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Review—Electrochemical Biosensors for Alpha-Fetoprotein Detection: Recent Advances and Future Perspectives

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Given the multitude of global health concerns, it is crucial to promptly and precisely identify biomarkers like alpha-fetoprotein (AFP) in order to facilitate the early identification and treatment of diverse illnesses, with a special emphasis on cancer. Conventional detection techniques often exhibit limitations in terms of intricacy, temporal requirements, and ease of use, underscoring the pressing want for inventive resolutions. The use of electrochemical biosensors has shown great potential in the field of AFP detection, because they provide efficient, highly responsive, and economically viable detection capabilities. This study examines current advancements in electrochemical biosensors specifically designed for the detection of alpha-fetoprotein (AFP), with a focus on the incorporation of state-of-the-art materials, sophisticated manufacturing methods, and novel biorecognition approaches. This study seeks to meet the urgent need for dependable and easily available diagnostic tools in the worldwide battle against cancer and other illnesses by offering a thorough examination of the current advancements in electrochemical biosensors. By making progress in AFP detection technologies, our goal is to have a positive impact on healthcare outcomes, improve illness management, and eventually reduce the global burden of disease.

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Electrochemical biosensors are sensors based on electrochemical interactions among the analytic (the compound that we need to measure) and the electrodes.[1–5] These sensors normally contain electrodes that are impressionable to chemical changes that are accompanied by electrical changes. For instance, electrochemical sensors can diagnose diseases, measure glucose levels in blood, detect environmental pollution, and control industrial processes. As maintained by the electrochemical characteristics of these sensors, they have high sensitivity, accuracy, speed, and real-time measurement capabilities. In addition, using these sensors can reduce detection and monitoring costs. Electrochemical biosensors are used in various fields, including medicine, biology, environment, and industry. These sensors can detect and measure the concentration or presence of different analytes. The phrase "biosensor" relates to a robust and cutting-edge analytical tool that uses a biological sensing component and has a range of uses, including drug development, diagnostics, biomedicine, food safety and processing, environmental monitoring, defence, and security.[6] According to Fracchiolla et al.[7] and Turner,[8] the first biosensor developed by Clark and Lyons[6] to quantify glucose in biological samples used the method of the electrochemical detection method of oxygen or hydrogen peroxide utilizing an immobilized glucose oxidase electrode. Since then, remarkable advancements have been made in both the technology and applications of biosensors using cutting-edge strategies, including electrochemistry, nanotechnology, and bioelectronics.[8] This study aims to provide several technological tactics used to produce biosensors to present fundamental information and the current scientific illustration of biosensor technology in light of the astounding biosensor advancements. The performance of biosensors has progressed from traditional electrochemical to optical/visual polymers, silica, glass, and nanomaterials to increase the detection limit, sensitivity, and selectivity, emphasizing the research methods that clarify this evolution. Interestingly, label-based biosensors were significantly influenced by microorganisms and bioluminescence,[9] while label-free biosensors used transistor- or capacitor-based technology and nanomaterials. For quantitative biologists, biosensors serve as a foundation for understanding technical advancement in instrumentation, including sophisticated high-throughput machines and portable qualitative or semiquantitative devices for non-specialists. As shown in Fig. 1 which is related to 2023, the use of biosensors in countries that are professionals in the medical field has increased in order, and can even intensify in the subsequent.[10–14]

The first electrochemical biosensor was discovered in 1962 by Clark and Lyons[6] using a glucometer based on a glucose oxidase-based biosensor. Since glucose biosensors are crucial for ongoing blood glucose monitoring, hospitals, and diagnostic centers frequently use them. However, inhomogeneity or unstable enzyme activity often causes problems for glucose biosensors, necessitating further calibration.[15] Due to these possible limitations, various biomolecules with various electrochemical characteristics were developed,[8,16] opening the door to developing more effective glucose biosensors. Recently, biomaterials such as enzymes, antibodies, or DNA have been used to change the surface of metal and carbon electrodes to create electrochemical biosensors.[17] The output signal of a biosensor is often produced by particular binding or catalytic reactions of biomaterials on the surface of an electrode. Since early identification or monitoring of diseases appears to be crucial, developing electrochemical sensors has become necessary to diagnose diseases.[18] In this regard, using synthetic materials instead of proteins is frequently considered for developing nonenzymatic biosensors. It is interesting to note that different types of biomolecules exhibit variable electrode selectivity and stability, eventually aiding in the creation of novel electrochemical biosensors for various applications. On the basis of their use, many types of electrochemical biosensors were created. As mentioned above, glucose biosensors[18] have rapidly since their creation.

The sensitivity limit of biosensors has improved due to recent developments in biological approaches and technology incorporating fluorescent tags on nanomaterials.[19–23] Aptamers and nucleotides, affibodies, peptide arrays, and polymers with imprinted molecules are instruments for creating novel biosensors instead of conventional techniques. The multifunctional glycoprotein known as alpha-fetoprotein (AFP) is a member of the albuminoid gene family, which also includes albumin, vitamin D binding protein (Gc), alpha-albumin, and AFP-gene-associated protein (ARG). This protein, which is generated by the liver and yolk sac throughout foetal development, is the most prevalent plasma protein. When a newborn is born, their bloodstream typically contains high levels of AFP, which diminish to very low levels by the first year of life. A single-chain glycoprotein called AFP is separated into three domains by intramolecular loops that are configured according to disulfide-bridging regions, resulting in a tertiary V-shaped conformational

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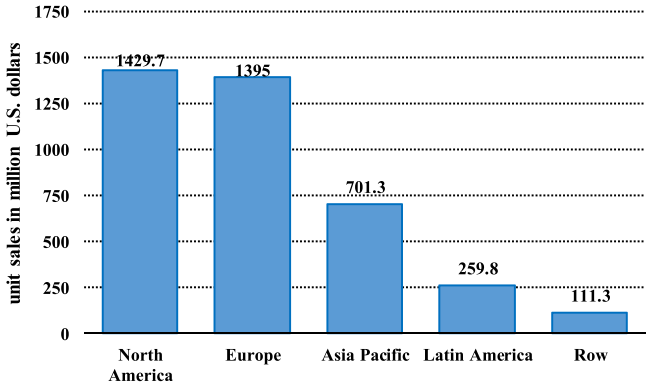

Figure 1. The market distribution of biosensors in different nations (www.statista.com).

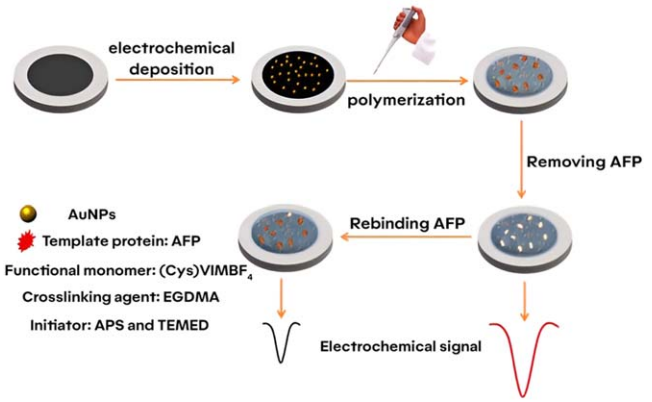

Figure 2. AFP imprinted sensor construction and electrochemical responses.[41]

structure. The AFP molecule has a molecular mass of 67–72 KD, 609 amino acids, 3%–4% glycan content, and an isoelectric pH range of 4.7–5.1. It also contains 19 amino acids as the leader signal sequence. The AFP gene is found on the q arm of chromosome 4. A glycan molecule at asparagine (Asn) makes up human AFP.[24]

Due to the striking performance of electrochemical biosensors in detecting alpha-fetoproteins, several related studies[25–39] have been provided so far.[40] For example, using an ionic liquid as a functional monomer, Wu et al. in 2019.[41] The ionic liquid has several great qualities that may enhance the detecting abilities of the imprinted electrochemical sensor. This was demonstrated by creating an imprinted polymerized ionic liquid film with AFP on a surface of a glassy carbon electrode (GCE) surface using the ionic liquid 1-[3-(N-cystamine)propyl]−3-vinyl imidazolium tetrafluoroborate [(Cys) VIMBF4] as a functional monomer at room temperature as shown in Fig. 2. The imprinted or non-imprinted film electrode was the working electrode in the electrochemical works. A platinum wire electrode was the counter electrode, and a saturated calomel electrode was the reference electrode. $K3[Fe(CN)_6]/K_4[Fe(CN)_6]$ were used as redox probes for the cyclic voltammetric and electrochemical impedance at a concentration of 5.0 mM. Overall, using biomarkers is crucial for detecting and diagnosing diseases such as cancer.[24] Therefore, developing sensitive and precise techniques for measuring alpha-fetoprotein is crucial. Biosensors, which have the benefits of high sensitivity specificity, simple operation, and instrumentation, provide a prospective role among the different approaches that have been created to detect biomarkers. In 2022, Liu et al. examined the development of AFP biosensors with improved sensitivity and selectivity through nanoparticles.[42] Representative examples were also considered to clarify the nanotechnologies used in the early detection of AFP, and significant results were obtained. In a review of 2018, Gui et al.[43] comprehensively listed the most recent developments in MIP-based ECBSs published in recent years concerning the setup, structures, and elements of sensing systems.[44–47] The authors specified the sensing applications for many important biomolecules (proteins, antibiotics, pesticides, neurotransmitters, hormones, etc) and the sensing process and detection capabilities. Logical summaries, current issues, and potential future developments in the area of MIPs-based ECBSs were also reviewed.

Moreover, Li et al.[48] proposed a rapid and accurate approach to detect AFP-L3. The first step was the creation of biotinylated Lens culinaris agglutinin-integrated silver nanoparticles (B-LCA-AgNPs). Due to the unique interaction between AFP-L3 and Lens culinaris agglutinin, AFP-L3 can be detected immediately via the electrochemical signal readout of AgNP, omitting the additional processes often used in clinical practice. A considerable number of AgNPs can also be accumulated at the binding site via avidin-biotin interactions after recognition between B-LCA-AgNPs and AFP-L3 and may increase the signal. Yang et al.[49] proposed a new bidirectionally amplified ratiometric electrochemical aptasensor based on exonuclease-assisted target recycling and bioconjugate probe. The ferrocene-labelled capture probe (Fc-CP) was used to connect the aptasensor to the electrode surface by hybridization with thiolated DNA1. The identification of AFP led to the dissociation of Fc-CP from the DNA1/Fc-CP duplex, which in turn set off the RecJf exonuclease cleavage process and the recycling of AFP. As a consequence, a great number of Fc labels, as well as a lot of exposed DNA1, were released onto the electrode surface in preparation for hybridization with a bioconjugate probe containing a great deal of methylene blue (MB) molecules.

In 2022, Chen et al.[50] used liquid exfoliation and ultrasonic techniques to create black phosphorus nanosheets (BPNS) and Fe^{3+} to add iron. Techniques such as TEM, AFM, Raman spectroscopy, XPS, and CV were used to study the morphology, structure, and electrochemical characteristics of the resulting $Fe3^{+}$/BPNSs. The load of Fe3+ improved electrochemical performance and detection sensitivity. The BPNSs also showed high biocompatibility. AFP immunosensors were developed using BPNS, with a 1.2 pg/ml detection limit and a linear relationship between 0.005 ng/ml and 50 ng/ml. The findings suggested that surface modification with metal ions is a quick and efficient technique to enhance BPNSs' electrochemical characteristics, increasing their potential in the electrochemical sector. The mineral makeup of bones and teeth is comparable to that of the inorganic complex hydroxyapatite (HA) based on calcium phosphate. Biomolecule absorption may be facilitated by the enormous surface area provided by the nanoscale HA and its structure, which includes positive and negative charges on the sidewalls. Good qualities, including HA's biocompatibility, stability, and bioactivity attributes, have sparked widespread research for internal support materials in the creation of electrochemical biosensors. In addition to serving as an immobilization matrix, the HA in the electrochemical biosensor may also be used as a signal probe. Hartati et al. discussed many uses of hydroxyapatite in the last ten years, including its use in aptamers, antibodies, enzyme-based biosensors, and nucleic acids, and obtained striking results. Alizadeh and Salimi discussed current advancements and achievements in bioaffinity electrochemical sensors, focusing on a subclass that includes molecularly imprinted sensors, aptasensors, immunosensors, and genosensors.[51] Recent advances in electrochemical methods and innovative manufacturing procedures have led to the development of biosensors that can detect a variety of target analytes. Despite this remarkable interest, the research can still overcome many gaps and shortcomings.

Due to the remarkable importance of AFP highlighted in the literature,[52–76] the study in this regard is essential. Some studies[77–90] focused on AFP treatment techniques,[91–113] and the rest examined its characteristics. AFP levels decrease significantly after birth and remain low in healthy individuals. It is essential to note that elevated levels of AFP in the blood can indicate the presence of certain cancers in adults. AFP has proven to be a valuable biomarker for

HCC, as elevated levels of AFP are frequently observed in patients with HCC. Measurement of AFP levels in the blood is commonly used as a screening tool for HCC, helping to detect early and improving treatment outcomes. Additional diagnostic tools and imaging techniques are often employed to complement AFP measurements in the clinical assessment of these cancers. The use of AFP as a biomarker has its limitations, one limitation is the possibility of false positive or false negative results to interferences from other biomolecules in blood samples. Another area for improvement is the cost of the equipment required for electrochemical biosensing, which can be expensive and unaffordable for small clinics or hospitals in developing countries.[2] However, AFP remains a valuable tool in the early detection and monitoring of HCC and germ cell tumours. Recent nanotechnology and materials science advances have produced more sensitive and selective electrochemical biosensors for AFP detection with improved performance characteristics. Another aspect of recent research on electrochemical biosensors for AFP detection has been the integration of bioinformatics and artificial intelligence (AI) algorithms for better data analysis and interpretation. This has enabled researchers to understand better the complex interactions between AFP and other biological components in a sample, leading to more accurate and reliable AFP detection. Providing real-time, accurate, and reliable AFP measurements could help clinicians diagnose and monitor HCC better, facilitating earlier intervention and improving patient outcomes.[3]

Within the rapidly growing healthcare industry, there is an urgent need for diagnostic equipment that are quick, precise, and cost-efficient. The detection of AFP plays a crucial role in the timely identification and treatment of many medical conditions, with a special emphasis on cancer. Although there have been notable advancements in medical technology, conventional detection techniques often lack in terms of intricacy, time requirements, and availability, hence emphasising a huge market need. Efficient electrochemical biosensors have emerged as viable alternatives, presenting notable benefits like enhanced sensitivity, rapidity, and straightforwardness. Nevertheless, despite the significant potential of these biosensors, there is a noticeable deficiency in research pertaining to their complete utilisation and the resolution of obstacles such as false positives/negatives and cost-effectiveness. The objective of this study is to close this divide by combining current progress in electrochemical biosensors specifically designed for AFP detection, with a particular emphasis on tackling worldwide health issues. This review aims to offer insights into the improvement of disease diagnosis and patient outcomes on a global scale by examining the integration of cutting-edge materials, advanced fabrication techniques, and innovative biorecognition strategies in AFP detection technologies. The objective is to enhance the performance, accessibility, and affordability of these technologies.

Experimental Issues in AFP Detection

Significant progress has been made in the field of biosensors throughout the years, characterized by notable milestones in their development. The chronology begins with the groundbreaking contributions of Clark and Lyons in 1962, whereby they produced the first electrochemical biosensor designed for the detection of glucose. This seminal study established the groundwork for later investigations in this particular domain. Significant progress was made throughout the 20th century with the emergence of enzyme-based biosensors and the incorporation of nanotechnology to improve sensitivity and selectivity. The introduction of label-free biosensors and the integration of sophisticated materials like graphene and carbon nanotubes were notable developments in the early 21st century. In recent years, there has been a notable increase in scholarly investigations centered on the integration of artificial intelligence and machine learning algorithms to enhance the analysis and interpretation of data.[22] Additionally, there has been a growing interest in the advancement of point-of-care biosensors, which aim to facilitate quick and decentralized testing. The future of biosensors has the potential to revolutionize healthcare diagnostics and monitoring via ongoing innovation, propelled by developments in materials science, biotechnology, and digital technologies.

In the dynamic and ever-changing field of bio sensing technology, the incorporation of sophisticated methodologies and state-of-the-art materials has significant potential for the advancement of sensors in the future. Recent studies, such as the investigation of 2D MXenes and Borophene in the context of sustainable intelligent sensors, highlight the transition towards sensor platforms that are more advanced and environmentally friendly. Furthermore, research on the difficulties and possibilities of creating electrochemical biosensors for point-of-care diagnostics emphasizes the urgent need for economic viability in the healthcare industry. The integration of 5th generation AI and IoT-enabled sensors with electrochemical biosensing methods has significant potential for driving disruptive developments. By using cutting-edge technologies and incorporating them into electrochemical biosensors, it is possible to augment sensitivity, precision, and the ability to monitor in real-time. This approach effectively tackles significant deficiencies in the diagnosis and treatment of diseases. The objective of this review is to examine the convergence of these advancements, offering a thorough examination of the most recent progress in electrochemical biosensing methods and their incorporation with cutting-edge technology for future sensors that have the potential for commercialization in the point-of-care diagnostics industry. This synthesis aims to elucidate strategies for surmounting current obstacles and capitalizing on emerging prospects for the progression of healthcare technology.[24]

Biosensors that identify AFP are an essential weapon in the fight in defiance of diseases. The materials that make up these biosensors work harmoniously to achieve unparalleled performance and accuracy. We have arranged the materials used in AFP detection into electrodes and surface modifications. To boost the sensor's performance, electrodes can be improved by adjusting them, such as through sensitivity or improving stability. Also, distinctive materials can be used for surface modifications to further elevate electrodes and enhance biosensor performance.[12] Fabrication of AFP detection biosensors utilizes various materials containing polymers, carbon-based materials, metal oxides, and nanomaterials. For example, carbon nanotubes have been demonstrated to enhance sensitivity and selectivity in AFP detection, while graphene oxide has exhibited the power to enhance stability. Also, gold nanoparticles have shown sensational potential in increasing the biocompatibility of AFP detection biosensors.[13]

Electrodes: the foundation of AFP detection.—The electrodes involved, including the working, counter, and reference electrodes, are constructed from materials that boast excellent electrical conductivity and stability. When it comes to the working electrode (WE), it serves as the primary site where the electrochemical reaction between the AFP and the electrode surface is made and the generation of measurable signals takes place. The objective is to amplify the interaction between the target AFP molecules and the electrode surface, resulting in stronger signal generation or increased specificity for AFP detection. These materials have electrical solid conductivity, biocompatibility, and ease of surface modification, enabling efficient electron transfer and heightening the sensitivity of the detection system. The counter electrode (CE) completes the electrical circuit in the electrochemical cell, ensuring charge neutrality.[19] Because of their exceptional electrical conductivity and stability, noble metals like gold or platinum are utilized as counter electrodes. These materials facilitate efficient current flow during electrochemical reactions, ensuring the precise measurement of the electrochemical signal associated with AFP. Last but not least, reference electrodes (RE) play a critical role in establishing a fixed potential reference point in electrochemical measurements. Common reference electrodes used in AFP detection include the Ag/AgCl electrode, the saturated calomel electrode (SCE), and the standard

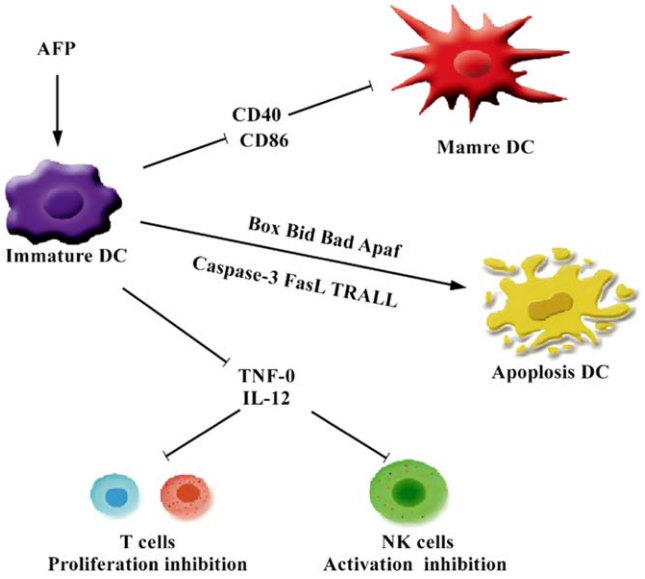

Figure 3. The function of AFP in immune evasion of liver cancer cells.[114]

hydrogen electrode (SHE). These reference electrodes provide a stable and well-defined reference potential against which the potential on the working electrode can be accurately measured.[20] Nevertheless, the most common electrode used in Alpha-Fetoprotein (AFP) detection biosensors is screen-printed electrodes (SPEs), including all three electrodes, which are a type of electrode fabricated using a screen-printing process. SPEs are typically made by depositing a conductive ink or paste onto a substrate through a screen mesh, creating a patterned electrode. The screen printing process allows the production of disposable and mass-produced electrodes, making them suitable for rapid and cost-effective sensing platforms. The role of AFP in liver cancer cells' immune evasion is indicated in Fig. 3. AFP contributes to the immunosuppression of cancer cells by altering the function of immune cells. AFP reduces dendritic cell maturation and cytokine release, increases dendritic cell death, and prevents T cell proliferation and activation of NK cells.

Surface modifications: enhancing electrodes for improved AFP detection.—In the field of biomarker detection, the performance of electrodes in detecting alpha-fetoprotein (AFP) can be improved through surface modifications. CNTs, with their high surface area and excellent electrical conductivity, can increase the active surface area of the electrode, resulting in improved sensitivity and electron transfer kinetics. Additionally, conducting polymers provide the advantage of selectively adsorbing or immobilizing AFP antibodies or aptamers onto the electrode surface, thereby enhancing the specificity and selectivity of the detection system. These surface modifications have great potential for enhancing the precision and sensitivity of AFP detection in clinical settings.[21] Here is a brief review of the most utilized material to develop biosensors.

Gold nanoparticles (AuNPs).—Because gold nanoparticles (AuNPs) have unique electrical and optical properties that greatly enhance their suitability for biosensor applications, this industry has extensively used them. They have optical characteristics that enable the sensitive detection of biomolecular interactions, such as strong plasmon resonance and high scattering efficiency. Controlling the size, shape, and surface chemistry of AuNPs allows for precise tuning of their localized surface plasmon resonance (LSPR), allowing optimization for particular biosensing applications.[22] In addition to their optical qualities, AuNPs have a larger surface area than their size, giving biomolecules like antibodies or DNA probes plenty of room to immobilize. This makes target analytes, like AFP,

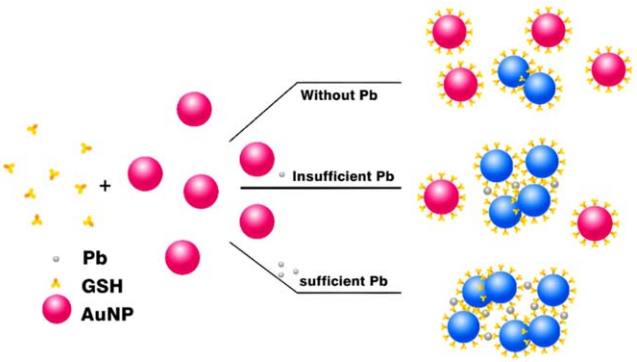

Figure 4. Time-dependent Pb^{II} concentrations in water can be determined using this schematic idea.[115]

easier to capture and recognize, improving biosensor detection's sensitivity and selectivity. AuNPs can also function well as signal amplification components in biosensors. The presence of AuNPs can significantly amplify the signal produced by the target-analyte interaction through various amplification techniques, such as enzyme-mediated reactions, DNA hybridization, or nanoparticle aggregation, enabling ultrasensitive detection of AFP. The sensitivity and selectivity of AFP biosensors have improved significantly due to using AuNPs in their construction. Biosensors can perform better by utilizing the special qualities of AuNPs, allowing early detection and accurate monitoring of AFP levels for clinical applications.[21] Changming Shen et al. aimed to develop an immunosensor for detecting AFP in serum samples in their study. To label the anti-AFP antibody, Au nanocubes were synthesized, and horseradish peroxidase (HRP) was used for antibody detection. GO-MB-AuNP nanocomposites were used as the platform for the immobilization of AFP and the immune complex. As the immunosensor generates a signal proportional to the logarithmic value, differential pulse voltammetry (DPV) was used to measure this current. The proposed sandwich-type immunosensor showed good sensitivity, selectivity, and stability performance. The linear detection range for AFP was 0.005–20 ng ml^{-1}, with a low detection limit of 1.5 pg ml^{-1}. This immunosensor demonstrated good precision and selectivity for AFP determination in clinical tests. The principal of the label-free method for the detection of lead in water using AuNPs is presented in Fig. 4.[27]

Graphene oxide (GO).—In Fig. 5, one monomolecular layer of graphite with multiple oxygen-containing functions, including epoxide, carbonyl, carboxyl, and hydroxyl groups, makes up the unusual substance known as graphene oxide (GO). Two-dimensional carbon-based material generated a great deal of interest in the creation of biosensors because of its exceptional electrical conductivity and large surface area. These qualities make GO an excellent option for immobilizing biomolecules, enabling the sensitive and precise detection of analytes such as alpha-fetoprotein (AFP). The value of GO in the detection of AFP was demonstrated by Wang et al. using a microfluidic immunosensor. In their study on a microfluidic platform, GO and gold nanoparticles were combined to increase the sensitivity of AFP detection. Because GO has a large surface area, antibodies could be effectively immobilized, which improved the binding and recognition of AFP molecules. The synergistic combination of these promising techniques for early cancer diagnosis. The synergistic interaction of GO and gold nanoparticles, which resulted in the achievement of ultrasensitive AFP detection, made this promising method for early cancer diagnosis possible. Electrical conductivity, surface area, and biomolecule immobilization are all improved when GO is used in biosensors for AFP detection. These characteristics support the general sensitivity and efficacy, allowing for early detection of AFP and potentially improving patient outcomes.[26] In the study by

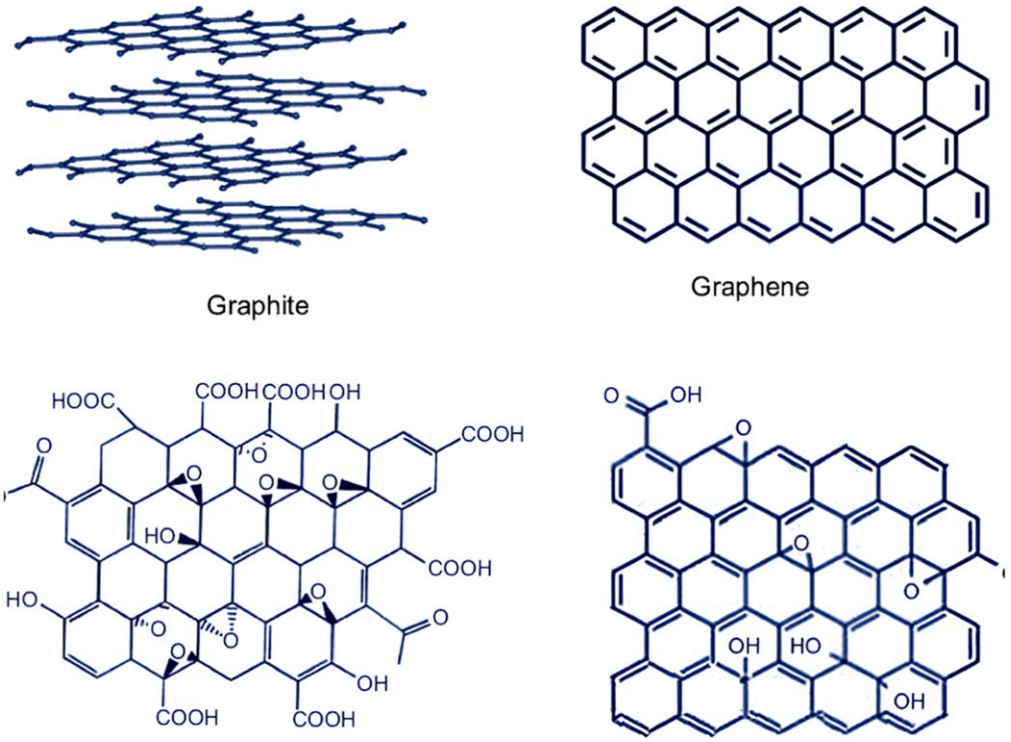

Figure 5. Structures of graphite and Graphene in the oxide conditions.[116]

Jisan Sun and colleagues, GO was incorporated to enhance the conductivity and intensity of the electrochemical current. They developed a label-free electrochemical immunosensor to detect the cancer biomarker α-fetoprotein (AFP). Hydroxyapatite (HAP) and graphene oxide (GO) are used to modify the electrode surface. The formation of an immunocomplex layer on the electrode hinders the reaction between hydroxyapatite NPs and molybdate, decreasing the electrochemical current. The magnitude of the current change is proportional to the concentration of AFP present in the sample. The immunosensor demonstrated a wide linear detection range of 0.01–10 ng ml^{-1} AFP with a low detection limit of 5 pg ml^{-1}. In a study by Geu et al., a label-free electrochemical aptasensor for the detection of alpha-fetoprotein (AFP) was used using a combination of thionine (TH), reduced graphene oxide (RGO), and AuNPs as immobilization platform, AFP aptamer being used as a detection molecule.[116] To achieve this, they fabricated the aptasensor by modifying a screen-printed electrode (SPE) with TH, RGO, and AuNPs, and then immobilising the AFP aptamer on the electrode surface. Observations showed changes in the intensity of characteristic peaks such as Au4f, C1s, N1s, and O1s, indicating the presence and binding. Then, the analytical performance of the aptasensor was evaluated by detecting different concentrations of AFP using differential pulse voltammetry (DPV). The current response of the aptasensor increases with an increase in the AFP concentration, indicating the successful formation of the AFP-aptamer complex. In this investigation, the researchers evaluated the repeatability, specificity, and stability of the aptasensor to evaluate its reliability. The aptasensor showed good reproducibility with similar current responses among multiple electrodes (interassay RSD 3.9%). It also showed good specificity, responding selectively to AFP compared to other interfering biomolecules. The stability of the aptasensor was demonstrated by storage at four °C for 7 and 14 days, with activity at 95.76% and 90.86% of the initial response, respectively. Finally, the practical applicability of the aptasensor was verified by analyzing human serum samples using the standard addition method.[112]

Carbon nanotubes (CNTs).—Because of their special qualities, CNTs (Fig. 6) can be used to create highly sensitive and stable biosensors for detecting AFP. Cao et al. utilized the beneficial properties of CNTs in an immunosensor created to detect AFP. They improved the sensitivity and stability of their sensor platform by incorporating CNTs. Also, CNTs are innovative Nano carrier systems with numerous uses in engineering, science, and the environment. Because CNTs can be functionalized (i.e., have their surfaces engineered), which involves adding specific chemical groups, their physical or biological properties can be changed for various uses. The enormous surface area and flexibility of CNTs have been exploited to use them in thermal conductivity to kill cancer cells photo thermally and their capacity to act as carriers for various medicinal compounds. The superior electrical conductivity of CNTs made the electron transfer of electrons more effective, which enhanced signal transduction.[36]

Furthermore, the large surface area of CNTs made it possible to effectively immobilize antibodies, which improved the capture and recognition of AFP molecules. The performance improved because of the integration of CNTs, making it a promising method for detecting AFP. These characteristics improve the sensitivity, stability, and performance in detecting AFP, facilitating the early detection and monitoring of cancer.[23] Ke Gao et al. used prussian blue (PB) as an electron mediator, a typical hexacyanoferrate with a face-centered cubic lattice structure, in electrochemical systems to detect biomolecules. The researchers loaded PB onto carbon nanotubes (CNTs) to increase the stability of PB and improve the electrical conductivity of the nanocomposites as the bottleneck of PB usage is quickly leaking, and the activity is destroyed or lost activity in weak alkaline environments, resulting in the short lifetime of these PB-based electrochemical sensors. Additionally, dopamine (DA) was used as a coating on PB-CNTs to form polydopamine (PDA) coating, which can prevent the unstable and outflow of PB on the surface of the electrode and prevent PB from losing activity in weak alkaline environments. The PDA coating not only serves as an extremely versatile platform for the immobilisation of biological molecules, but also possesses excellent reduction ability. AuNPs were in situ synthesized on the surface of PB-CNTs@PDA, based on the excellent reduction ability of the PDA coating without any other reducing agents. This provided a favourable microenvironment to

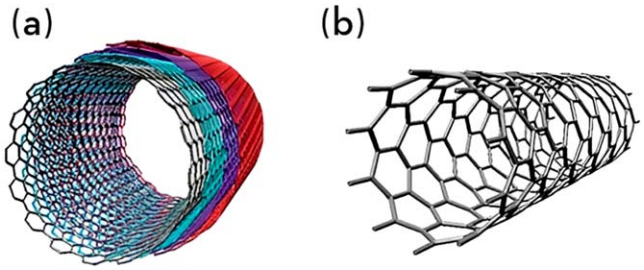

Figure 6. A typical structure of CNTs with details.[117]

increase the loading capacity of the antibody without deactivation and enhance the conductivity of the composite. The researchers then fabricated an electrochemical immunosensor using a glassy carbon electrode modified by PB-CNTs@PDA-AuNPs nanocomposite and incubated in anti-AFP solution to detect AFP. Under optimal experimental conditions, the immunosensor showed excellent detection performance with a wide dynamic response range of AFP concentration from 0.005 to 80 ng ml^{-1} and a detection limit of 0.001 ng ml^{-1} (S/N = 3). The proposed electrochemical immunoassay method provides potential applications for other antigens or tumour markers in clinical diagnosis. Overall, this study demonstrates the importance of developing innovative and efficient detection methods for cancer screening and detection.[28]

Conducting polymers.—Conducting polymers (CPs) and their composites are widely used (Fig. 7), including in the development of cancer biomarkers. Their large surface area, porosity, and presence of functional groups provide CPs with binding sites suitable for capturing biomarkers and enabling easy and sensitive detection. Researchers are currently devoting great efforts to achieve biosensors' maximum sensitivity and selectivity and avoid the biofouling effect. CP-based surfaces have been found to limit the biofouling effect, allowing for sensitive detection in real patient samples.[96] The antifouling property of a sensor material is a supreme character in the practical applications of biomarker detection. In addition, CPs provide direct cell capturing and detection, which will make cancer diagnosis more accurate and efficient. Researchers have now reached the stage where a single biosensor can detect multiple biomarkers simultaneously. In this way, it is possible to provide a detailed encryption of cancerous initiation and growth. However, in addition to laboratory methods, no devices have been reported elsewhere for the point-of-care diagnosis of cancer biomarkers. The fabrication of stable, sensitive, selective, and antifouling cancer biomarker biosensing devices, particularly those based on CPs, is expected to be a positive new development in the field of cancer diagnosis to replace conventional analytical methods.[89]

In summary, CP-based electrochemical sensors show great potential for the early and accurate detection of cancer biomarkers. CPs, including polypyrrole and polyaniline, are widely utilized in biosensing platforms due to their inherent electrical conductivity and ease of integration. CPs have been extensively investigated as intrinsic electrocatalysts and photo catalysts due to their distinctive one-dimensional (1D) delocalized conjugated structures and remarkable electrical, electrochemical, and optical characteristics. These polymers are compatible with biomolecules and can be easily functionalized for specific detection applications. Their conductivity allows for efficient charge transfer, making them valuable components in biosensors for detecting alpha-fetoproteins (AFP). Jain et al. (2017) demonstrated the use of polypyrrole in an impedance-based AFP biosensor. By electropolymerizing a nanostructured polypyrrole film on the sensor surface, they achieved label-free and sensitive detection of AFP. The conducting polymer film acts as a transducer, facilitating the transduction of the binding events into electrical signals. This approach allowed the detection of AFP with high sensitivity while eliminating the need for additional labels or markers.

Figure 7. Biomedical Applications of Conducting Polymer.[118]

Nanocomposites.—Nanocomposites, which involve combining multiple materials (Fig. 8), offer tremendous potential to enhance the performance of biosensors. Integrating different materials into a single structure can achieve synergistic effects, leading to improved sensitivity and selectivity in detecting alpha-fetoprotein (AFP). Zhang et al. (2018) conducted a study in which they developed a biosensor for highly sensitive AFP detection by integrating AuNPs, graphene oxide (GO), and a molecularly imprinted polymer (MIP) in a nanocomposite. The AuNPs contributed to signal amplification and facilitated the immobilization of biomolecules. GO provided a great surface area and enhanced electrical conductivity. MIP acted as a selective recognition element for AFP, ensuring high specificity. The combination of these materials in the nanocomposite structure resulted in a biosensor with significantly improved sensitivity and detection limits for AFP. The integration of multiple materials in nanocomposites offers complementary advantages, including signal amplification, enhanced surface area, improved electrical conductivity, and discerning recognition. Using these synergistic effects, biosensors can achieve highly sensitive and particular detection of AFP, qualifying early diagnosis and monitoring of cancers.[45] In the study conducted by Wang et al., a novel label-free electrochemical immunosensor based on multifunctionalised graphene nanocomposites was developed to design a highly sensitive and selective immunosensor for the detection of Alpha-Fetoprotein (AFP). They used a combination of graphene, gold nanoparticles (Au NP), iron oxide nanoparticles (Fe_3O_4 NPs), and toluidine blue (TB) to construct the immunosensor. The reproducibility of the immunosensor was evaluated by preparing five electrodes and measuring 1.0 ng ml^{-1} AFP, resulting in a dependent average deviation (RSD)

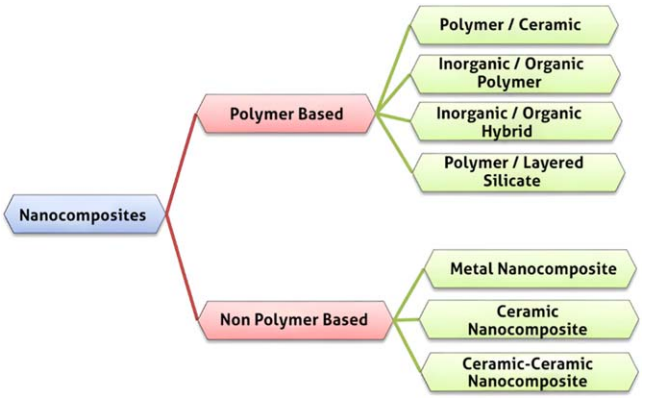

Figure 8. Categorisation of NCs in detail.

of less than 5%. The good stability of the immunosensor was attributed to the biocompatibility of the graphene nanocomposites. The consequences demonstrated good agreement between the two methods, indicating the feasibility of the designed immunosensor for clinical applications.[47]

Challenges and alternate solutions.—Electrochemical biosensors encounter many obstacles that impede their extensive implementation and clinical use, despite their considerable promise. An important obstacle is the possibility of interference from other biomolecules in intricate biological samples, which may result in inaccurate positive or negative outcomes and undermine precision.[119] In order to tackle this issue, scholars are now investigating novel approaches to signal amplification, including the utilisation of nanomaterials or signal amplification enzymes, with the aim of augmenting the precision and responsiveness of biosensors. One further obstacle is to the financial implications associated with the acquisition of equipment and reagents necessary for electrochemical biosensing, which may pose a significant barrier, especially in settings with low resources. In order to surmount this obstacle, there are ongoing endeavours to create affordable and easily transportable biosensor systems that use smartphone technology for the processing and transmission of data. This would provide decentralised testing and diagnostics at the point of care. Furthermore, the presence of stability and reproducibility concerns in the performance of biosensors presents significant obstacles for the implementation of long-term monitoring applications. Researchers are now investigating novel manufacturing processes and materials engineering methodologies in order to improve the stability and reproducibility of biosensors. This is done with the aim of assuring consistent and dependable performance over an extended period of time. To fully harness the promise of electrochemical biosensors in revolutionising disease diagnosis, environmental monitoring, and industrial processes, it is crucial to tackle these problems and adopt alternative methods.[120]

Working Principles of AFP Sensor

Electrochemical techniques, hailed as potent diagnostic instruments, have become increasingly prevalent in the field, providing a sensitive, rapid, and economical means of early detection. This article delves into the utilization of electrochemical methods to prompt the prompt identification of Alpha-Fetoprotein (AFP), a biomarker linked to multiple diseases, such as liver cancer. Here are three commonly used electrochemical techniques for the detection of AFP.[59]

Amperometry for AFP detection.—The amperometry technique electrifies the detection of AFP, a commonly pursued electrochemical approach. The resulting current is measured by imposing a steady potential between the working and reference electrodes. Immunosensors dependent on amperometry detection infuse AFP-specific antibodies immobilized on the working electrode surface. Once the AFP molecules fuse with these antibodies, there is a signal shift in the current, and AFP levels can be quantified. Various studies have shown the acute sensitivity and selectivity of amperometry-based AFP detection, presenting a hopeful possibility for early diagnosis. Huan Wang et al. used Pd nanoplates to create an electrochemical immunosensor for the detection of the cancer biomarker AFP. The nanoplates facilitated the effective immobilization of AFP antibodies and improved electron transmission, resulting in enhanced sensitivity. The label-free immunosensor detected AFP in a wide range (0.01–75.0 ng ml^{-1}) with a low detection limit (4 pg ml^{-1}). It successfully determined AFP in human serum. In their study, Shuo Liu et al. developed a sensitive and selective electrochemical sensor to detect alpha-fetoprotein (AFP). They used a three-dimensional (3D) macroporous polyaniline (PANI) material doped with poly (sodium 4-styrene sulfonate) (PSS). The sensor was fabricated using a hard-template method. They employed a conversational three-electrode system for electrochemical measurements and used scanning electron microscopy (SEM) to characterize the materials. The fabrication process involved assembling polystyrene macrospheres on a glassy carbon electrode (GCE), depositing PANI into the interstitial spaces through electropolymerization, and dissolving the macrospheres to create a porous PANI/GCE structure. The porous PANI/GCE was then incubated with AFP antibodies in phosphate buffer saline (PBS) solution to construct the immunosensor. The performance of the immunosensor was evaluated using various electrochemical techniques, including electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV), and differential pulse voltammetry (DPV). The results demonstrated that the porous PANI-based immunosensor exhibited satisfactory sensing capabilities for AFP detection, with a wide linear detection range and a low detection limit. Importantly, the 3D macroporous structure of PANI (Fig. 9) provided a higher response sensitivity compared to a planar PANI-modified electrode.[22]

Voltammetry techniques for AFP detection.—The techniques of voltammetry, including the illustrious cyclic voltammetry (CV), the striking square wave voltammetry (SWV), and the awe-inspiring differential pulse voltammetry (DPV), are vastly employed in the pursuit of detecting AFP. These techniques require the application of

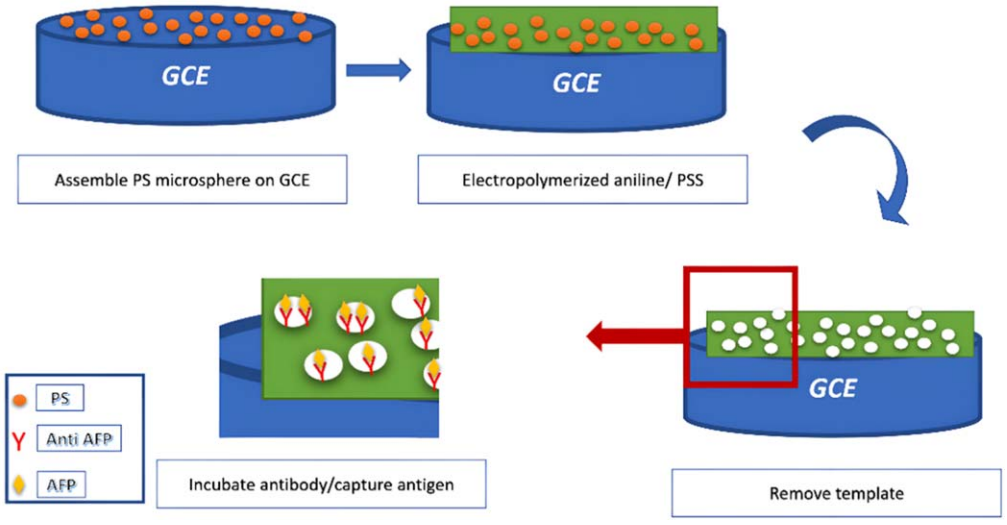

Figure 9. Fabrication process of the AFP immunosensor based on macroporous PANI.

a voltage waveform to the working electrode, followed by the measurement of the resulting current. The detection of AFP through voltammetry techniques is based on the redox properties of the analyte.[36] Its concentration can be established by vigilantly observing the electrochemical response of AFP at particular potentials. Voltammetry-based AFP detection methods offer unparalleled sensitivity and exceptional ability to differentiate between AFP and interfering species, enabling early disease detection. Upan et al. developed a label-free electrochemical aptasensor for sensitively detecting alpha-fetoprotein (AFP) using platinum nanoparticles on carboxylated graphene oxide (PtNPs/GO-COOH). The GO-COOH was synthesized to increase the surface area and facilitate the immobilization of the AFP aptamer. PtNPs were decorated on GO-COOH to enhance electrical conductivity and the oxidation current of the hydroquinone electrochemical probe. The aptasensor operates on the selective interaction between the AFP aptamer and AFP molecules, which leads to a decrease in the peak current of the hydroquinone probe. Binding of biomolecules on the electrode surface impedes the electron transfer of the redox probe, resulting in a measurable change in the current response. The developed aptasensor exhibited a linear detection range of 3.0–30 ng ml^{-1} of AFP with a detection limit of 1.22 ng ml^{-1}. Integrating PtNPs and GO-COOH nanocomposites improved the electrochemical response by increasing the electrode's electron transfer rate. The aptasensor demonstrated excellent performance characteristics and has promise for clinical applications in the early diagnosis and monitoring of HCC.[21]

Modern analytical equipment can precisely identify samples with only a few materials to be characterized. Raman spectroscopy can evaluate and identify materials using their unique molecular information and has the benefit of great specificity.[122] However, Raman spectroscopy has a poor sensitivity, making it ineffective for the study of substances with low concentrations. The research suggests that SERS (surface-enhanced Raman spectroscopy), a potential remedy for this issue, involves using metal nanostructures or particles to strengthen the intrinsically weak Raman effect. SERS analyses samples with the lowest analyte concentrations by combining Raman spectroscopy's selectivity and high sensitivity. Its benefits include straightforward operation, excellent sensitivity, rapid detection, and remarkable reproducibility. It is regarded as a desirable tumor marker analysis and detection.[123] Due to the lengthy experimental duration and intricate techniques, most traditional SERS analysis is based on the reaction between the biomarker antigen and antibody, necessitating expensive antibodies.[124] A DNA hydrogel with good flexibility and stability is used in the new type of SERS biosensing platform by Wang et al.[30] to detect AFP with great sensitivity (Fig. 10).

Recent research has demonstrated the value of resonant light scattering (RLS) for identifying and describing prolonged aggregates of chromophores. The scattering strength of these substances at or close to the absorption wavelength will increase by several orders of magnitude when there is strong electronic coupling between the chromophore units.[125] The RLS technique has been widely used in detecting various biomolecules, including DNA,[126] protein,[127] metal ions, and pharmaceuticals, due to its high sensitivity, simplicity, and quick performance. Through the electrical interaction of methyl violet (MV) and dsDNA, the Chen et al.[128] aptamer biosensor leverages LPS technology to detect AFP with excellent sensitivity (Fig. 11).

Impedance spectroscopy for AFP detection.—As an electrochemical technique, electrical impedance has shown a glimmer of hope in detecting AFP in its early stages. This technique requires measuring the electrical impedance across a range of frequencies. The detection of AFP via impedance depends on the changes in the interface properties of the electrode surface upon the binding of AFP to specific recognition elements. The presence and concentration of AFP can be guessed through the analysis of impedance spectra, including alteration of resistance and capacitance.[84] The work of Haiying Yang et al. investigates the development of an electrochemical impedance spectroscopy (EIS) biosensor capable of detecting and distinguishing alpha-fetoprotein (AFP) without the need for labels. They used wheat germ agglutinin (WGA) lectin as a recognition factor to do this. The biosensor was made by adding carboxyl-functionalized single-wall carbon nanotubes (SWNTs) to a screen-printed carbon electrode (SPCE) and attaching WGA to the electrode's surface. When AFP binds to the biosensor, it increases resistance to electron transport. In general, it can be used to build early-stage cancer detection. A comprehensive table is given below for biosensors (Table I).[97]

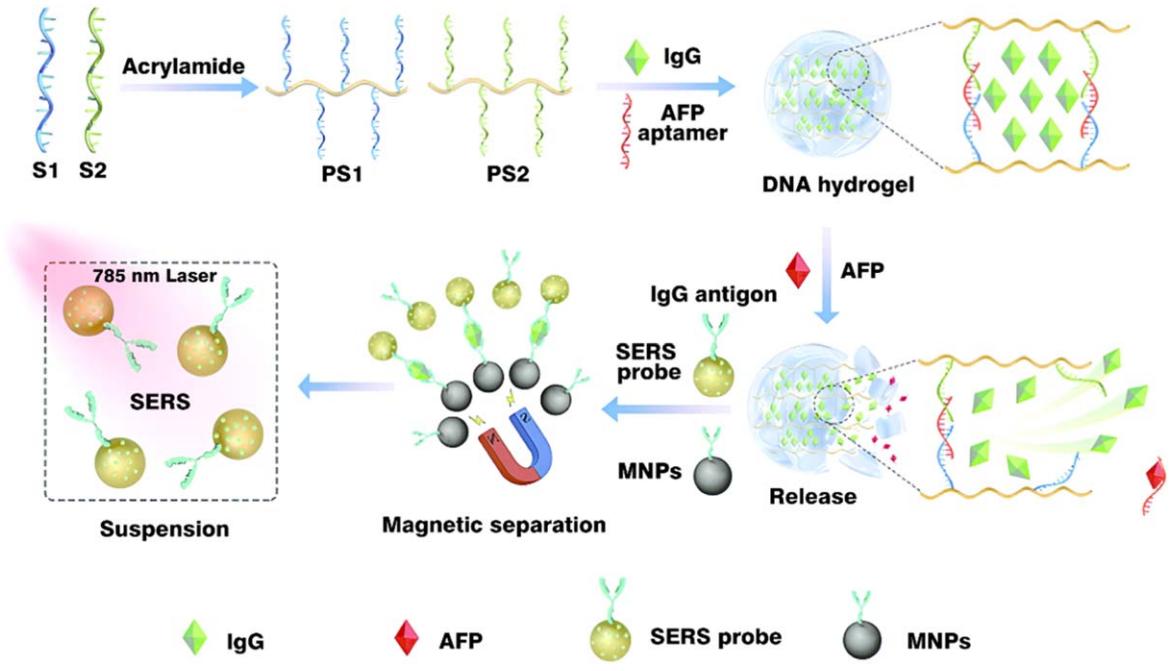

Figure 10. Aptamer biosensor for AFP detection based on the SERS schematic diagram.[121]

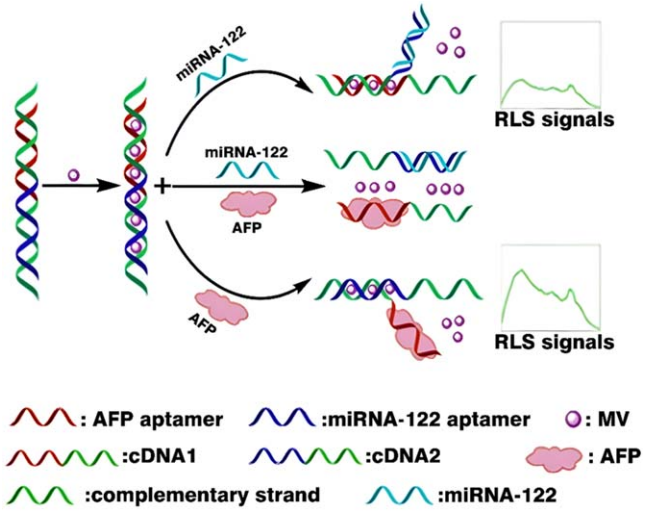

Figure 11. Schematic of an aptamer biosensor.[128].

Applications and Importance of Electrochemical Biosensors for Alpha-Fetoprotein Detection

The mentioned materials offer unique properties, such as high surface area-to-volume ratios, enhanced electron transfer, and biocompatibility, making them ideal candidates for electrochemical biosensor development for AFP detection. Furthermore, combining these nanomaterials with molecular recognition elements such as antibodies, aptamers, and enzymes can improve their sensitivity and selectivity for AFP detection. These devices can also be easily integrated into point-of-care testing platforms, allowing them to be used in rural places with inadequate laboratory infrastructure. The continuous development of electrochemical biosensors for AFP detection opens up intriguing possibilities for personalized medicine, in which personalized diagnosis and therapy can be based on an individual's biomarker profile.[50]

Early diagnosis of primary liver cancer.—The prevalence of primary liver cancer, including menacing hepatocellular carcinoma (HCC), is a formidable global health threat. Detecting HCC is the cornerstone of effective treatment and increased patient survival. These biodetectors possess the uncanny ability to ferret out infinitesimal amounts of AFP, thereby facilitating the early detection of HCC, even in its nascent stages when remedial options are more efficacious. Prudent diagnosis plays a pivotal role in advancing the prognosis and survival rates of primary liver cancer patients. Notably, Smith et al. study reported the creation of an electrochemical biosensor predicated on a gold electrode modified with graphene oxide nanocomposites. This biosensor demonstrated remarkable sensitivity, detecting AFP concentrations as low as 1 ng ml^{-1}. The early detection of liver cancer using such biosensors has critical potential for timely intervention and escaleted patient outcomes.[46]

Monitoring of the efficacy of treatment.—The capability of liver cancer treatment depends on the patient's response to therapy with precision. Electrochemical biosensors are a game changer in this regard, as they provide a dynamic tool for real-time evaluation of AFP levels. By accurately measuring AFP concentrations during treatment, clinicians can make informed decisions regarding the course of therapy, improving patient outcomes and minimizing needless interventions. Innovative research by Chen et al. showcases the potential of electrochemical immunosensors. The label-free biosensor, featuring a gold nanoparticle-modified electrode, enables sensitive and selective AFP detection in clinical samples. This novel technology offers a reliable means of assessing treatment efficacy and optimizing therapy for superior patient outcomes. With electrochemical biosensors, clinicians can stay ahead of the curve in monitoring AFP levels, aiding in evaluating treatment feedback and optimizing therapy.[35]

Point-of-care testing.—The advent of compact and downsized electrochemical biosensors has expedited point-of-care examination for AFP detection. These handheld devices expedite on-site scrutiny, abrogating the necessity of dispatching samples to centralized labs and abridging the turnaround time for outcomes. Point-of-care electrochemical biosensors for AFP detection can potentially change healthcare delivery, particularly in resource-strapped locations, by providing timely diagnosis and monitoring without requiring extensive infrastructure or specialized training.[45] Point-of-care testing (POCT) has garnered substantial attention as it enables rapid on-site recognition of AFP without requiring intricate laboratory infrastructure. Electrochemical biosensors, because of their miniaturization and portability, proffer tremendous potential for POCT applications. For example, Johnson et al. concocted a handheld electrochemical biosensor for AFP detection grounded on screen-printed electrodes. This portable gadget allowed for swift measurement of AFP levels in blood samples with high sensitivity and specificity. The availability of such portable electrochemical biosensors provides opportunities for convenient and timely AFP testing in resource-limited settings or remote areas, enabling early detection and facilitating immediate medical decisions.[124]

Personalised medicine.—Electrochemical biosensors unlock personalized medicine's potential by detecting AFP in individual patients. These biosensors possess exceptional sensitivity and specificity, ensuring precise measurement of AFP levels. This enables clinicians to tailor treatment strategies to individual patients by closely monitoring AFP levels and adjusting treatment regimens based on the patient's response. Personalized medicine seeks to deliver tailored healthcare based on an individual's unique characteristics. In AFP monitoring, customizing the electrochemical biosensor design and detection parameters according to patient-specific needs achieves personalized approaches. Li et al. developed a personalized electrochemical biosensor for the detection of AFP by incorporating patient-specific antibodies on the electrode surface.[47] This innovation allowed for the precise recognition of AFP isoforms and improved the monitoring accuracy in patients with variant forms of AFP. Personalized medicine strategies enable more accurate AFP monitoring by customizing biosensors to individual patients. This ensures optimized treatment plans and improved patient care.[115]

Discussion

According to former information, AFP levels in the blood could indicate various types of cancer, so this property can be used in early cancer detection. Electrochemical biosensors are a promising technology for detecting AFP in blood samples, especially now that advances in micro- and nanofabrication are allowing for the development of small and portable electrochemical biosensors. Combining electrochemical biosensors with other technologies makes detecting many biomarkers in a single experiment possible. Electrochemical biosensors for AFP detection have a promising future with various potential applications. These devices use biological recognition elements, such as antibodies or enzymes, to selectively bind to AFP and generate an electrical signal that can be measured and quantified.[4–6] With a look to the future, several exciting developments are on the horizon for electrochemical biosensors for AFP detection. One key area of research is the development of new and more selective biological recognition elements, like aptamers or molecularly imprinted polymers, which can improve the accuracy and specificity of AFP detection. Electrochemical biosensors can be customized to detect specific biomarkers in individual patients, allowing targeted treatment plans. In addition, electrochemical biosensors have the potential to be

Table I. A comprehensive review of the related works.

No	Auther	Aim	Method	Result
1	42	Explore efficient biosensors for detecting AFP. Enhance sensitivity and selectivity using nanomaterials.	• Nanoparticles were utilized to improve AFP biosensor performance. • The study reviewed various nanotechnologies employed in early AFP detection. • Representative examples of these enhanced biosensors were discussed.	Super sensitivity: Detecting trace AFP concentrations. High selectivity: Specifically targeting AFP. Reduced time consumption: Faster detection.
2	129	Develop highly sensitive and selective biosensors for detecting AFP.	Utilize nanomaterials (such as nanoparticles) due to their exceptional optical, electrical, and chemical properties. Employ functional nanotechnology to enhance sensitivity, selectivity, and speed in AFP detection. Explore various techniques, including surface-enhanced Raman scattering (SERS), enzyme-linked immunosorbent assay (ELISA), and aptamer-based sensors. Investigate how nanoparticles can improve the performance of AFP biosensors.	• Enhanced sensitivity and selectivity achieved through nanotechnology. • Representative examples of successful AFP biosensors using nanoparticles have been documented. • Challenges related to clinical application have been identified, along with opportunities for future development in this field
3	130	Explore graphene's potential in early pancreatic cancer detection.	Use graphene-based biosensors targeting K-Ras gene, CEA, and MicroRNA. Employ GFETs for sensitive electrochemical sensing.	Promising sensitivity to GPC-1, enabling early diagnosis.
4	131	Develop sensitive electrochemical sensors for detecting hematological malignancies.	Utilize graphene-enhanced sensors and bioelectrochemical techniques.	Improved detection accuracy, potential for personalized treatment strategies.
5	132	Investigate risk factors related to prognosis and treatment of AFP-producing epithelial ovarian carcinoma (EOC).	Reviewed 24 cases of AFP-producing EOC.	
6	133	Investigated clinical and pathologic differences between AFP-positive and AFP-negative HCC patients.	Observed patients from Eastern and Southern China, comparing features.	• AFP-positive patients had larger tumors, advanced stages, and elevated liver markers. • Differences in clinical pathologic characteristics can aid in disease management and prognosis.
7	134	Develop a low-cost, sensitive method for AFP detection.	Used a paper-based chip with sandwich-type immunoassay.	Successful AFP detection in serum samples.
8	135	Develop a sensitive Nano sensor for detecting alpha fetoprotein (AFP).	• Synthesize NIR fluorescent carbon quantum dots. • Modify them to create a turn-on fluorescent Nano sensor specific to AFP. • Assess biocompatibility and photo stability. • Determine a low detection limit of 3.0 pg/ml for AFP. • Apply the Nano sensor for quantification and imaging.	• High sensitivity to AFP. • Accurate quantification in human serum. • Potential for early-stage liver cancer diagnosis.
9	136	The aim was to achieve a lower limit of detection (LOD) of 100 fg/mL, showcasing the biosensors' exceptional sensitivity for cancer biomarker detection.	The biosensors were utilized to detect AFP, demonstrating a direct correlation with AFP antigen concentrations ranging from 100 fg/ml to 1 μg ml^{-1}.	The biosensors exhibited remarkable efficacy in the detection of AFP, displaying a direct relationship with the concentration of AFP antigens within the range of 100 fg/ml to 1 μg/ml. Furthermore, they achieved a lower limit of detection (LOD) of 100 fg/ml, thereby highlighting their highly sensitive capabilities for the detection of cancer biomarkers.
10	132	The aim was to highlight the biosensors' potential for precise and sensitive detection in clinical settings, emphasizing their ability to meet stringent clinical requirements.	The biosensors were employed to detect AFP, achieving detection levels below the clinically significant threshold of 20 ng ml^{-1}.	The biosensors demonstrated exceptional efficacy in the detection of AFP, attaining detection levels that fell below the clinically meaningful threshold of 20 ng ml^{-1}. This underscores their promise for precise and sensitive detection in clinical environments.
11	137	The aim was to demonstrate the exceptional sensitivity and accuracy of the biosensor in identifying liver cancer		The present work showcases the exceptional performance of the Au biosensor in the detection of AFP. The biosensor exhibits a notable

Table I. (Continued).

No	Auther	Aim	Method	Result
		biomarkers, emphasizing its potential for clinical applications in early cancer detection.	The Au biosensor was utilized to detect AFP, showcasing a remarkable limit of detection of 1.5 ng ml^{-1} in a saline buffer.	limit of detection of 1.5 ng ml^{-1} in a saline buffer, hence highlighting its high sensitivity and accuracy in identifying liver cancer biomarkers.
12	138	The aim was to highlight the immunosensor's exceptional performance in detecting AFP with high sensitivity and accuracy across a wide concentration range, demonstrating its potential for precise quantification in clinical applications.	Linear range spanning from 10–5 ng ml^{-1} to 50 ng ml^{-1}	The electrochemiluminescence (ECL) immunosensor exhibited remarkable sensitivity and precision in the identification of AFP, displaying a broad linear range spanning from 10–5 ng ml^{-1} to 50 ng ml^{-1}.
13	121	The aim of this study was to develop a fast, accurate, and flexible method for detecting AFP, the specific marker of hepatocellular carcinoma (HCC), to improve diagnosis and treatment of cancer.	A novel SERS biosensing platform combining a target-responsive DNA hydrogel and surface-enhanced Raman scattering (SERS) was constructed for the sensitive detection of α-fetoprotein (AFP), utilizing the specific recognition of AFP by an aptamer within the DNA hydrogel, controlled release of encapsulated immunoglobulin G (IgG), and formation of sandwich-like structures with SERS probes and biofunctional magnetic beads.	The proposed method exhibited a wide detection linear range (50 pg ml^{-1} to 0.5 μg ml^{-1}) and a low detection limit of 50 pg ml^{-1}, demonstrating ultrahigh sensitivity and promising potential for detecting tumor markers with convenience and cost-effectiveness.
14	128	The aim of this study was to develop a novel method for one-spot simultaneous detection of AFP and miRNA-122, key markers of hepatocellular carcinoma, using a label-free sensor approach, aiming at practical applications in cancer research.	An intelligent and label-free sensor utilizing resonance light scattering (RLS) was employed for simultaneous detection of hepatocellular carcinoma markers AFP and miRNA-122, where cDNA1 hybridized with cDNA2 to form double-stranded DNA (dsDNA) and interacted electronically with methyl violet to form the RLS sensor.	The detection limits of AFP and miRNA-122 were determined to be 0.94 $\mu g\, l^{-1}$ and 98 pM respectively, with good linear ranges achieved (5 to 100 μg/l for AFP and 200 pM to 10 nM for miRNA-122). In the presence of AFP and miRNA-122 mixtures, the RLS signal increased due to AFP binding to its aptamer and decreased due to miRNA-122 binding to its complementary strand, enabling one-spot simultaneous detection of both markers and indicating potential practical applications in hepatocellular carcinoma research.

integrated into wearable devices for continuous monitoring of health markers. This could allow for early detection and prevention of diseases, as well as real-time treatment monitoring. In general, the future of electrochemical biosensors for detecting AFP is bright, with the potential for practical, sensitive, and specific diagnostic devices that could revolutionize the field of clinical diagnosis and personalized medicine. These devices can potentially revolutionize how AFP and other biomarkers are detected and monitored, particularly in resource-limited settings where admission to traditional laboratory testing is limited. Persistent research and development in this field are projected to consequence in even more advantages in the future, with the potential to enhance patient outcomes and protect lives.[64]

Via extensive academic study and commercial promotion, the Internet of Things (IoT) has significantly enhanced human life across several fields via the development of diverse applications such as smartphones, intelligent monitoring systems, home security systems, and wearable electronic gadgets. The notion of IoTs, which refers to the connection of objects or individuals to the Internet via operational nodes, has seen significant growth across diverse domains such as intelligent transportation, smart environment, urban development, industrial production, augmented reality (AR), virtual reality (VR), and others.[139] The wireless sensor network serves as the fundamental infrastructure of the IoT, often consisting of over one billion sensors and electrical devices.[140] The proliferation and utilization of IoTs have led to a significant rise in the quantity of wireless sensor nodes, resulting in a substantial increase in the overall power consumption of electronic devices and batteries inside IoT networks.[141] Self-powered sensors/systems without power supply have emerged as the most viable and sustainable solutions to address the growing need for multiple sensors in the IoT and the associated high-power-consuming concerns. The integration of Internet of Things (IoT), Artificial Intelligence (AI), Machine Learning (ML), and Green Nanotechnology with sensor technologies signifies a groundbreaking domain that has significant ramifications for diverse sectors. The convergence described herein has the potential for unparalleled capabilities in the domains of data collecting, processing, and sustainability. The purpose of this introduction is to examine the future potential of these technologies, using references to papers that emphasize their potential synergies and applications.[140,142]

In addition, the integration of Green Nanotechnology is a viable and environmentally conscious strategy for the advancement of sensor technology. The field of Green Nanotechnology is centered on the use of ecologically sustainable methods in the development, production, and utilization of nanomaterials. Through the use of nanotechnology, it is possible to design sensors that exhibit enhanced efficiency, decreased energy usage, and diminished environmental effect.[143]

In order to further investigate these revolutionary patterns, we shall consult papers that examine the mutually beneficial connection between these technologies. These articles provide insightful viewpoints on the transformative impact of IoT-enabled sensors, which are augmented by AI and ML algorithms and constructed using environmentally friendly nanomaterials. These sensors are revolutionizing several sectors and promoting sustainable advancements.[144] This introduction establishes the foundation for a thorough examination of the incorporation of Internet of Things (IoT), Artificial Intelligence (AI), Machine Learning (ML), and Green Nanotechnology into sensor technologies. It highlights the potential future consequences of this integration and draws upon pertinent academic literature to provide valuable insights.[145]

Conclusions

Electrochemical biosensors have emerged as highly promising implements for the detection of alpha-fetoprotein (AFP). Conventional AFP detection methods often need more need more complex and time-consuming procedures, limiting their clinical applicability. However, current advances in electrochemical biosensors have defeated these conditions by integrating innovative materials, advanced fabrication techniques, and novel recognition strategies. The incorporation of materials has significantly improved the performance of electrochemical biosensors for AFP detection. AuNPs possess unique optical properties and an excellent surface area, enabling effective biomolecule immobilization and improving sensitivity and selectivity. With its exceptional electrical conductivity and massive surface area, GO facilitates precise immobilization and improves detection sensitivity. CNTs offer excellent electrical properties, high surface area, and biocompatibility, contributing to biosensor sensitivity and stability. Conducting polymers, known for their porosity and functional groups, provide binding sites for AFP capture, enabling sensitive detection. Integrating nanotechnology, materials science, and bioinformatics with electrochemical biosensors has further propelled the detection of AFP. Nanotechnology allows the fabrication of biosensors with enhanced sensitivity and selectivity. Despite significant progress, challenges persist in electrochemical biosensors for AFP detection. Interference from other biomolecules in blood samples can lead to false-positive or false-negative results, which affects accuracy. The cost of equipment required for electrochemical biosensing may be a barrier in resource-limited settings. Addressing these challenges requires further research and development. Electrochemical biosensors for AFP detection hold immense potential in personalized medicine and targeted therapies. Ongoing advances in electrochemical biosensors, along with improved affordability and accessibility, can contribute to improved patient outcomes and overall health care. Amperometry, voltammetry, and impedance spectroscopy are precious electrochemical techniques for AFP detection. These techniques enable sensitive and rapid analysis, which holds promise for developing point-of-care devices and screening platforms that facilitate early intervention and improved patient outcomes. Continued research and advancements in electrochemical techniques will further enhance their utility in early disease detection, ultimately leading to improved health outcomes.[44] Additionally, the versatility of electrochemical biosensors extends beyond AFP detection, encompassing a wide range of applications in healthcare, environmental monitoring, and industrial processes. These biosensors have been utilized for the detection of various biomolecules, including proteins, nucleic acids, hormones, and neurotransmitters, enabling early diagnosis and monitoring of diseases such as cancer, infectious diseases, and metabolic disorders. Moreover, their potential for rapid, on-site analysis makes them invaluable for environmental monitoring, detecting pollutants, toxins, and pathogens in water, soil, and air. In the industrial sector, electrochemical biosensors play a crucial role in quality control, process optimization, and safety monitoring, facilitating real-time analysis of chemical compounds, gases, and contaminants. As technology continues to advance, the integration of electrochemical biosensors with artificial intelligence and IoT technologies holds promise for the development of smart, autonomous sensing systems capable of continuous monitoring and data analysis. This convergence of technologies opens up new opportunities for personalized medicine, precision agriculture, and smart manufacturing, revolutionizing how we monitor and manage our health, environment, and industrial processes.

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