentiometric, or measurable changes in the conductive characteristics of the material between the electrodes that called conductometric. Alternative electrochemical detection techniques have been studied extensively. These include field-effect, which measures current resulting from a potentiometric effect at a gate electrode by using transistor technology, and impedimetric, which measures impedance (reactance and resistance). Voltammetry is one of the most widely used methods for electrochemical biosensing of natural substances. With this method, an electric potential is adjusted to yield

information about an analyte, and the resulting electric current is then measured [51-55]. For this reason, it's called an amperometric method. Due to the numerous methods available for altering a potential, there exist various types of voltammetry, such as differential staircase, linear sweep, normal pulse, differential pulse, reverse pulse, and others. A direct correlation exists between the concentration of electroactive chemicals and the greatest magnitude of electric current detected across a given range of electric potential [56,57]. In electrochemical processes, protein analytes are not naturally able to function as redox partners. Consequently, in order to facilitate the analyte's electrochemical reaction at the working electrode, these devices predominantly use facilitated electrochemistry. This approach is known as indirect sensing systems. Despite this limitation, amperometric instruments are said to retain greater sensitivity than potentiometric instruments. The remarkable capacity of the Electrochemical Impedance Spectroscopy (EIS) approach to quantify molecular interactions of electrochemically inert substances present on the electrode surface is well known. EIS is a straightforward and efficient technique used to detect and quantify organic compounds, including protein biomarkers. This method is also useful for tracking changes in electrical characteristics brought about by biorecognition events on electrodes with altered surfaces. However, in spite of the advantages indicated above, EIS measurements are carried out using iron ferrocyanides as a probe. Additionally, this method cannot distinguish between nonspecific and specific binding, both of which raise impedance. In fact, EIS is extremely sensitive to electrode contamination in any form. The salient features of electrochemical methods indicate their appropriateness for real-world uses and biosensing studies. Electrochemical methods have a wide range of applications, and their improvements in adjacent domains make them a highly active area of research [54, 58-64].

4. NANOPARTICLES-BASED SENSOR FOR MYOGLOBIN DETECTION

An emerging class of sensing devices called nanostructure-based electrochemical sensors is used for illness diagnosis, environmental monitoring, and food analysis [65-70]. Different nanomaterials such as inorganic nanoparticles [71-78], quantum dot [79], and carbons based nanostructures [80, 81] can be employed in a variety of applications because of their superior optical and electrical qualities. High surface-to-volume ratio nanoparticles has unique and remarkable characteristics that frequently differ from bulk material properties [82-87]. The exceptional and distinctive characteristics of nanoparticles have led to their widespread utilization in diverse domains including energy, photocatalysis, biotechnology, materials science, and virtually all other practical and technological fields [88-90]. In recent years, there have been various methods developed to harness the creative potential of nanostructures and apply them in various scientific fields, including sol-gel [91-95], co-precipitation [96-100] solid-state [101], and sonochemistry [102-106] have been developed for the synthesis of nanostructures. Scientists have attempted to synthesize various nanostructures with different

sizes and morphologies by manipulating the conditions that impact the properties of these nanostructures. This is due to the fact that a nanostructure's size and shape are critical factors in determining how well it performs in various applications [107,108]. By varying the reactant content, temperature, and addition of surfactants, the co-precipitation approach allows for the manipulation of the shape and size of the nanostructures that are created [109-112].

Today, highly precise and innovative biosensors have been fabricated for the purpose of myoglobin determination. This review presents a comprehensive overview of different types of electrochemical biosensors that utilize various nanomaterials for the purpose of designing biosensors capable of detecting myoglobin (Figure 1). In order to achieve this objective, an extensive examination of various sources in the field was conducted. The examination encompassed a wide range of topics, such as metal nanoparticles, semiconductor nanoparticles, mesoporous silica nanoparticles, semiconductor quantum dots, molybdenum disulfide nanosheets, carbon nanomaterials, and polymeric nanomaterials.

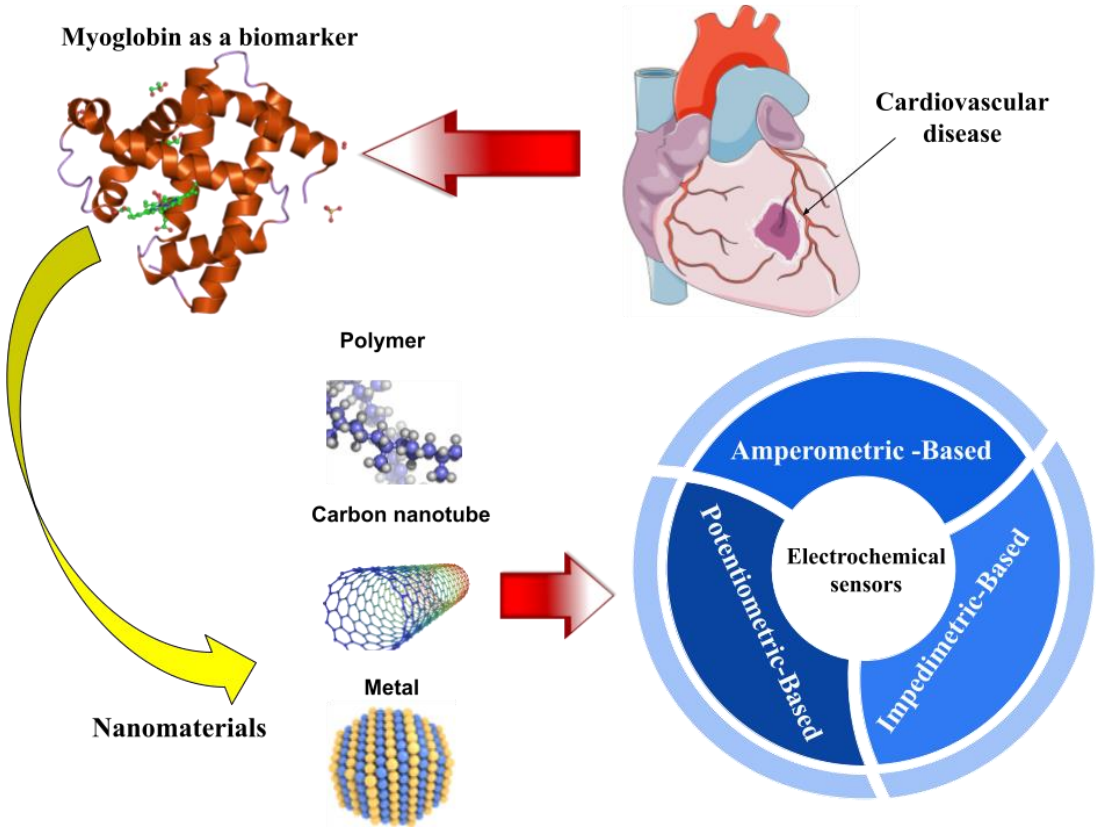

Figure. 1 Schematic representation of nanomaterial-based bio-interfaces for cardiac biomarker Myoglobin electrochemical biosensing.

4.1. Metal nanoparticles-based sensor for myoglobin detection

Gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) are widely researched nanomaterials [113,114]. Noble metal nanoparticles possess distinctive physicochemical

characteristics, including biocompatibility, high conductivity, ease of functionalization through straightforward chemical processes, high surface-to-volume ratio, and enhanced catalytic properties. These properties make them suitable as immobilising platforms to facilitate electron transfer [115-118]. Important parameters in biosensors that impact the bioactivity of the biological and natural recognition elements stabilized on the sensor surface are their amount and stability. In most cases, when biomolecules are adsorbed onto the surfaces of bulk materials without any additional coating, they often undergo denaturation and lose their bioactivity. However, AuNPs are highly suitable for immobilising biomolecules. Because AuNPs have a high surface free energy and are biocompatible, biomolecules can adhere to their surfaces and maintain their stability and bioactivity [119]. AuNPs, in comparison to flat Au surfaces, possess a significantly greater surface area, enabling the loading of a larger quantity of consequently and, bioreceptors, exhibiting higher potential sensitivity. Noble metal nanoparticles can serve as electrochemical signal tags, in addition to immobilising the bioreceptors and amplifying the signal. This purpose has involved the utilization of gold and silver nanoparticles. The use of the deposited AuNPs' electrochemical stripping signal for biosensing different analytes has been reported in a number of research [120,121]. This strategy involves selectively depositing silver on recognition probes to further enhance the electrochemical signal. Electrochemical oxidation of AuNPs in HCl can result in electroactive tetrachloroaurate, which can then be reduced to yield a detectable signal. Examining the conductivity of noble metal nanoparticles and using them as conductive markers to act as a "switch" in an electrical circuit is an alternate electrical technique for biosensing. Following this idea, other groups developed electrode-gap-based DNA biosensors. These biosensors relied on the interaction between noble metal nanoparticles (NPs) that were modified with oligonucleotides. The binding of these modified NPs to the target DNA caused changes in conductivity, indicating the occurrence of binding events between the target DNA and the probe [121].

Scientists created a composite material called AuNPs@rGO, which is made up of AuNPs and reduced graphene oxide (rGO), in order to create an immunosensor for myoglobin. By immobilizing an antibody specific to cardiac myoglobin on the electrode surface, the immunosensor was produced. Using differential pulse voltammetry (DPV) to monitor the immunosensing response, a decreased peak was observed at about 0.5 V *vs.* Ag/AgCl. The reduction peak appears as a result of the iron component in myoglobin's heme group being reduced [detection limit of 0.67 $ng.mL^{-1}$]. For cardiac myoglobin, the immunosensor showed a dynamic linearity range of 1 $ng.mL^{-1}$ to 1400 $ng.mL^{-1}$. The obtained result exhibited an approximately eightfold improvement in detection limit when compared to ELISA tests utilizing the same antibodies, which had a detection limit of 4 ng/mL^{-1} [12].

AuNPs were used in the alternative investigation to modify the electrode surface, antibodies, and a synthetic substance called didodecyl dimethyl ammonium bromide (DDAB) to facilitate the detection of human cardiac myoglobin. As long as myoglobin was present in

the samples, the electrochemical reduction of myoglobin heme was seen. EIS was also used to study the process of myoglobin binding to antibodies that were adsorbed on the electrode surface. The analysis of electroanalytical properties indicates that the developed approach exhibits a great level of sensitivity and specificity. The biosensor exhibits a broad operational range encompassing measured values ranging from 17.8 to 1780 ng/mL^{-1} (equivalent to 1 to 100 nM), and possesses a low limit of detection. The utilization of stripping voltammetry enabled the identification of signals [122].

Zhang and colleagues developed a new electrochemical aptasensor based on meso-tetra (4-carboxyphenyl) porphyrin-functionalized graphene-conjugated gold nanoparticles (TCPP–Gr/AuNPs) for the selective and sensitive detection of myoglobin. This aptasensor is capable of detecting myoglobin with high sensitivity and accuracy. Because of its significant specific surface area, outstanding electrical conductivity, and superior mechanical qualities, TCPP-Gr/AuNPs is a useful sensor for myoglobin detection. During the meantime, it provides a practical framework for immobilising myoglobin-binding aptamer. The electrochemical aptasensor has a linear range of 2.0 ×10^{-11} M to 7.7 ×10^{-7} M and a detection limit of 6.7×10 $^{-12}$ M [S/N = 3]. The technique is advantageous due to its low cost, high specificity, and great sensitivity [123].

Zhu and colleagues developed a highly sensitive and efficient self-powered aptasensing system using an enzyme biofuel cell (EBFC) to detect myoglobin. The self-powered electrochemical aptasensor consisted of a CNT-AuNP-aptamer (biocathode) containing carbon nanotube-Au nanostructures aptamer and a CNT-AuNP-GOx (bioanode) containing carbon nanotube-Au nanostructures-glucose oxidase. The CNTs with Au nanostructures were selected as the electrode nanomaterial for the self-powered aptasensor due to their exceptional biocompatibility, chemical stability, and high electrical conductivity. The biocathode was constructed by functionalizing CNTs with Au nanostructures through the formation of an Au-S bond. When the myoglobin was present, the aptamer was able to specifically recognize it on the biocathode, and the substantial steric hindrance effectively prevented the redox probe $[Fe(CN)_6]^{3-}$ from being electronically transported to the biocathode, leading to a sharp drop in the open-circuit voltage (EOCV). The sensor exhibits exceptional selectivity, repeatability, and stability, with a detection limit as low as 0.011 ng mL^{-1}. This self-powered aptasensor exhibits great promise due to its exceptional sensitivity in detecting myoglobin across a wide concentration range of 0.1-1 104 ng mL^{-1}. The recovery results (99.27-101.34%) and relative standard deviation (RSD) tests demonstrate that the aptasensor is capable of accurately measuring Mb in complex biological matrices. This study provides a sophisticated approach for highly sensitive detection of myoglobin, which has the potential to be used as a model for a portable and on-site biomedical sensor. Monitoring myoglobin in real-time is crucial for clinical diagnosis [124].

In a separate investigation, the identification of cardiac myoglobin relied on the direct transfer of electrons between the electrode surface and Fe(III)-heme that had been altered with metal nanostructures that were stabilized by antibodies and didodecyldimethylammonium bromide. Au, Ag, and Cu nanostructures were examined for the Fe^{3+}/Fe^{2+} electrode process. Blood plasma samples from both healthy donors and individuals with a diagnosis of myocardial infarction were used in the experiments. The suggested method does not necessitate the labeling of secondary antibodies. The immunoassay sensor exhibits a detection threshold of 5 nanograms per milliliter and a wide range of concentrations. This entire process takes twenty minutes to complete and can be used to diagnose AMI [125].

Shorie M. and colleagues developed a nanohybrid-mediated surface-enhanced Raman spectroscopy (SERS) substrate by directly creating and assembling Au nanostructures on exfoliated nanosheets of tungsten disulfide [WS_2] to form plasmonic hotspots. The functionalization of the nanohybrid surface with particular aptamers led to a notable improvement in the surface's selectivity for the cardiac marker myoglobin. The developed aptasensor exhibited significant signal amplification when analysed using SERS with a 532 nm laser, enabling the quantification of myoglobin within the concentration range of 10 fg. mL^{-1} to 0.1 µg. mL^{-1}. The paper presents a technique for enhancing multiple SERS signals by leveraging the unique electromagnetic and chemical properties of both Au nanostructures and tungsten disulfide [126].

The study employed a new electrochemical aptasensor that utilized a Y-shaped structure consisting of a gold electrode, an exonuclease I [Exo I], and dual-aptamer (DApt)-complementary strand of aptamer (CS) conjugate to achieve specific detection and highly sensitive of myoglobin. The aptasensor that was developed possesses the advantageous characteristics of Au, such as a large surface area and excellent electrochemical conductivity. Additionally, the Y-shaped DApt-CS conjugate serves as a gate and barrier, preventing the redox probe from reaching the electrode surface. The aptamer exhibits high specificity and sensitivity towards its target, while the enzyme Exo I selectively degrades the 3' end of single-stranded DNA (ssDNA). The Y-shaped structure persists in the absence of myoglobin. As a result, a subpar electrochemical signal is observed. The addition of the target causes the dissociation of the DApt from the CS and its binding to myoglobin, resulting in the disassembly of the Y-shaped structure. Upon the addition of Exo I, a pronounced electrochemical signal becomes evident. The manufactured aptasensor demonstrated exceptional selectivity for myoglobin, with a limit of detection as low as 27 pM. Furthermore, the developed aptasensor was effectively employed for the detection of myoglobin in human serum [127].

4.2. Carbon nanomaterials-based sensor for myoglobin detection

Carbon nanostructures have been widely used to create electrochemical biosensing platforms that can detect biomarkers with high sensitivity, even when they are present in very

small quantities in various clinical samples. The integration of carbon nanostructures into sensor platforms is currently an expanding field in sensor and biosensor design. Carbon nanostructures present appealing prospects for enhancing biosensor capabilities, elevated specific surface area and primarily owing to their exceptional mechanical and electrical characteristics. Graphene, Graphene oxide (GO),carbon nanotubes (CNTs) and its derivatives, graphene quantum dots (GQDs), carbon nanohorns (CNHs), and carbon quantum dots (CQDs) are the most commonly utilized carbon nanostructures in bioassay applications [128].

GO is regarded as a promising carbon nanostructures in the list of carbon nanomaterials due to its exceptional characteristics such as superior mechanical strength, high surface area, biocompatibility, and low density [129]. The GO sheets have a 2D structure with a basal plane that is adorned with different functional groups such as epoxy, carboxyl, and hydroxyl. These allow for a variety of surface-functionalization reactions, which can be applied to create graphene oxid-based nanobiosensors. Although GO lacks the charge carrier transport commonly found in nearly flawless graphene, its processing ease and adaptability make up for it. With relation to carbon nanomaterials, GO is considered a promising structure because of its remarkable mechanical strength, high surface area, biocompatibility, and low density [129]. The GO sheets have a 2D structure comprising a basal plane adorned with epoxy, carboxyl, and hydroxyl functional groups. When creating sensors and biosensors based on GO, these functional groups create a diversity of surface-functionalization reactions. Conical carbon nanostructures called carbon nanohorns, also called nanocones, are created by stacking sp2 carbon sheets. Carbon nanohorns are composed of individual graphene sheets arranged in a tubular structure. These tubes have diameters ranging from 2 to 5 nanometers and lengths of 40 to 50 nanometers. The ends of the tubes have a conical shape. Nanohorns can be synthesized without the need for a metal catalyst and can be manufactured in large quantities for industrial purposes. Additionally, their distinct conical morphology contributes to their individual behavior. However, a number of issues have hindered their research and development progress, especially during the synthesis phase. A few thousand carbon nanohorns combine to form quasi-spherical aggregates with a diameter of around one hundred nanometer, like a "dahlia flower". This constraint has recently been surpassed by implementing a novel method of isolating these dahlia-like clusters into distinct nanocones [130]. Carbon nanohorns offer a practical and valuable substitute for graphene and carbon nanotubes across various applications.

Carbon quantum dots, since their discovery in 2004, also known as CDs, have been extensively studied as an environmentally friendly alternative to traditional CQDs for the development of sensors. The high biocompatibility and low toxicity of carbon dots, demonstrated in both in-vitro and in-vivo scenarios, make them highly good for sensing applications. They usually have carboxylic acid groups on their surface, which give them

remarkable water solubility and allow for additional modification with other biological materials [131].

GQDs are a carbon nanostructures in class of zero-dimensional (0D) that possess properties originating from both CDs and graphene. They can be thought of as extremely tiny fragments of graphene. Combining the edge effects and quantum confinement of CDs with the graphene structure yields GQDs' distinct optical and electrical features. GQDs, unlike CDs, unequivocally exhibit a graphene structure within the dots, irrespective of the dot size. This characteristic imparts upon them certain distinctive properties associated with graphene [28]. Researchers have developed very sensitive surfaces for electrochemical nanobiosensors by taking advantage of the strong interaction between graphene-based materials and functionalized bioreceptors. These biosensors involve the use of electrodes modified with GQDs and specific bioreceptors. Furthermore, the exceptional electrical conductivity, favorable biocompatibility, and minimal toxicity render GQDs optimal frameworks for electrochemical biosensing assays. Nevertheless, the progress of graphene quantum dots is constrained by their synthetic methodology.

Tabish et al proposed that electrochemical biosensors incorporating GQDs can offer valuable predictive insights into AMI at an initial, reversible, and potentially treatable phase [132]. GQDs have several advantages compared to other nanomaterials used for electrochemical detection of AMI. These advantages include strong interactions between cardiac troponin I (cTnI) and GQDs, low consumption of biomarkers, and the ability to reuse the electrode. Due to the conductive nature of graphene and other special characteristics of GQDs, the improved sensor shows good electrochemical responses. These features include a π–π interactions with the analyte, high specific surface area, size-dependent optical properties, easy electron-transfer mechanisms and electrochemical luminescence emission capability, the interplay between bandgap, photoluminescence, ease of functionalization, and biocompatibility. Additional benefits arise from the existence of functional groups like epoxide, hydroxyl, carboxyl, and carbonyl groups, which improve the ability of GQDs to dissolve and disperse in various solvents and biological substances. This review examines the current understanding of the early detection of AMI using electrochemical sensors based on GQDs. Additionally, it investigates how this sensor technology might enhance AMI patient care [132].

Yoo et al. presented a new method to enhance the sensitivity and electroactive surface area of graphene electrode-based EIS biosensors, without relying on external structuring techniques like patterning or pore formation. To modify the spacing between sheets, a technique was employed to synthesize stacked films using graphene oxide nanosheets that were functionalized with octadecylamine groups. Subsequently, in order to enhance the electroactive surface area and sensitivity, the GO films underwent thermal reduction to generate a textured surface. The sensing capabilities of GO electrodes with different inter-sheet distances were assessed using

EIS measurements to demonstrate the detection of myoglobin as a proof of concept. The deliberate control of interlayer alkylation in stacked GO nanosheets has proven to be an effective method for creating GO electrodes with a significantly increased and/or controlled surface area. This has resulted in a detection limit of 2.37 pM for myoglobin [19].

Researchers have developed an extremely sensitive label-free chemiresistive sensor using single-walled carbon nanotubes (SWNT) to detect myoglobin (Ag-cMb). To immobilize a cardiac myoglobin antibody that targets a specific region, a polymer with carboxyl groups attached to it was deposited onto an electrophoretically aligned SWNT channel using an electrochemical process. The device exhibited a linear response in the SWNT channel, with a change in conductance, over a concentration range of 1.0 to 1000 ng mL^{-1}. The sensitivity was measured at 118% per decade, indicating a strong ability to detect changes in concentration. Additionally, the device demonstrated good specificity, meaning it accurately distinguished between different substances [133].

Wang and his colleagues created a thin layer with specific molecular structures to detect myoglobin using the ionic liquid through electrochemical means. The film deposition on a GCE modified with MWCNT involved the use of an ionic liquid 1-3-((2-aminoethyl)amino)propyl-3-vinylimidazole bromide as the functional monomer, N,N′-methylenebisacrylamide as the crosslinker, myoglobin as the template, and N,N,N′,N′-tetramethylethylenediamine as the initiator and a redox system comprising ammonium persulfate. The effectiveness of the modified electrode in sensing was examined using an electrochemical redox probe (hexacyanoferrate). The outcomes showed that the biosensor has a high degree of sensitivity and great selectivity. The myoglobin content and the observed relationship were linear, ranging from 60.0 nM to 6.0 μM, and the oxidation peak current at a voltage of 0.3 V *vs.* SCE. The detection limit was determined to be 9.7 nM, with a signal-to-noise ratio of 3. The sensor was employed to quantify myoglobin in serum samples that had been artificially enriched, and it exhibited an average recovery rate (for a sample size of 5) of 96.5% [134].

A study investigated the production, analysis, and use of MWCNTs embedded in SU-8 elec trospun nanofibers for highly sensitive detection of cardiac biomarkers such as creatine kinase MB (CK-MB), cardiac troponin I (cTn I), and myoglobin (Myo), using EIS. EIS, Electrochemical Impedance Spectroscopy, a superb method for studying the adsorption kinetics, was used to identify the synthesised nanofibers after they had been functionalized with the biomarker antibodies. The addition of MWCNTs imparts the composite nanofibers with remarkable electrical and transduction properties, while SU-8 enhances their biocompatibility and facilitates functionalization. The linearity range of this nanobiosensor is from 1 to 50 ng/mL. The achieved detection threshold was 0.1 ng/mL [135].

A study was conducted where a composite material consisting of CNT and PtSn nanostructures (PtSnNP/CNTs) was created and utilized for the electrochemical analysis of myoglobin. The electrochemical probe used in this study was hexcyanoferrate, and a

myoglobin-aptamer was fixed onto a GCE. The PtSnNP/CNTs were synthesized through the application of a microwave-assisted ethylene glycol reduction technique. Myoglobin causes the myoglobin-aptamer to fold and change in conformation, which prevents electron transmission and serves as the foundation for detection. The level of myoglobin content directly influences the interaction between myoglobin and the aptamer on the GCE, ultimately determining the amperometric signal for hexacyanoferrate. This signal is most effectively detected at a voltage of 0.2 V compared to the reference electrode Ag/AgCl. This method exhibits selectivity and sensitivity for myoglobin due to the aptamer's highly specific recognition capability, the potent electronic properties of CNT, the organized decoration of carbon nanotubes with PtSn nanostructures, and the superior electron transfers to hexacyanoferrate. The assay exhibits a minimum detectable concentration of 2.2 0.1 pM and demonstrates linear correlations within the ranges of 0.01- Nano molar and 200 Nano molar. The altered glassy carbon electrode was employed to quantify the quantity of myoglobin present in samples of artificially enriched human serum [136].

The researchers, Almehizia et al., developed a biomimetic antibody that targets myoglobin and is attached to the surface of carboxylated multiwalled carbon nanotubes (MWCNT-COOH). This was achieved using the molecular imprint technique. To accomplish this, myoglobin was affixed to carboxylated MWCNT surfaces, and the voids were filled by gently polymerizing acrylamide in N,N-methylenebisacrylamide and ammonium persulphate. The surface modification of the MWCNTs was confirmed through FTIR and SEM measurements. Fluorinated alkyl silane (CF10)-coated hydrophobic paper substrate has been joined to an all-solid-state printed Ag/AgCl reference electrode. The sensors that were suggested exhibited a linear range from 5.0×10^{8} to 1.0×10^{4} M, a detection limit of 28 nM at pH 4, and a potentiometric slope of -57.1±0.3 mV decade. The sensor exhibited high selectivity for myoglobin in comparison to sucrose, galactose, thiamine, alanine, uric acid, glucose, troponine T, and glutamine. The average relative standard deviation of 4.5% indicates a consistent and reliable performance in identifying myoglobin in various fictitious serum samples, with recovery rates ranging from 93.0% to 103.3% [137].The results of these investigations could be utilized as a valuable analytical instrument for the creation of cost-effective, disposable paper-based potentiometric sensing devices for the detection of myoglobin. Clinical analysis enables the mass production of such types of analytical equipment.

4.3. Polymeric nanomaterials-based sensor for myoglobin detection

Recently, there has been a rise in interest from researchers in employing molecularly imprinted polymers [MIPs] to produce electrochemical sensor recognition elements. This has prompted them to develop new formats for these sensors [138,139]. Molecular imprinting is a highly adaptable technique that produces synthetic materials with the ability to identify and, in certain instances, interact with specific biological and chemical substances, such as proteins

[17-19], substituting [or enhancing] inherent receptors. A flexible MIP has the ability to imitate the biological antibody model, but the characteristics of the polymer may result in durability and long-lasting properties that are typically found in a rigid polymer [140,141].

In order to detect prostate-specific antigen (PSA) and myoglobin simultaneously in human blood and urine samples, Karami and colleagues created a new immunosensor based on MIP and a nanostructured biosensing layer. On a gold screen printed electrode (SPE), 3,3′-dithiodipropionic acid di[N-hydroxysuccinimide ester) (DSP) was self-assembled in the first stage. Next, the DSP-SPE and the target proteins were covalently bonded. N, N′-methylene bisacrylamide was used as a crosslinker, acrylamide as a monomer, and PSA and myoglobin as templates to create the imprinted cocktail polymer ((MIP(PSA, myoglobin)-SPE)) at the SPE surface. Following, using decorated magnetite nanoparticles, graphene oxide, multi-walled carbon nanotubes, and a particular PSA antibody (Ab), a nanocomposite (NCP) was created. After that, NCP was incubated with MIP (myoglobin, PSA)-SPE. For PSA and myoglobin, the linear dynamic ranges were determined to be 0.01–100 and 1–20000 ng mL^{-1}, respectively, indicating the limits of detections of 5.4 pg mL^{-1} and 0.83 ng mL^{-1}, respectively. The suggested biosensor's exceptional sensitivity and specificity in detecting both myoglobin and PSA at the same time presents a significant possibility for the next generation of biosensors. For integration with lab-on-a-chip kits to assess a broad panel of biomarkers present at ultralow levels during early stages of illness progression, this dual-analyte specific receptors-based device is particularly desirable [142].

A study was conducted to create thin films of polyethylenimine (PEI)-functionalized rGO(PEI-rGO) for the purpose of developing an extremely sensitive electrochemical aptamer-based sensor [aptasensor] that does not require labeling, specifically for the detection of myoglobin. The researchers utilized a positively charged polymer known as PEI for reduction of GO. This process led to the generation of a highly positive charge on the surface of rGO. Consequently, the negatively charged single-strand DNA aptamers against myoglobin were able to be directly immobilised on the rGO surface through electrostatic interactions, without the need for linkers or coupling chemistries. Using differential pulse voltammetry, which measured the current change brought on by the direct electron transfer between the electrodes and myoglobin proteins (Fe^{3+}/Fe^{2+}), myoglobin was found on myoglobin aptamer-modified electrodes. The detection limits were 2.1 pg mL^{-1} with 10-fold-diluted human serum and 0.97 pg mL^{-1} with phosphate-buffered saline, exhibiting a linear relationship with logarithmic myoglobin concentration. Furthermore, the specificity and repeatability of the aptasensors were examined. This electrochemical aptasensor, which utilizes polymer-modified rGO, showcases the potential for early myoglobin assessment in point-of-care testing applications [143].

The suspension polymerization method was employed by researchers to create myoglobin-imprinted microspheres. The complex functional monomer chosen for this purpose is N-methacryloylamino folic acid-Nd^{3+} (MAFol-Nd^{3+}). Modifications to the pH, temperature, and

myoglobin concentration of the medium were implemented during the optimization trials. The optimal pH for maximum myoglobin binding was determined to be 7.0 in the prepared imprinted microspheres. The binding capacity achieved was the highest at 623 mg. The selectivity studies revealed that the imprinted microspheres displayed a high degree of selectivity for myoglobin, even in the presence of potentially competing proteins such as hemoglobin, lysozyme, and cytochrome c [144].

Table 1. Nanocomposite-based electrochemical sensors for Myoglobin biosensing

Number	**Electrode**	**Linear range**	**Limit of detection**	**Ref.**
1	Nafion/Mb/AuNRs/CILE	0.01- 720 μM	19.25 and 0.446 mM	[146]
2	Ag/Mb/AuNPs/APTES/ITO	10 ng/mL-1 μg/mL	2.7 ng/mL	[147]
3	antibody fragments/4-ATP SAM/Au electrode	0.02 to 1 μg/mL	5.5 ng/mL	[148]
4	Au/RGD/GR-COOH/GCE	0.0001 to 0.2 g L^{-1}	26.3 ng mL^{-1}	[149]
5	GCE/TCPPG/AuNPs/Aptamer	20 pM- 0.77 μM	6.7 pM	[123]
6	SPE/GO-Auric acid/goldNPs/Ab	1- 1400 ng mL^{-1}	~ 0.67 ng mL^{-1}	[12]
7	GCE/APTES/PPy-Au Aptamer	0.0001- 0.15 g L^{-1}	30.9 ng mL^{-1}	[150]
8	ITO/APTES/MPA capped PtNPs/Ab	0.01- 1 μg mL^{-1}	1.7 ng mL^{-1}	[151]
9	Bio-functionalized Pt NPs	0.01 μg/mL-1 μg/mL	4 ng/mL	[151]
10	SCE/ Ag-DDAB, Au-DDAB, Cu-DDAB/Ab	10- 400 ng mL^{-1}	5 ng mL^{-1}	[125]
11	TiO_2 nanotubes	----	50 nM	[152]
12	Fe_3O_4@SiO_2 microsphere/carbon ionic liquid electrode	0.2- 11 mM	0.18 mM	[153]
13	Nafion/Mb-SA-TiO2/CILE	5.3- 114.2 mmol/L	0.152 mmol/L	[154]
14	Nafion/Mb/Co/CILE	0.4- 12.0 mmol/L	0.2 mmol/L	[155]
15	Mb/ZrPNS/GCE	0.1- 2.2 mmol/L	0.025 mmol/L	[156]
16	Nafion/Mb/NiO/GR/CILE	0.69- 30.0 mmol/L	0.23 mmol/L	[157]
17	Mb-HSG-SN-CNTs/GCE	0.002- 1.2 mmol/L	0.0036 mmol/L	[158]
18	Nafion/Mb-SA-Fe3O4-GR/CILE	1.4- 119.4 mmol/L	0.174 mmol/L	[159]
19	Nafion/Mb-Co3O4-Au/IL-CPE	0.04- 0.26 mmol/L	0.01 mmol/L	[160]
20	SA-Mb-IL-Fe2O3/CILE	-	1.3 mmol/L	[161]
21	Mb/AgNPs-APS-CPE	0.03- 0.15 mmol/L	0.0058 mmol/L	[162]

Farahani and colleagues employed the reversible addition-fragmentation chain transfer polymerisation (RAFT) technique to conduct a controlled radical polymerisation process. This method resulted in the synthesis of two distinct types of amphiphilic copolymers, namely poly(styrene)-block-poly(acrylic acid). The findings indicate that PS596-b-PAA61 exhibits lower loading and releasing capacity for myoglobin compared to PS61-b-PAA596. This study effectively created an aptasensor for the detection of low concentrations of myoglobin by utilizing the synthesized methylene blue-loaded polymersome. The aptasensor that was suggested exhibited an exceptionally low detection limit of 0.73 aM and a wide linear range spanning from 1.0 aM to 1.0 μM. The utilization of this amplification technique yielded successful results in the determination of myoglobin in real samples [145]. Table 1 lists the reports on the detection of myoglobin by Nanocomposite based electrochemical.

5. CONCLUSION

This review presents a short overview of important successes in the field of electrochemical nanobiosensors for the detection of Myoglobin. These biosensors are used to detect myoglobin, a biomarker for cardiovascular health, with great accuracy and sensitivity. The review also focuses on the use of nanomaterials in this context. CVD is widely recognized as a significant threat to global public health. There has been a growing demand for a biosensing device that can quickly and economically detect CVD in recent years. The commercial implementation of point-of-care nanobiosensors for the simultaneous detection of cardiovascular biomarkers remains a significant challenge in the prompt diagnosis of cardiovascular diseases. This technology aims to eliminate the requirement for expensive and time-consuming laboratory analyses that are currently limited to hospital settings. Furthermore, the improvement of sensitivity and specificity poses an additional obstacle. Currently, nanomaterials are being widely used to detect biomarkers through electrochemical biosensing techniques, showing a wide range of potential applications. An important advantage of using nanomaterials is the significant increase in the surface-to-volume ratio, which improves the concentration of bioreceptors and can potentially result in increased sensitivity. The use of nanomaterials to improve the sensitivity of biosensing devices for point-of-care applications and early diagnosis has attracted considerable interest .

Declarations of interest

The authors declare no conflict of interest in this reported work.

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