Novel Electrochemical Biosensing Strategy for Antibody IgG detections

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Abstract

The human immune system is a complex network of cells, tissues, and organs that work together to protect the body from harmful pathogens. Antibodies, also known as immunoglobulins (Ig), are small proteins that play a vital role in the immune system’s defense mechanism. Among the five classes of immunoglobulins, Immunoglobin G (IgG) is the most abundant and widely studied. This article provides an in-depth overview of the basics of IgG, including their chemical and physical properties and their roles in the human immune system. The article then focuses on the critical biosensor working principles with an emphasis on electrochemical biosensors. Biosensors are analytical devices that convert a biological response into an electrical signal, allowing for rapid and sensitive detection of specific analytes. The use of biosensors for IgG detection has gained significant attention in recent years due to their sensitivity, specificity, and ability to detect IgG in real-time. This article also discusses many novel strategies that have been reported in the literature for sensitive IgG detection. These strategies include the use of different biorecognition elements, such as antibodies, aptamers, enzymes, and biomimetic materials. Moreover, the article concludes by highlighting recent research advances and future directions for sensitive IgG detection, such as the use of nanomaterials and adaptable machine learning models, leading to a more efficient method of IgG detection.
What is IgG (Immunoglobin G)?

Antibodies, also known as immunoglobulins (Ig), are small proteins that play a vital role in the human immune system. They are produced by plasma cells upon exposure to foreign substances called antigens. These antigens could be viruses, bacteria, toxins, or any other foreign entity that enters the body. Antibodies recognize and bind to these antigens, leading to their elimination from the body. In fact, antibodies constitute 20% of plasma protein in the human body. The discovery of antibodies is attributed to Emil von Behring, who discovered antitoxins for diphtheria, tetanus, and anthrax.

Antibodies are classified into five different types based on their structure and function. These types are Immunoglobulin M (IgM), Immunoglobulin D (IgD), Immunoglobulin G (IgG), Immunoglobulin A (IgA), and Immunoglobulin E (IgE). IgG is the most abundant antibody and accounts for 10-20% of plasma proteins and 70-75% of the total immunoglobulins in plasma. IgG is further classified into subclasses based on their abundance in the body, which include IgG1, IgG2, IgG3, and IgG4. These subclasses differ in their primary amino acid sequences and structural differences in the hinge and heavy chain constant regions.

The basic structure of an antibody consists of two light chains and two heavy chains. The heavy chains are indistinguishable from each other and form the Y-shaped compact
structure along with two similar light chains. The constant region and variable region of light chains differ, and the heavy chains vary for each subtype of an antibody. Antibodies are composed of 82-96% protein and 4-18% carbohydrate. The specific effector functions of antibodies vary based on their heavy chain structure. In summary, antibodies are essential components of the immune system that provide protection against foreign substances, and their classification and structure enable targeted responses to specific antigens.
IgG Basics

Immunoglobulin G (IgG), one of the most abundant antibodies in the human body, has a molecular mass of approximately 160 kDa, which is due to the presence of two light chains and two heavy chains. The Fab region present within the IgG structure contains a paratope and can help in pathogen inhibition through recognition events, while the Fc region is responsible for interacting with different accessory molecules to regulate indirect effector operations.\(^{12,14–16}\) These operations include the complement-dependent cytotoxicity mechanism (CDC), antibody-dependent cellular cytotoxicity, and phagocytosis (ADCC and ADCP). The structure of IgG subtypes and allotypes has been extensively studied for more than a half century, and the differences have been illustrated in other literatures, with the different regions and various components of IgG.\(^{17–19}\) Schematics for the structure of other immunoglobins such as IgE, IgD, IgA, and IgM have also been provided in literature.\(^{17,19–21}\) This detailed understanding of the structure of immunoglobulins has led to the development of therapies such as monoclonal antibodies, which target specific antigens and have shown promising results in the treatment of various diseases.

The human immune system is a complex network of cells, tissues, and molecules that work together to defend against foreign invaders such as bacteria, viruses, and parasites. Based on the type of molecules involved in providing the defense mechanism, human immunity
can be categorized into two types, cell-mediated immunity and humoral immunity.\textsuperscript{22–25} Cell-mediated immunity involves the role of T cells, which directly attack infected cells, while humoral immunity works through the use of antibodies. Antibodies, also known as immunoglobulins, are small proteins produced by the immune system that bind to foreign substances known as antigens, and play a crucial role in defending against infections. Of all the immunoglobins present in the plasma, IgG is an important class of antibodies that constitutes 75\% of them, and plays a key role in humoral immunity.\textsuperscript{25–27} IgG has the ability to bind with a diverse number of molecules due to the primary amino acid sequences, which diversifies its capability to tackle pathogens. The defense mechanism of IgG against pathogens is complex and involves multiple mechanisms, including neutralization of toxins, opsonization, and complement activation. Neutralization of toxins involves the ability of IgG to bind to toxins and render them harmless, while opsonization involves the coating of pathogens with IgG, which then facilitates their recognition and phagocytosis by immune cells. Complement activation involves the binding of IgG to pathogens, which then triggers the activation of the complement system, leading to the destruction of the pathogen. These mechanisms show the different ways in which IgG interacts with pathogens to protect the host.\textsuperscript{28,29} In addition to its role in defending against infections, IgG also plays a vital role in various other physiological processes such as placental transport, immune complex formation, and inflammation. The high abundance of IgG in plasma and its diverse mechanisms of action have made it an important target for
diagnostics and therapeutics. IgG-based serological tests have become important biomarker tools for diagnosing and monitoring infectious diseases such as COVID-19, and the therapeutic potential of IgG has been demonstrated in various diseases such as autoimmune disorders and cancer. The importance of IgG in improving immunity, treating pathological diseases, and serving as a biomarker, as well as its role in vaccines and biosensors for diagnostics, underscores its critical role in human health.

In addition to its role in defending against infections, IgG also plays a vital role in various other physiological processes such as placental transport, immune complex formation, and inflammation. The high abundance of IgG in plasma and its diverse mechanisms of action have made it an important target for diagnostics and therapeutics. IgG-based serological tests have become important biomarker tools for diagnosing and monitoring infectious diseases such as COVID-19, and the therapeutic potential of IgG has been demonstrated in various diseases such as autoimmune disorders and cancer. The importance of IgG in improving immunity, treating pathological diseases, and serving as a biomarker, as well as its role in vaccines and biosensors for diagnostics, underscores its critical role in human health.
**IgG as critical biomarkers for disease diagnosis**

The National Institute of Health provides a definition of biomarkers as a "characteristic used to measure and evaluate objectively normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention." This definition highlights the potential of biomarkers to personalize medicine. They offer a variety of benefits, including the early detection of diseases like cancer, and providing diagnostic, prognostic, and predictive information. Biomarkers also support the assessment of risk and disease monitoring in high-risk populations, and can be applied to guide targeted therapy and differential diagnosis. Additionally, biomarkers can be used to monitor the response to therapy, providing valuable information for adjusting treatment plans and improving patient outcomes. The use of biomarkers is an exciting area of research that offers a wealth of opportunities for improving the practice of medicine.

Discovering protein biomarkers in patient blood is ideal due to easy access to the sample. However, the high dilution of protein biomarkers released into the circulation from disease-targeted organs or tumors makes it challenging to measure relevant protein biomarkers in blood (serum or plasma) with the required sensitivity. Consequently, this has hindered protein biomarker discovery in serum or plasma. To overcome this constraint, biomarker discovery can be directly performed in tissue biopsies where
expression levels are highest, and biomarkers can be later translated into higher sensitivity technologies in plasma, as described in the following section. There are several antibody-based technologies that can be used for biomarker discovery and validation. One of the most attractive options is based on highly multiplexed protein immunoassays.\textsuperscript{46-49} These immunoassays use antibodies specific to multiple biomarkers, allowing for the simultaneous measurement of numerous proteins in a single sample. These assays are sensitive, precise, and can measure protein biomarkers in various sample types, including serum, plasma, and tissue. Therefore, they are widely used in biomarker discovery and validation, allowing for the identification of potential diagnostic and therapeutic targets for various diseases, including cancer.

Numerous studies have highlighted the importance of IgG as a biomarker for various pathological conditions. For instance, the outbreak of SARS-CoV-2 has once again brought attention to IgG serum levels as they fluctuate upon infection. Sun et al. investigated the response of IgG and IgM glycoproteins in COVID-19 patients and found elevated levels of N and S protein-specific IgG and IgM after the onset of symptoms in non-ICU patients.\textsuperscript{50} While serological methods cannot be used in the initial stages of infection, they can diagnose the disease in later stages. Hence, IgG can be used to identify people who have had the disease without showing symptoms. Hou et al. reported that quantifying IgG antibodies could be a useful tool to assess the prognosis and severity of
COVID-19 infection. \textsuperscript{51} Zhang et al. suggested that immune response isotyping based on IgG response and NLR could help differentiate COVID-19 patients based on disease severity. \textsuperscript{52} In conclusion, IgG is a vital biomarker associated with various diseases. It plays a crucial role in humoral immunity and diversifies its capabilities to combat pathogens by binding to a diverse range of molecules. With advancements in serological tests, IgG has become an important tool for diagnosis, prognosis, and evaluation of disease severity, and its significance in vaccine development and biosensing cannot be overlooked.
Immuno-biosensor Basics

Biosensors are devices that are both sensitive and compact, and they measure biomolecules (called analytes) using various chemical or biological reactions to generate multiple output signals.\textsuperscript{53,54} For several decades, biosensors have been used in the healthcare, food, and environmental monitoring industries.\textsuperscript{55–59} In the healthcare sector, biosensors are used for disease diagnosis and treatment, as well as in research labs.\textsuperscript{32} The basic components of a typical biosensor include:

**Analyte or substrate:** The biomolecule to be identified, such as the IgG antibody, which can be detected using various biosensors.

**Receptor or biorecognition element:** These molecules have an affinity to bind to the analyte. Examples include enzymes, antibodies, antiantibodies, aptamers (DNA or RNA), and proteins. The binding of an analyte to a receptor is known as biorecognition. Biosensors can be classified based on the specific biorecognition element used.

**Transducer:** These devices help convert the biorecognition phenomenon into a measurable output signal, a process known as transduction or signalization. Biosensors can be categorized based on the various transduction events they employ.
**Electronic signal output**: This part of a biosensor processes the transduction mechanism and prepares it for display. Complex electronic circuits amplify the transduction signals from analog to digital form.

**Results display** to users: This combination of hardware and software presents the data in a readable form, such as graphs, numeric values, images, or tables. Figure 6 shows a complete schematic of a biosensor's components.
**Novel Electrochemical IgG Biosensors**

Numerous studies have investigated the potential of electrochemical biosensing to detect IgG with unique, selective, sensitive, and cost-effective characteristics.

**Impedance Spectroscopy IgG Biosensors**

Impedimetric biosensors are a type of electrochemical sensor that can detect changes in capacitance and conductance on the surface of an electrode. These biosensors can be classified into different types based on the biorecognition element used, including cell-based impedimetric biosensors, immunobinding-based biosensors, nucleic acid-based biosensors, and enzyme-based biosensors. The distinct characteristics of each type of impedimetric biosensor, such as sensitivity, selectivity, and reproducibility, enable them to detect multiple biological molecules. Thanks to their unique properties, impedimetric biosensors have found widespread use in a variety of applications.

A new polydopamine-silver nanoparticle-polydopamine-gold (PDA-AgNPs-PDA-Au) film based surface plasmon resonance (SPR) biosensor for horse IgG detection is reported. The biosensor was constructed via layer-by-layer assembly and built on Au-film. Ag ion was reduced in situ to AgNPs in the presence of PDA. The top PDA layer was used to
prevent AgNPs from being oxidized and connect with antibody directly via Schiff alkali reaction. The morphology and thickness of the modified gold film were characterized using scanning electron microscope and Talystep. The experimental results showed that the PDA-AgNPs-PDA-Au film sensing platform is stable, regenerative, and sensitive for horse IgG detection. The detection limit of horse IgG obtained with the present biosensor is 0.625 μg mL\(^{-1}\), which is 2-fold and 4-fold lower than that obtained with biosensor based on PDA modified Au film and conventional biosensor based on MPA, respectively. Furthermore, when challenged to real serum samples, the sensor exhibited excellent specificity to horse IgG, indicating its potential for industrial application. In this study, the researchers used non-toxic polydopamine for more effective immobilization of the antibody onto the sensing platform, instead of conventional chemical reagents. AgNPs were used as a signal-enhancing label, and the antibody was immobilized via Schiff alkali reaction directly. The new PDA-AgNPs-PDA-Au film-based SPR biosensor exhibited 4-fold and 2-fold lower detection limit compared with that of the PDA modified Au film-based biosensor and MPA-based conventional SPR biosensor, respectively. Furthermore, when challenged with chicken serum samples spiked with horse IgG, the sensor exhibited acceptable recoveries. The new sensor is remarkably stable and regenerative. The findings of this study show the potential of this new SPR biosensor to be used for practical purposes. However, further investigation is required to build sensor chips based on this new sensor for industrial application.
Gold electrodes are commonly used in electrochemical biosensors due to their well-described functionalization process with thiols and good chemical stability. However, the use of the commonly used redox-pair in electrochemical impedance spectroscopy, Hexacyanoferrate (II)/(III), can lead to corrosion of the gold electrodes, which damages the surface modification and alters the sensing signals. To address this issue, the authors introduced the in-situ generation of Hexaammineruthenium (II)/(III) as a redox-pair during the impedimetric measurement by applying a DC-bias. The DC-bias was chosen to supply Hexaammineruthenium (II) in a suitable concentration at the electrode surface by reducing Hexaammineruthenium (III). In this study, the stability of photolithographically fabricated thin-film and screen-printed gold electrodes was compared in Hexacyanoferrate and Hexaammineruthenium solutions. Long-time characterization of the electrochemical properties with cyclic voltammetry and electrochemical impedance spectroscopy revealed that Hexaammineruthenium (II)/(III) was an excellent redox-pair for stable impedimetric measurements with gold electrodes. To demonstrate the suitability of Hexaammineruthenium for biosensing, the authors applied it for the impedimetric detection of human-IgG. This biosensor exhibited a linear range from 11.3 ng/mL to 113 μg/mL, which is a suitable range for diagnostic applications. The use of in-situ generated Hexaammineruthenium (III) as an alternative redox-probe to replace HCF in electrochemical impedance spectroscopy for the detection of biomolecules using gold
electrodes significantly improved the stability of the sensor and reduced the risk of false sensor signals. 67 Furthermore, Hexaammineruthenium (III) was applied as a redox-probe to realize a human-IgG biosensor based on EIS, and the measured signals were stable, showing a good linear correlation to the logarithm of IgG-concentrations over five decades. This study has successfully introduced Hexaammineruthenium (III) as an alternative redox-probe to replace HCF in electrochemical impedance spectroscopy for the detection of biomolecules using gold electrodes. The use of a DC-bias during the EIS measurement generated Hexaammineruthenium (II)/(III) and did not cause a degradation of the gold electrodes, allowing recording of stable EIS signals for at least 60 minutes. The RCT value decreased only by 10%, in comparison to a variation between 100% and 140% when HCF was used as a redox-pair measuring a constant analyte concentration. Neither the production method nor the surface coverage of the sensor resulted in sufficiently stable gold electrodes for EIS measurements with HCF as a redox-probe. The authors believe that additional optimization of the functionalization and the DC-bias can improve the sensor performance even further. An important next step will be the application of Hexaammineruthenium for EIS biosensing in biological samples such as serum and saliva, which will further validate the effectiveness of this alternative redox-probe. 67 Overall, this approach could lead to the employment of the EIS technique as a reliable biosensing method for point-of-care testing applications.
Glycosylphosphatidylinositol anchored proteins (GPI-APs) are a type of natural conjugate found in the plasma membrane of eukaryotic cells. They are formed by attaching a glycolipid to the C-terminus of many proteins and play a crucial role in cell signaling and adhesion, with implications for health and diseases. On the surface of the protozoan parasite Toxoplasma gondii, both GPI-APs and GPls without protein (free GPls) are found in abundance. Serological assays have been developed to detect anti-GPI IgG and IgM antibodies to differentiate between toxoplasmosis patients and healthy individuals. However, these methods have limitations such as poor efficiency, cross-reactivity, and the need for sophisticated laboratory equipment and qualified personnel. To mitigate these limitations, a label-free electrochemical glycobiosensor has been established for the detection of anti-GPI IgG and IgM antibodies in serum from toxoplasmosis seropositive patients. The biosensor uses a synthetic GPI phosphoglycan bioreceptor immobilized on screen-printed gold electrodes through a linear alkane thiol phosphodiester. The antigen-antibody interaction is detected and quantified by electrochemical impedance spectroscopy (EIS). The glycobiosensor has a linear dynamic range of anti-GPI antibodies in serum ranging from 1.0 to 10.0 IU mL−1, with a limit of detection of 0.31 IU mL−1. This method not only allows for the detection and quantification of IgG and IgM anti-GPI antibodies for the diagnosis of toxoplasmosis, but also holds great potential for the detection of IgG antibodies related to other medical conditions characterized by overexpression of antibodies. The electrochemical method offers new opportunities for
diagnosis in decentralized settings. The high performance of the glycobiosensor has been demonstrated by detecting a concentration of antibodies of clinical relevance in serum samples and determining positive serostatus reactive against T. gondii. An augmented glycobiosensor response was related to an increase of toxoplasma antibodies, thus demonstrating that the method is suitable for the diagnosis of acute toxoplasmosis. In summary, the glycobiosensor provides a promising approach for the diagnosis of various medical conditions based on antibody detection, including toxoplasmosis.

Another recent study discussed the development of a novel impedimetric immunosensor design for detecting dengue virus (DENV) IgG antibodies using plant-derived antigenic glycoprotein. The electrochemical immunosensor platform was constructed using a screen-printed carbon electrode (SPCE) modified with a graphene/titanium dioxide (G/TiO2) nanocomposite to enhance the electrode's electrochemical performance and specific surface area. The researchers used a plant-derived dengue envelope domain III (EDIII) protein as the antigenic probe protein in their immunosensing strategy. The immunosensor demonstrated high sensitivity towards DENV IgG in a wide linear working range (62.5–2000 ng/mL) and a limit of detection of 2.81 ng/mL under optimised sensing conditions. The immunosensor showed high specificity for distinguishing DENV IgG from antibodies of other infectious diseases, including the closely related Zika virus (ZIKV). To verify the reliability of the immunosensor in serological diagnosis, the team challenged it
against serum samples and compared the results to conventional enzyme-linked immunosorbent assay (ELISA). Based on its remarkable performance throughout the study, the team proposes the devised immunosensor as a reliable and practical diagnostic tool for serological detection of dengue in realistic applications. In summary, the development of an impedimetric immunosensor based on the G/TiO2 nanocomposite modified electrode platform for detecting DENV IgG was reported. The researchers optimized various sensing parameters to improve sensitivity and selectivity, using a plant-based cEDIII peptide as a probe. The immunosensor demonstrated high sensitivity towards DENV 1–4 IgG detection, with a limit of detection of 2.81 ng/mL within a linear working range of 62.5 ng/mL to 2 μg/mL. Additionally, the immunosensor exhibited high specificity for discriminating DENV IgG against antibodies of other infectious diseases, including the similarly structured ZIKV. The immunosensor was stable and had an acceptable shelf-life, with only a minute signal decrease over 15 days. The researchers also investigated the analytical performance of the immunosensor in detecting dengue antibodies in serum samples with the aid of an optimised blocking protocol. The immunosensor produced distinctive readings to identify DENV IgG-positive samples in a wide working range from a dilution factor of 1:500 up until 1:32,000, and the results were comparable to readings from conventional ELISA assays. The key elements of the immunosensor offer attractive merits on their own, including the large specific area and excellent catalytic properties of G/TiO2 nanocomposites, the scalability and low cross-
reactivity of plant-based cEDIII, and the rapid response, miniature size, and low costs of electrochemical biosensors. Combining all these qualities, a promising analytical tool with good sensitivity and specificity could be developed for dengue diagnosis, which holds great potential to be further developed into a point-of-care diagnostic assay for clinical use.
**Amperometric IgG Biosensor**

Amperometric biosensors typically use a working electrode made of a noble metal such as gold, titanium, or nickel, or coated carbon with bioreceptor elements. Indium tin oxide (ITO) is also a commonly used material for this purpose. When a potential is applied, the enzymatic conversion or absorption of proteins on the surface of the electrode generates current. This current is directly proportional to the concentration of the analyte and can be used to quantify its presence. The sensitivity and selectivity of the biosensor depend on the bioreceptor used and the properties of the electrode material. The amperometric biosensor has found wide applications in clinical diagnostics, environmental monitoring, and food safety due to its high sensitivity, rapid response, and ease of use.

In a recent study, the construction of immunosensors based on graphite-epoxy with RlG incorporated into the composite matrix is presented. To improve the electrochemical properties of the immunocomposite electrodes, electrochemical impedance spectroscopy and cyclic voltammetry were used for characterization and optimization. The optimal proportion of the transducer material (graphite-epoxy ratio) was determined to be between 16% and 17% with a constant amount of RlG. The optimal composition range provides high electron-transfer rate, high signal-to-noise ratio, and suitable sensitivity.
The analytical properties of the immunosensors were evaluated by measuring RIgG using a competitive assay and alkaline phosphatase-labeled antibody. Amperometric measurements were performed using hydrogen peroxide as a substrate. For the first time, an optimization of the antigen-antibody ratio used in the assay was performed, which significantly reduced the immunological species used. An electrochemical immunosensor based on graphite-epoxy-RIgG was constructed, characterized, and optimized using EIS and CV techniques. Superior sensors were obtained with respect to analytical properties using these electrochemical techniques. The optimized immunosensors showed an enhanced analytical signal, better stability, and higher signal-to-noise ratio compared to the non-optimized ones. Although the sensitivity was reduced, the detection limit achieved was one order of magnitude lower. The optimized immunosensors also allowed the detection of lower RIgG concentrations (0.0069 µg/mL and 0.0014 µg/mL) compared to the standard composition of 20% graphite loading (not detectable) when GaRIgG-HRP concentration was 0.017% (v/v). Based on the results obtained in this work, future studies will focus on the development of immunosensors based on composites for determining human IgG at low concentrations for the diagnosis of diseases. Overall, this study provides valuable insights into the optimization of electrochemical immunosensors and their potential applications in the field of disease diagnosis.
Potentiostatic IgG Biosensors

Potentiostatic biosensors are a type of electrochemical biosensor that utilize a potentiostat to detect the voltage difference between the working electrode (WE) and reference electrode (RE). This is achieved by adjusting the potential difference of the counter electrode with respect to the working electrode, while the resultant potential difference is determined by the concentration of an analyte. A new light-addressable potentiometric sensor (LAPS) system was developed for detecting the model biomarker, human immunoglobulin G (hIgG). The system utilized goat anti-human immunoglobulin G antibody as a recognition element that was covalently immobilized on the surface of the LAPS chip. The light-addressable capability of the LAPS system enabled the detection of hIgG dissolved in the supporting electrolyte solution by monitoring potential shifts of the sensor. To produce a stable photocurrent, a laser diode controlled by a field-programmable gate array was used as the light emitter to drive the LAPS. The potential shift response showed a linear correlation with the concentration of hIgG, with a regression equation of \(\Delta V (V) = 0.00714ChIgG (\mu g/mL) - 0.0147\) and a correlation coefficient of 0.9968 over a range of 0–150 \(\mu g/mL\). The X-ray photoelectron spectroscopy data confirmed that the hIgG antibodies were successfully immobilized on the LAPS chip surface, and hIgG was captured by the goat anti-human IgG. Specificity tests proved a highly specific interaction between hIgG and its antibodies. Furthermore, the LAPS system
demonstrated acceptable stability and reproducibility, making it more applicable to the
detection of disease biomarkers. This study proposed a promising strategy for the
detection of multi-biomarkers by extending the sensing surface membrane lifetime and
fabricating array LAPS biosensors in the future. The LAPS system reported here offers a
simple operation and multiple-sample format, which could have great potential in various
environmental, food, and clinical applications.
Conductometric IgG Biosensors

Conductometric sensors are a type of biosensor that detect changes in conductivity resulting from a biological event in an analyte solution [65]. These sensors have the ability to detect both electroactive and non-electroactive species, and the conductivity electrodes can either be in direct contact with the solution or insulated by a thin layer [66]. Conductometric sensors have found a wide range of applications, particularly as gas sensors and for evaluating the quality of engine oil. Due to their ability to detect a wide range of analytes and their ease of use, conductometric sensors have become increasingly popular in many fields. They have proven to be useful in detecting changes in the properties of a wide range of substances, making them a valuable tool in research, industry, and medical diagnostics.  

Conductometric biosensors are highly advantageous due to their ability to operate at low working voltages, which not only saves energy, but also reduces the risk of electrochemical reactions that can interfere with the accuracy of measurements. Furthermore, they do not require the use of a reference electrode, which simplifies their design and operation. These sensors can quickly analyze samples, which is a critical feature in time-sensitive applications such as medical diagnostics. Conductometric biosensors are also known for their high sensitivity and precision for detection of biological molecules, including both electroactive and non-electroactive species. Another advantage is that they can be produced using low-cost and
easily accessible technology. These features make conductometric biosensors highly suitable for various applications, including gas sensing, food quality control, and medical diagnostics.

The Virus BioResistor (VBR) is an innovative biosensor that can detect small protein disease markers in human urine within one minute with high sensitivity and precision. The VBR uses engineered virus particles as receptors to selectively bind the protein of interest, which are entrained in a conductive PEDOT channel. The sensitivity of VBRs is inversely related to the molecular weight of the protein target, and large proteins, such as IgG antibodies, cannot be detected even at high concentrations. To address this limitation, the PEDOT channel of the VBR is subjected to a simple electrochemical process called potentiostatic overoxidation, which reduces the conductivity of the polymeric channel. The resulting biosensors, known as O²VBRs, exhibit enhanced sensitivity to both small and large proteins. For instance, two undetectable antibodies at normal VBRs can be detected using O²VBRs with a limit of detection of 40 ng/mL and a dynamic range for quantitation extending to 600 ng/mL. The increased sensitivity of O²VBRs is likely due to the strong and disproportionate increase in resistance of the PEDOT-PSS base layer of the channel relative to the virus-PEDOT sensing layer. However, the study does not exclude the possibility of the influence of significant morphological changes caused by the oxidation process on sensitivity, such as increased porosity or mean pore diameter of the virus-
PEDOT layer. The SEM and AFM images show significant changes in the topography of the virus-PEDOT layer caused by overoxidation, but it is uncertain if they affect the ability of large proteins to permeate the layer. In conclusion, the Virus BioResistor is an excellent biosensor for detecting small protein disease markers, and the O\textsuperscript{2}\textsubscript{VBRs} with enhanced sensitivity offer a promising approach to detecting larger proteins such as antibodies. The electrochemical process of potentiostatic overoxidation can dramatically increase the sensitivity of the VBRs, and further studies are needed to explore the mechanism and morphological changes caused by this process.

Researchers have developed a biosensor that uses a single polyaniline (PANI) nanowire to detect the cardiac biomarker myoglobin (Myo) and immunoglobulin G (IgG). The single PANI nanowires were grown using an electrochemical method between a pair of patterned electrodes. The nanowires were then functionalized with monoclonal antibodies (mAbs) specific to Myo or IgG using 1-ethyl-3-(3-dimethyaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimde (NHS) to form strong covalent bonds between the mAbs and the PANI nanowires. The researchers verified the functionalization using Raman spectroscopy and fluorescence microscopy. By measuring the conductance change of the functionalized single PANI nanowires, the researchers were able to detect the target proteins of IgG and Myo. The biosensor demonstrated excellent specificity towards the expected target detection, with no response observed when exposed to a
non-specific protein. The detection limit was found to be 3 ng/mL for IgG and 1.4 ng/mL for Myo, and the biosensor exhibited a fast response time of a few seconds. The single PANI nanowire-based biosensor is a promising technology for the detection of cardiac markers and other proteins, owing to its high sensitivity, good specificity, and fast response time. The surface immobilization technique employed in this research could also be applied to the detection of other mAbs for various protein detection tests, including cancer markers and other cardiac markers. Overall, this study demonstrates the potential of PANI nanowires as a highly sensitive and specific biosensor platform for protein detection.

In response to the urgent global need for precise, sensitive, and rapid diagnostic platforms capable of detecting contagious pathogenic viruses such as SARS-CoV-2, researchers have developed an ultra-precise fast diagnostic platform. 75 The platform is capable of detecting monoclonal IgG antibodies against the S1 protein of SARS-CoV-2 within infected patients’ blood specimens with COVID-19 in about 1 minute. The electrochemical-based nanosensor consists of a highly activated graphene-based platform in conjunction with Au nanostars, which can detect SARS-CoV-2 antibodies with a detection limit (DL) and sensitivity of $0.18 \times 10^{-19}\% V/V$ and $2.14 \mu A.\% V/V.cm^{-2}$, respectively, in human blood plasma specimens, even in the presence of a high amount of interfering compounds/antibodies. The nanosensor’s sensitivity/specificity is
remarkable compared with the gold standard (i.e., ELISA assay), confirming its superb performance. In this study, the researchers developed an improved label-free nanosensor composed of activated graphene oxide (GO) in conjunction with Au nanostars (Au NS) (G-Au NS) toward direct detection of the monoclonal IgM antibodies against S1 glycoprotein of SARS-CoV-2 within blood specimens of infected or suspected people with the infectious disease of COVID-19. They then used the nanosensors to enhance the glassy carbon electrode (GCE) and the working electrode of DRP C110 carbon-based screen-printed electrode. The obtained data were compared with the ELISA assay to evaluate the performance of the developed nanosensors toward label-free detection of monoclonal IgG antibodies against S1 glycoprotein of SARS-CoV-2 as confident COVID-19 detection metrics. The fast person-to-person transfer of pathogenic viruses such as SARS-CoV-2 throughout the world has raised healthcare authorities' requirements for developing precise, rapid, and sensitive diagnostic platforms capable of detecting SARS-CoV-2's biomarkers within biological samples. The developed diagnostic kit is a vital requirement for viral outbreaks, as it offers precise, rapid, and confident detection of infected people with the infectious disease of COVID-19. The outcomes justify the fantastic performance of the developed diagnostic kit and demonstrate its potential to address the global need for rapid and sensitive diagnostic platforms for pathogenic viruses.
Concluding marks and future perspectives

The current review article focuses on the significance of various biosensors for detecting IgG antibodies and their working principles, highlighting the importance of IgG as a biomarker tool for diagnostic applications in various pathological conditions. The review discusses the different types of biosensors, their classification based on transduction properties, and biorecognition elements. Specifically, the comparison is made between electrochemical biosensors, such as impedimetric, amperometric, potentiostatic and conductometric, which have evolved over the years with major developments in their initial use as glucose sensors for IgG detection in a variety of diseases. However, the current demand for IgG biosensors in disease prevention, such as diagnosis of prostate cancer, tumor markers, autoimmune diseases, and COVID-19, is significant. Despite the advantages, IgG biosensors have some limitations, such as detection limit, detection time, selectivity, and difficulty in maintaining a high throughput system. Advances in nanomaterial production, such as carbon nanotubes, nanoparticles, and metallic organic frameworks, have the potential to address some of these limitations. Moreover, the article highlights the balance between specificity and versatility as a major challenge in IgG biosensor development. While expensive biosensors that utilize single IgG molecules maintain specificity, making them affordable requires expanding IgG detection with protein molecules using adaptable machine learning models that are programmed
iteratively. With more suitable nanomaterials and well-designed software, handheld devices could be developed for point-of-care detection of IgG, making it possible for people to perform diagnostic tests in their own homes. Therefore, future research should focus on improving the sensitivity, selectivity, and cost-effectiveness of IgG biosensors for widespread use in disease prevention and diagnosis.
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