Advanced Non-Invasive Nanobiosensors for Point-of-Care Diagnostics: Nanomaterial-Based Detection in Saliva

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ABSTRACT

Saliva, as a non-invasive biofluid containing a wide range of biological markers, offers unique opportunities for rapid and non-invasive disease detection. Despite its complex composition and presence of impurities, the development of nanobiosensors based on graphene, gold nanoparticles, quantum dots, magnetic nanoparticles, and two-dimensional nanomaterials has enabled sensitive and rapid detection in saliva samples. These sensors include electrochemical, voltammetric, impedimetric, SPR, and field-effect transistor (FET)-based platforms, with potential integration into portable devices and nanochips. The use of aptamers, nanobodies, and peptide-based bioprobes enhances diagnostic performance.

Various nanomaterial synthesis and surface modification strategies, Cas12a-based sensors, and lateral flow (LFA) and ELISA assays were applied for the detection of proteins, viruses, and miRNAs in saliva. Results demonstrated that the developed sensors can detect targets such as SARS-CoV-2 within 20 minutes. Nanobiosensors pave the way for smart devices and personalized clinical applications, providing high potential for non-invasive detection of bacterial, viral, cancer, and cardiovascular biomarkers.

Keywords: Salvia, Nanobiosensors, Non-invasive diagnostic

1. INTRODUCTION

The focus on precision medicine enhances the need for timely diagnosis and frequent monitoring of chronic diseases. For example, the recent SARS-CoV-2 pandemic has created a strong demand for rapid detection and surveillance of viral infections [1].
The detection of protein biomarkers and antigens in saliva allows for the rapid identification of diseases or their changes in scenarios where the test result at the point-of-care is required [1].

Moreover, recent advancements in nanotechnology and biomedicine have led to the utilization of nanomaterials in various medical applications, including the detection of infections such as Helicobacter pylori, which is also carcinogenic [2].

These advances have become the focus of public health control systems, not only to prevent the spread of infectious diseases but also to enable the early detection of critical diseases through continuous health monitoring technologies [3].

This context highlights the importance of nanosensors, particularly those that are non-invasive and patient-friendly, in modern diagnostics.

1.1 The Importance of Saliva as a Diagnostic Medium *(Style: Times New Roman, 10pt, Bold, Title Case)*

Saliva is a non-invasive biological fluid that contains a wide range of biochemical markers; however, the presence of various impurities makes direct detection challenging [4]. Saliva is a complex fluid composed of proteins, urea, uric acid, bacteria, and enzymes. Studies have shown that the concentrations of certain biomarkers in saliva strongly correlate with their levels in blood, which has led researchers to describe saliva as an "extracellular blood surrogate" [4].

Saliva is an excellent candidate for continuous monitoring of electrolytes and metabolites within the oral cavity [3]. As a highly valuable biofluid, it has attracted significant attention from researchers in medical diagnostics and biosensor engineering [5]. Unlike blood, saliva collection is non-invasive, painless, rapid, low-cost, and highly suitable for routine monitoring. It contains a broad spectrum of biomarkers associated with inflammation, infectious diseases, cancer, metabolic disorders, and even pharmaceutical compounds; therefore, it is considered a promising medium for real-time diagnostics and at-home health monitoring [5].

Despite these advantages, the use of saliva faces several challenges, including the presence of large particles, cells, bacteria, bulky biomolecules, and the lack of standardized protocols for dilution or preprocessing. Under these circumstances, the development of highly sensitive and selective sensors is essential [5].

1.2. Biosensors and Their Advantages

Biosensors consist of a biologically responsive element coupled with a transducer that converts the recognition event into a measurable signal, whose magnitude is proportional to the amount of the target pathogen [2].

Traditional methods such as ELISA, despite their high accuracy, are not suitable for rapid point-of-care testing [1]. Electrochemical biosensors offer high sensitivity, rapid detection, low cost, and excellent miniaturization capability, making them ideal for detecting proteins in saliva [1].

1.2.1.Emerging Types of Biosensors:

• Electrochemical Transistors (EGTs): These devices offer high sensitivity, a simplified assay format, rapid detection, and the ability to operate with unprocessed saliva samples [1].

• Photoelectrochemical (PEC) Sensors: Owing to their high signal-to-noise ratio, PEC sensors provide a remarkably broad detection range—from femtograms per milliliter (fg/mL) to nearly milligrams per milliliter (mg/mL) [1].

• One-step Detection: The direct conjugation of redox probes to the bioreceptor reduces the number of assay steps and eliminates the need for additional reagents [1].

•Multiplexed Detection: Although simultaneous detection of multiple biomarkers is advancing, its precision still does not match that of optical methods; however, integration with microfluidics and nanocomposites shows promising potential [1].

• Microfluidic Systems and Paper-based Platforms: These technologies enable automation, sample-in–answer-out operation, device miniaturization, and user-friendly functionality [1].

1.3. Application of Nanomaterials in Diagnostics

• Nanomaterials such as gold nanoparticles, carbon nanotubes, graphene, and metal oxides are widely utilized to enhance sensor sensitivity, lower the limit of detection, and improve overall stability [1].

1.3.1.Nanomaterial-Based Strategies:

• Gold nanoparticles, carbon-based structures, and graphene derivatives (GO, MWCNTs, g-C_3N_4), along with copolymer-based materials, significantly improve electrode performance [1].

• Heterostructures and nanocomposites enhance charge-transfer efficiency at the electrode/electrolyte interface [1].

• Signal amplification methods—such as doping, two-dimensional nanomaterials, and nano-branched electrodes—facilitate more efficient mass transport and electron transfer [1].

• Nanotechnology-based sensing platforms, using mechanisms such as fluorescence, colorimetry, electrochemistry, and Surface-Enhanced Raman Scattering (SERS), provide substantial signal enhancement and improved detection capabilities [3].

1.3.2. Gold Nanoparticles (AuNPs)

Gold nanoparticles possess a characteristic red color and generate optical signals through localized surface plasmon resonance. They enhance sensitivity, specificity, and biostability. Gold nanorods, in particular, can increase analytical accuracy by up to 30-fold, and when used for SARS-CoV-2 detection, they can achieve sensitivities ranging from 95% to 100% [3].

1.3.3. Quantum Dots (QDs)

Quantum dots are semiconductor nanocrystals with strong and tunable fluorescence. They can significantly enhance detection sensitivity and are highly suitable for identifying neutralizing antibodies against SARS-CoV-2 [3].

1.3.4. Magnetic Nanoparticles (MNPs)

Magnetic nanoparticles can generate electrochemical signals and enable the separation and concentration of biomarkers through an external magnetic field [3].

1.3.5. Silver Nanowires (AgNWs) for SERS

Silver nanowires generate strong electromagnetic "hot spots" that can enhance Raman signals by several orders of magnitude, enabling highly sensitive SERS detection [3].

• Gold nanoparticles (AuNPs): Produce distinct colorimetric signals and serve as strong signal amplifiers.
• Quantum dots (QDs): Generate highly intense fluorescence signals and are well suited for detecting SARS-CoV-2 antibodies.
• Magnetic nanoparticles (MNPs): Provide electrochemical signal generation and enable sample concentration under an external magnetic field.
• Silver nanowires (AgNWs): Create SERS hot spots that dramatically enhance detection sensitivity.

With the advancement of nanosensors, diagnostic assays have become faster, more accurate, and suitable for point-of-care use—including at home or in clinical settings. These technologies have become critically important for controlling infectious diseases and enabling the early detection of serious health conditions [3].

1.4. Biorecognition Probes

Nanobodies, aptamers, and peptide-based proteins are employed for ultra-sensitive detection within the constraints of the Debye length [1]. Electroactive labels (such as polydopamine and ferrocene) enhance charge-transfer efficiency and improve overall sensor performance [1].

2. TYPES OF NANOSENSORS FOR SALIVA-BASED DIAGNOSTICS

2.1. Graphene-Based Sensors

A typical graphene-based biosensor consists of three main components:
1. Transducer: A layer of graphene or its derivatives that converts the biological interaction into an electrical signal.
2. Bioreceptor: Functional elements such as antibodies, DNA, enzymes, or aptamers.
3.Target molecule: Examples include PSA, IL-6, IL-8, and various cancer or inflammation biomarkers.
This architecture enables rapid and accurate detection without the need for additional labeling agents, offering a fully label-free sensing approach [5].

2.2. Amperometric Sensors

Amperometric sensors are capable of detecting proteins in saliva samples with minimal preprocessing. By using sensors functionalized with redox reporters on their surface, the assay can be reduced to a single step procedure. Advantages: simplicity, compatibility with on-chip integration, low cost, rapid signal transduction, and suitability for minimally prepared saliva samples [1].

2.3. Potentiometric Sensors

Potentiometric sensors, often using nanostructured electrodes coated with molecularly imprinted polymers (MIPs), are employed for measuring ions and proteins [1].
•Detection is based on changes in the open-circuit potential upon binding of the target protein.
•Rapid detection (<5 minutes) is feasible for viral proteins and protein biomarkers.
•Advantages: fast, simple, and minimal sample preparation required.
•Disadvantages: low sensitivity, limited linear range, susceptible to pH and temperature variations, requires frequent calibration, and depends on a stable reference electrode [1].

2.3.1.Further Advantages:
1.Very broad detection range.
2.Low detection limit suitable for physiological protein concentrations in saliva.
3.Rapid signal generation due to redox reactions at the electrode/electrolyte interface.
4.Design versatility and compatibility with various bioreceptors.

2.3.2.Further Disadvantages:
1.Complexity in electrode fabrication to achieve optimal photocatalytic efficiency.
2.Stability affected by pH and electrolyte ionic strength.
3.Requirement for additional active materials for the sensing mechanism, limiting automation.
4.Necessity for saliva sample preprocessing before measurement (1).

2.4. Impedimetric Sensors

These sensors employ metal oxide, carbon, or gold electrodes, which are sometimes nanostructured or graphite-based.Detection is based on steric hindrance that affects electron transfer, measured via electrochemical impedance spectroscopy (EIS).

Advantages: compatible with a variety of bioreceptors (antibodies, aptamers, MIPs), low-cost electrodes, and flexible electrode design[1].

2.5. Voltammetric Sensors
•Techniques such as differential pulse voltammetry (DPV) and square wave voltammetry (SWV) generate high signal-to-noise ratio signals, enable rapid assays, and allow straightforward signal processing.
•Gold nanoparticles (AuNPs) enhance catalytic activity, electrode conductivity, and surface area for antibody/aptamer immobilization.
•Capable of detecting proteins (e.g., SARS-CoV-2 S1, RBD, CRP) at fg–ng/mL levels.
•Graphitic nanomaterials (graphene, CNTs) are also suitable for high sensitivity and facile surface modification.
•Typical analysis time is 20–30 minutes.

2.5.1.Advantages:
1.Wide availability of nanoelectrocatalytic materials for DPV and SWV electrodes.
2.Flexibility in assay design using one or multiple bioreceptors.
3.Reduced sample preparation compared to impedimetric sensors.
4.Ability to analyze minimally processed saliva samples.

2.5.2.Disadvantages:
1.Dependence on the redox activity and diffusion kinetics of the probe.
2.Reduced charge-transfer efficiency due to surface coatings such as PEG or casein.

3.Influence of target protein orientation and polarity on electron transfer (1).

2.6. Surface Plasmon Resonance (SPR) Sensors

Surface plasmon resonance (SPR) technology has become a key tool in biosensing.
This technology enables real-time monitoring of molecular interactions without the need for labeling, offering very high sensitivity. Its applications are extensive, spanning biomedical research, pharmaceutics, disease diagnostics, and monitoring the quality of water and food [6].

The performance of these sensors relies on detecting very small changes in the refractive index. Designing optimized multilayer structures is particularly important for enhancing sensitivity and response quality [6].

2.6.1.SPR sensors have been widely applied in the detection of:
1.Diabetes biomarkers
2.Kidney disease markers
3.Biochemical compounds in clinical samples

They hold significant value for the development of next-generation diagnostic technologies [6].

3. General Comparison of Electrochemical Sensors
•Amperometric and Potentiometric Sensors: Sensitive to pH and temperature variations.
•Impedimetric and Voltammetric Label-Free Sensors: Utilize steric hindrance for protein detection, offering high accuracy even with minimally processed samples.
•EGTs and PECs (Electrochemical Transistors /Photoelectrochemical Sensors): Exhibit exceptional sensitivity, rapid response times, and allow direct analysis of saliva with minimal preprocessing.
•Analysis Time: Voltammetric sensors ~30 minutes, PEC sensors 30–40 minutes, and EGTs <15 minutes [1].

4. Applications of Nanosensors in Diagnostics

4.1.1. Application of Nanosensors in Bacterial Detection

Helicobacter pylori (H. pylori) colonizes the gastric mucosa, increasing the risk of chronic gastritis, gastric ulcers, and gastric cancer. Traditional diagnostic methods have limitations in terms of accuracy, sensitivity, cost, and ease of use in healthcare settings [2].

Early detection and treatment of this infection are essential to prevent severe complications and reduce disease transmission. Traditional invasive methods, such as endoscopy and biopsy, are inconvenient and costly, while non-invasive approaches suffer from limited accuracy. Therefore, there is a critical need for rapid, sensitive, point-of-care (POC) biosensors, especially in resource-limited regions [2].

The use of two-dimensional (2D) nanomaterials facilitates the development of biosensors that are both reliable and rapid, while remaining cost-effective [2]. 2D nanomaterials can play a key role in constructing fast and accurate biosensors for detecting H. pylori [2]. These nanomaterials exhibit unique mechanical, chemical, and optical properties and, due to their extremely high surface-to-volume ratio, are highly valuable for applications requiring extensive surface interactions [2]. For example, graphene oxide enables effective immobilization of biomolecules such as antibodies or nucleic acid probes and allows specific recognition of H. pylori [2].

4.1.2. Application of Nanosensors in Viral Detection

Rapid detection of the coronavirus (SARS-CoV-2) is crucial for controlling disease transmission [7]. Detection has been enabled using probes functionalized with gold nanoparticles (AuNPs) [7].

The DNA probe is designed in a sandwich structure, with a gold nanoparticle linked to a magnetic particle to recognize the target DNA sequence. When the target sequence is detected, the released gold nanoparticles produce a visible color change. Cas12a (CRISPR-associated protein 12a) can be used to enhance specificity, while UV–Vis spectroscopy (Ultraviolet–Visible Spectroscopy) allows quantitative measurement [7].

4.1.3.This method offers several advantages:

•High Speed: Viral detection can be completed in less than 20 minutes using a simple handheld heater.
•High Sensitivity: Detection limit of 270 RNA copies per milliliter using GX/P2V samples.
•Versatility: By changing the CRISPR RNA (crRNA, CRISPR RNA), the system can be adapted for the detection of other viruses, bacteria, or nucleic acid sequences.

4.2. Application of Nanosensors in Biomarker Detection

4.2.1. Detection of Cardiac Biomarkers

Nanosensors, due to their extremely small size and high sensitivity, can detect biomarkers at very low concentrations. This feature has facilitated the transition from centralized laboratory testing to portable and home-based devices, enabling rapid point-of-care (POC) diagnostics [3].

Targeted rapid tests are typically based on immunoreactions and utilize nanomaterials for the recognition of antigens or antibodies in samples. Most studies focus on enhancing sensitivity, specificity, and multiplexing capability by employing various nanomaterials such as nanoparticles (NPs), quantum dots (QDs), nanowires (NWs), and nanotubes (NTs). These materials interact with target biomarkers and generate detectable signals, such as color change, fluorescence, or electrical current [3].

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide. Therefore, rapid detection of cardiac biomarkers is crucial for timely patient intervention [8]. N-terminal pro b-type natriuretic peptide (NT-proBNP) is secreted when blood pressure and volume increase in the heart, serving as a reliable indicator of cardiac function [8]. The level of this biomarker correlates directly with the severity of heart failure [8].

Recently, saliva has been proposed as an alternative sampling medium for monitoring NT-proBNP because it is non-invasive, easy to collect, and low-risk [8]. However, the concentration of this biomarker in saliva is approximately 1,000 times lower than in blood [8]. making femtomolar-level detection within a short timeframe challenging [8]. To achieve the necessary sensitivity, a label-free aptasensor was developed based on a reduced graphene oxide field-effect transistor (rGO-FET) with a liquid gate, utilizing a single-layer reduced graphene oxide (rGO) and a biomarker-specific aptamer [8].

Most sensors have traditionally employed specific antibodies; however, antibodies can suffer from variability in performance. In high-mobility field-effect transistors integrated with microfluidics (N3Oa), a single aptamer with high binding affinity for the biomarker was used [8]. Graphene-based biosensors are highly suitable for probing interactions between aptamers and biomolecules, providing rapid and accurate responses—a performance that conventional electrochemical sensors cannot achieve [8].

4.2.2. Detection of Cancer Biomarkers

MicroRNA-31 (miRNA-31) has emerged as a novel and rapid biomarker for detecting oral cancer in saliva, a key medium for cancer diagnostics. However, its concentration in saliva is low, and its sequence similarity with other RNAs poses a challenge [9].
To address this, researchers developed a three-dimensional dual DNA nanomachine combining a DNA walker and Cas12a (CRISPR-associated protein 12a). In this method, the target miRNA binds to magnetic nanoparticles functionalized with a DNA hairpin, initiating an ISAR (Iterative Strand-Displacement Amplification Reaction)-based DNA walker mechanism that produces multiple double-stranded DNA (dsDNA) molecules [9].

Cas12a then binds to the generated dsDNA and activates its trans-cleavage activity, forming a three-dimensional nano-hub. This system enables rapid, sensitive, and specific detection of miRNA-31 in saliva and shows high potential for early detection of oral squamous cell carcinoma [9].

5. Materials & Methods

Various advanced methods have been employed for the design and fabrication of biosensors and nanosensors for disease detection. For example, surface plasmon resonance (SPR) sensors can be fabricated as multilayer structures using inexpensive and readily available materials. Techniques such as sol-gel methods and physical vapor deposition (PVD) of silver (Ag) or gold (Au) have been used, often incorporating TiO_2/SiO_2 layers. The combination of phosphorus with Ag/Au enhances SPR sensitivity, while sensor chip preparation is simple and compatible with standard laboratory equipment, providing chemical stability and improved optical performance [6].

Various electrochemical methods have been employed, including amperometric sensors with gold electrodes or screen-printed electrodes (SPEs) functionalized with horseradish peroxidase (HRP) labels, electrode surface modification with nanoparticles (NPs), and potentiometric and voltammetric techniques such as differential pulse voltammetry (DPV), square wave voltammetry (SWV), electrochemical impedance spectroscopy (EIS), and electrochemical capacitance measurements. Biological recognition elements, including antibodies, aptamers, nanobodies, and molecularly imprinted polymers (MIPs), were utilized for specific protein detection [1].

Carbon screen-printed electrodes (SPEs) were modified with polypyrrole nanotubes (PPy-NTs) and carboxylated multi-walled carbon nanotubes (MWCNT-COOH) and functionalized with monoclonal anti-CagA antibodies using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxysuccinimide (EDC/NHS) chemistry. Structural and electrochemical characterizations were performed using scanning electron microscopy (SEM), cyclic voltammetry (CV), and electrochemical impedance spectroscopy (EIS). The sensor performance was validated by detecting CagA protein in phosphate-buffered saline (PBS) and clinical saliva samples [10].

Synthesis of two-dimensional (2D) nanoparticles was carried out using top-down methods (mechanical or liquid exfoliation), bottom-up approaches (atom-by-atom construction via chemical vapor deposition (CVD), solvothermal synthesis, or self-assembly), and hybrid strategies (combination methods) to achieve precise thickness control and high quality [2].

A reduced graphene oxide field-effect transistor (rGO-FET) functionalized with the aptamer N20a was capable of detecting N-terminal pro b-type natriuretic peptide (NT-proBNP) in artificial saliva with a limit of detection (LOD) of 41 fg/mL. Key materials included graphene oxide suspension, N-methyl-2-pyrrolidone (NMP), aptamer N20a, the linker molecule 1-pyrenebutyric acid N-hydroxysuccinimide ester (PBASE), ethanolamine, bovine serum albumin (BSA), and solvents such as dimethylformamide (DMF) and isopropanol (IPA) [9].

Targeted rapid tests were implemented using nanomaterials to enhance sensitivity and multiplexing capabilities and were applied in the form of lateral flow assays (LFAs) and enzyme-linked immunosorbent assays (ELISAs). These tests have been used for infectious, cancer, cardiovascular, and autoimmune diseases. Paper-based lateral flow assays (LFAs) operate via capillary movement of the sample and reaction with recognition elements, with detection through color change or ultraviolet–visible spectroscopy (UV–Vis) measurement. Advantages include speed, low cost, and portability, while limitations such as low sensitivity and specificity can be improved through nanotechnology [3, 7].

A sensor based on Cas12a (CRISPR-associated protein 12a) and a DNA walker has been designed, in which the target miRNA is captured by DNA hairpin-modified magnetic nanoparticles (DH-MNs). The DNA walker process, based on Iterative Strand-Displacement Amplification Reaction (ISAR), produces multiple double-stranded DNA (dsDNA) molecules. Cas12a then binds to the generated dsDNA and activates its trans-cleavage activity, forming a three-dimensional nano-harvester. This nano-harvester cleaves methylene blue-labeled hairpins on the sensor surface, producing an electrochemical signal change. The design of the dual three-dimensional nanorobot with DH-MNs enables enhanced signal generation and continuous target movement across the nanoparticle surface (9).

5.1.Summary of Methods:

These studies present a range of approaches including nanomaterial synthesis, electrode surface modification, use of aptamers and antibodies, electrochemical techniques, and the design of Cas12a- and DNA walker-based sensors. All methods are developed with the goals of enhancing sensitivity, lowering the limit of detection (LOD), enabling non-invasive detection, and providing usability in point-of-care (POC) and portable devices. Differences and strengths of each approach are defined based on the diagnostic target and sample type, whether saliva, artificial plasma, or clinical samples.

6. Results

Figure 1 shows the performance of the sensor for rapid virus detection in real samples. The graphs and tube images indicate that the sensor can detect viral RNA within 20 minutes using a hand warmer, and its detection limit and sensitivity are specified.

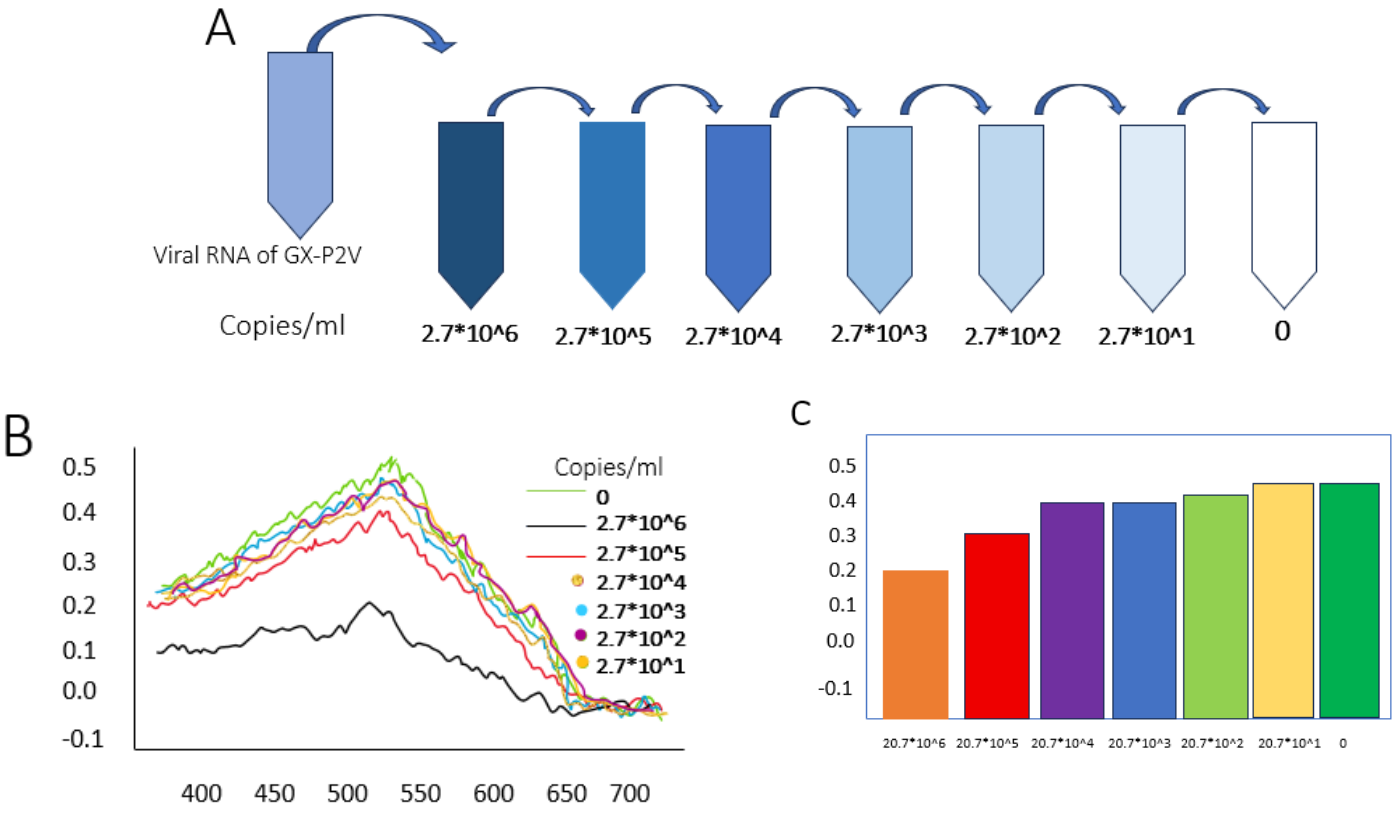

Figure 1. Applications on real samples (7).

- *(A) Extracted viral RNA.*
- *(B) Optical absorption curves at different concentrations.*
- *(C) Bar chart corresponding to the absorption peak at 521 nm at various concentrations.*

The optical absorption curves and tube images indicate that the sensor can detect viral RNA without laboratory equipment, using only a handheld heater within 20 minutes, achieving sensitivity up to 1 nM and a limit of detection (LOD) of 2.7×10^2 copies/mL. This system is suitable for rapid and portable viral detection[7].

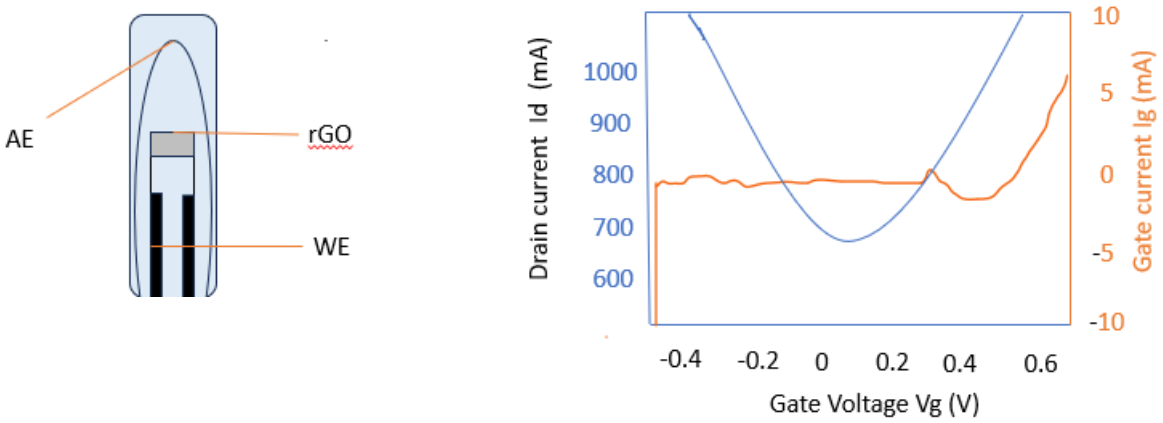

Figure 2. Fabrication and Characterization of the rGO-FET Sensor

Figure 2 illustrates the fabrication and characterization steps of the rGO-FET sensor on G-IDE222 electrodes. The images and graphs show the properties of the reduced graphene oxide (rGO) monolayer film, including the transfer current, leakage current. Comparison of the electrodes also indicates that G-IDE222 provides better performance for aptasensor construction.

The single-layer reduced graphene oxide (rGO) film was fabricated on G-IDE222 electrodes using the drop-casting method. The G-IDE222 electrodes exhibited lower leakage, higher threshold voltage, and superior performance for constructing the aptasensor [8].

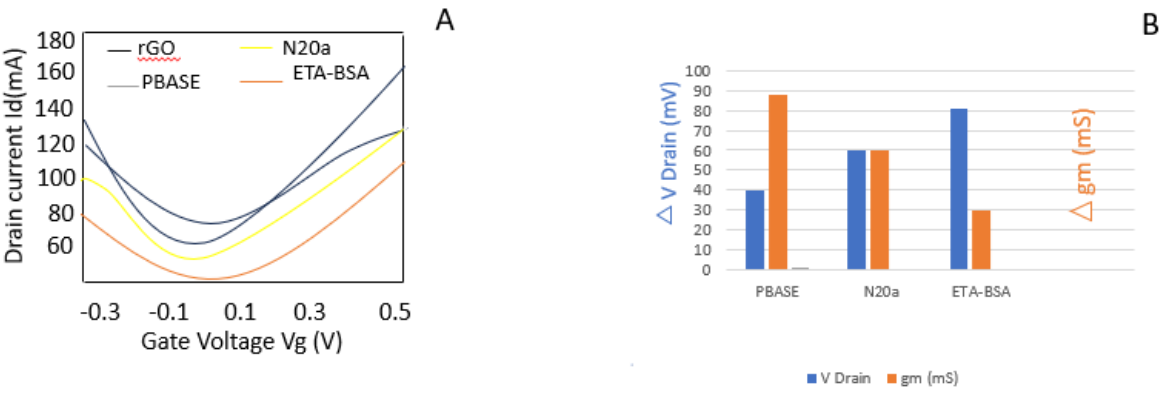

Figure 3 – Assembly and Electrical Changes of the NT-proBNP Biosensor

Figure 3 illustrates the assembly steps of the rGO-FET biosensor and the impact of each step on its electrical behavior. The attachment of PBASE to rGO increases the transconductance and shifts the Dirac point. Upon aptamer binding, conductivity decreases and the Dirac point shifts significantly toward positive values, indicating p-type doping caused by the abundant negative charges of the aptamer. Additional changes after introducing biomolecules (N20a and BSA) further enhance this p-doping and reduce conductivity. Overall, each assembly step produces clear changes in conductivity, doping, and Dirac point, reflecting successful biomolecule attachment on the sensor surface.

Some differences in the characteristics of the fabricated sensors were observed, mainly due to the rGO film structure and the type of substrate. PBASE attachment significantly affects the electrical properties of the rGO-FET, resulting in increased transconductance and a shifted Dirac point (Fig. 3A).

Aptamer binding to the rGO/PBASE surface decreases conductivity and shifts the Dirac point positively to 56±5 mV (Fig. 3B). [8].

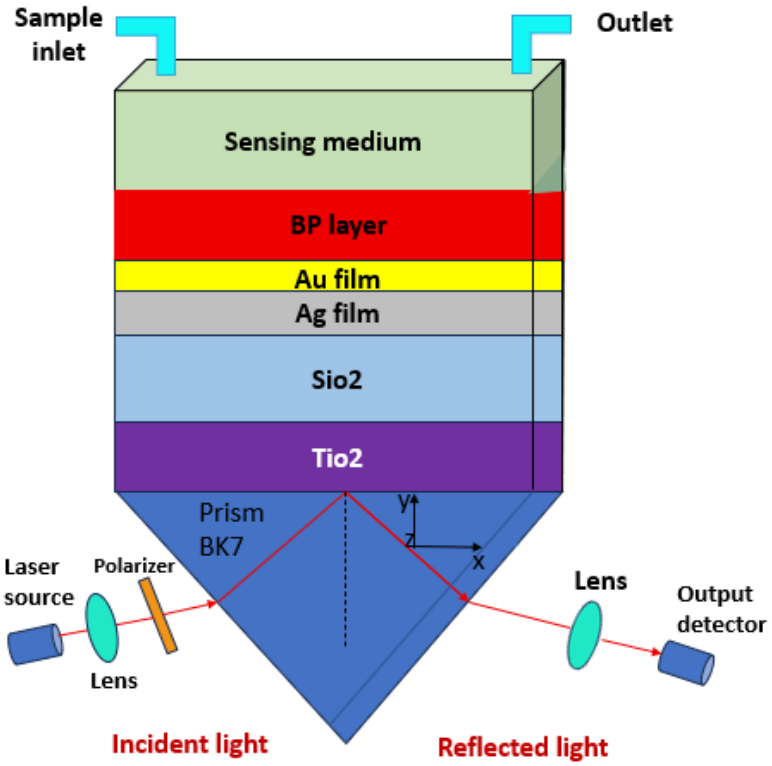

Figure 4. Schematic design of the proposed bimetallic SPR biosensor.

The hybrid multilayer structure BK7/TiO_2/SiO_2/Ag/Au/BP/SM is designed for biomedical biosensing. The Ag/Au bimetallic layer combines the strong plasmonic properties of silver with the optical and chemical stability of gold, while the TiO_2/SiO_2 bilayer improves light management in the SPR system. Adding a black phosphorus (BP) layer enhances sensor sensitivity, strengthens analyte adsorption and biomolecule binding, and expands the sensor's detection range [6].

7. Conclusion

Reasons for the diagnostic relevance of saliva:

•Easy collection without the need for a trained specialist
•Painless and stress-free (suitable for children and the elderly)
•Minimal risk of cross-contamination
•Contains biomarkers comparable to those found in blood
•Suitable for real-time monitoring
•Does not clot and is more stable than blood [1],[5],[9],[10].

Recent studies have shown that the incorporation of microfluidics into biosensors can significantly enhance the accuracy of saliva-based detection in complex environments, enabling rapid, sensitive detection with a low limit of detection (LOD) [4]. Furthermore, multilayer surface plasmon resonance (SPR) sensors, with intelligently combined dielectric-enhancing layers, bimetallic metals, and two-dimensional materials such as black phosphorus, have provided one of the most sensitive platforms for glucose detection [6]. Graphene-based biosensors, with features such as high sensitivity, low limit of detection (LOD), stability, and the capability for integration into portable devices, pave the way for non-invasive disease detection solely from saliva and play a crucial role in personalized medicine and real-time monitoring [3].

The combination of electrochemical sensors with nanomaterials and specific biorecognition elements enables rapid and accurate point-of-care (POC) detection of critical protein biomarkers in saliva, facilitating the development of next-generation diagnostic devices [1].

The use of nanostructured material-based biosensors for CagA detection enables non-invasive early diagnosis of H. pylori and its proteins, helping to prevent severe gastric diseases, including cancer. This platform has high potential for point-of-care (POC) applications, particularly for children, adolescents, and the elderly [10].

Two-dimensional (2D) nanomaterials, combined with advanced technologies such as point-of-care systems, artificial intelligence (AI), and machine learning algorithms, allow rapid, accurate, and personalized detection of H. pylori, potentially reducing disease burden and healthcare costs. However, for successful clinical and commercial translation, safety evaluations, scalable manufacturing, and regulatory considerations are essential [2].

Field-Effect Transistor (FET)–based sensors are transistors in which the current between the source and drain is controlled by the voltage applied to the gate. These sensors, with a wide dynamic range and the ability to simultaneously analyze Dirac point and transducer signals, exhibit high performance and accuracy. They can be used in the production of low-cost, disposable chips, although further evaluations are required before clinical application [8].

This technology enables rapid, accurate, and portable detection, and in the future, nano-biosensors, biochips, and lab-on-a-chip systems could be developed into smart wearable platforms that provide simultaneous detection, treatment, and monitoring [3].

Rapid and low-cost biosensors for SARS-CoV-2 nucleic acid detection have demonstrated the ability for quantification and generalization to other targets, offering affordable and accessible diagnostic approaches[7].

Finally, novel sensors based on Cas12a nano-harvester and DNA Walker enable the detection of miRNA-31 from saliva with high specificity and enhanced trans-cleavage activity of Cas12a, significantly amplifying the signal and opening new avenues for performance improvement [9].

In summary, advanced nano-biosensor technologies and electrochemical biosensors, with their capabilities for rapid, sensitive, non-invasive, and portable detection, have paved the way for the future of disease diagnostics, including bacterial infections, protein biomarkers, and disease-associated RNAs. These technologies also enable the development of smart wearable devices and personalized clinical applications.

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