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Circulating tumor DNA (ctDNA) Detection via electrochemical Biosensing Tools

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ABSTRACT

Cancer is characterized by the presence of mutated alleles in DNA, leading to the formation of tumors. A delayed diagnosis of this condition can result in fatal outcomes, making it a significant global cause of mortality. WHO has emphasized that early detection could significantly increase the chances of successful treatment and recovery. Traditional cancer diagnosis relies on invasive tissue biopsies, which pose risks to both patient's and healthcare professionals due to the use of formaldehyde, a known carcinogenic agent, for specimen preservation. In recent times, liquid biopsies have emerged as a promising alternative, particularly for the analysis of circulating tumor DNA (ctDNA), a fraction of which originates from tumor cells and circulates in the bloodstream. However, conventional molecular genetic tests for ctDNA analysis are often costly and time-consuming. Advancements in technology and the field of nanoscience offer the potential to develop cost-effective, rapid, highly sensitive, and selective diagnostic tools. Among these, biosensors stand out as a promising option. In this article, we delve into the quantification of ctDNA in plasma, discuss amplification techniques for ctDNA, and explore the development of electrochemical-based biosensors tailored for ctDNA detection. Finally, we highlight recent studies and innovations in the field of ctDNA detection.

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1. Introduction

Circulating tumor DNA (ctDNA) is a fraction of cell-free DNA (cfDNA) present in the bloodstream of cancer patients. Malignant tumors release ctDNA into the blood, typically in low quantities, either at the onset of the disease or following successful therapy [1]. In the year 2020, 19.3 million new cases of cancer worldwide were diagnosed (18.1 million excluding non-melanoma skin cancer) and around 10.0 million cancer-related deaths (9.9 million excluding non-melanoma skin cancer). All over the world, female breast cancer (2.26 million cases), lung cancer (2.21), and prostate cancer (1.41 million cases) were the most widely diagnosed cancers. Lung, liver, and stomach cancers were the most frequently reported causes of cancer-related deaths[2], WHO has underscored that early detection could enhance the curability of cancer, potentially saving 40 percent of affected individuals if diagnose at an earlier stage [3].

While numerous sensitive diagnostic methods and specimens have been employed for cancer detection, many of them pose risks to the patient's physical health. For instance, imaging techniques like tomography involve excessive ionizing radiation exposure. Solid biopsy methods, which analyze tissue samples, struggle to accurately capture dynamic tumor changes due to the heterogeneity of tumors and are inadequate for diagnosing brain and lung tumors [4]. Hence, liquid biopsies, utilizing bodily fluids such as peripheral blood, saliva, cerebrospinal fluid, and urine, depending on the tumor's. location, offer a safer alternative, as they contain cell-free DNA with quantifiable ctDNA levels. However, liquid biopsies still have certain limitations [5].

Liquid biopsies have emerged as a contemporary approach for diagnosing various diseases, ranging from genetic conditions to cancers, owing to their patient safety and ease of sampling [5]. Furthermore, liquid biopsies can be employed for diagnosing cancer and monitoring treatment efficacy by assessing cancer biomarkers (CB). These biomarkers encompass biologically active molecules, including proteins such as enzymes, nucleic acids like RNA and microRNA, immunoglobulins like IgG, or short amino acid chains. Notable examples include the identification of immunoglobulin IgG in 1847 as an indicator for multiple myeloma, elevated amylase enzyme levels in 1867 as an indicator for pancreatic cancer, and the discovery of

carcinoembryonic antigen (CEA) in 1965 as a marker for colon cancer. Subsequent biomarkers were developed in 1975. Testing these biomarkers allows differentiation between healthy and diseased tissues, early disease detection, and assessment of disease risk [5].

Liquid biopsies utilizing whole blood, serum, plasma, urine, sputum, or milk have gained prominence in recent years, particularly blood-based liquid biopsies for tumor localization, with plasma being the preferred choice for detecting solid tumor malignancies due to its higher cfDNA concentration [6]. Mandel and Metais published the first study on circulating cell-free DNA (cfDNA) in human blood in 1948. In 1970s notified that cfDNA in plasma rises during inflammation and injuries. Then, they know there will be a connection between cfDNA and tumors. ctDNA can be detected within the pool of cfDNA in plasma, where concentrations are higher than in serum, owing to its coagulation characteristics [7]. Table 1.1 below illustrates cfDNA concentrations for healthy persons and various cancer types, noting that these values may vary depending on the disease prognosis. These quantities are instrumental in cancer diagnosis and assessing the success of cancer therapies [8].

ctDNA offers several advantages, including straightforward sampling through a non-invasive approach, the ability to detect novel mutations, assess therapy effectiveness, surgical outcomes, and cost-effectiveness. ctDNA serves as a valuable molecular analysis tool for diagnostic, predictive, and prognostic purposes. Liquid biopsies, in particular, play a pivotal role in enhancing therapy response and identifying the development of resistance mutations.

Table 1: Comparison of cfDNA Concentration in Plasma Between Cancer Patient's and Healthy Individuals

Cancer type	cfDNA amount in total blood plasma
prostate cancer	115 ng/ml (range 13–339)
breast cancer	83-1414 ng/ml
lung cancer	35-173 ng/ml
stomach cancer	0-527 ng/ml
colon cancer	0-297 ng/ml
healthy women	15±13 ng/ml
healthy men	16±7 ng/ml

The identification of non-cancerous mutations in circulating tumor DNA (ctDNA) can be affected by the presence of mutant alleles in the DNA of hematopoietic stem cells or other early blood cells. In June 2016, the FDA approved the use of liquid biopsy in clinical practice for detecting EGFR mutations. Typically, an average concentration of ~10 ng/ml of cell-free DNA (cfDNA) in human plasma is considered realistic for diagnostic applications. Consequently, ctDNA accounts for less than 0.01% of the total cfDNA. The quantity of ctDNA can vary depending on factors such as tumor burden, proliferation rate, and disease stage. These variables must be taken into account when developing diagnostic systems or discussing the biological significance of ctDNA. As a result, considerable amounts of DNA from other sources frequently dilute ctDNA in the biofluids of cancer patients, which typically makes up 0.01-2% of the total cfDNA. Elevated cfDNA concentration is a sign of disease progression since it also tends to rise in advanced cancer stages and is noticeably higher in patients with metastatic cancer or big tumors [9].

Only 48% to 73% of patients with localized malignancies, such as colorectal cancer, gastroesophageal cancer, pancreatic cancer, and breast adenocarcinoma, have had ctDNA found in them. Despite being notable, these detection rates are not yet thought to be adequate for early cancer detection [10].

This information underscores the variability in ctDNA levels in plasma, which depends on factors such as cancer type, tumor location, disease stage, and whether the patient has undergone successful therapy. As an example, one study examined the concentration of ctDNA in plasma, focusing on colorectal cancer and considering disease prognosis. Only 48% to 73% of patients with localized malignancies, such as colorectal cancer, gastroesophageal cancer, pancreatic cancer, and breast adenocarcinoma, have had ctDNA found in them. Despite being notable, these detection rates are not yet thought to be adequate for early cancer detection as detailed in Table 2.

Circulating tumor DNA (ctDNA) is used to diagnose, treat, and predict the prognosis of numerous malignant illnesses and has undergone thorough analysis. ctDNA levels in blood plasma are typically low, with mutant allele fractions in locally advanced non-metastatic disease less than 1% and below 10% in cases of advanced metastatic disease, respectively. Furthermore, early-stage malignancies and those treated to cure them have substantially lower quantities of ctDNA, which frequently results in mutant allele fractions of less than 0.1%. Highly sensitive techniques are needed to detect ctDNA both during and after therapy [11].

The detection of aqueous solution samples can be accomplished through electrochemical transduction methods, which involve devices that convert information from one system (chemical) into another (physical). Electrochemistry techniques rely on ion transport, ion distribution, and redox reactions on solid conductors (electrodes).

In this review, we emphasize the utilization of electrochemical methods for ctDNA detection. Additionally, we touch upon biorecognition methods for ctDNA, amplification sequences, and the materials employed in designing electrodes for electrochemical techniques.

Table 2. ctDNA levels in various cancer types before and after surgery operations.

Stage	Surgery Number	Surgical procedure	Resection	Gene	Pre-surgery day 0	Post-operative days 13-56
IV	1	Hepatic metastasectomy	Complete	APC KRAS	99 ± 38 79 ± 35	16 ± 2.3 5.6 ± 0.9
IV	1	Hepatic metastecomy (First stage)	Incomplete	APC	2,952 ± 773	1,049 ± 254
IV	1	Sigmoid colectomy with hepatic metastectomy	Incomplete	TP53	14 ± 3	295 ± 71
	2	Hepatic metastectomy	complete	TP53	2,713 ± 775	1.8 ± 0.4
	3	RFA to hepatic metastases	complete	TP53	1,267 ± 243	39 ± 2
II	1	Sigmoid colectomy	complete	APC	2 ± 0.7	Neg
III	1	Sigmoid colectomy	complete	TP53	2 ± 0.6	Neg

2. Biorecognition Elements for Detection of ctDNA

Biosensors are specialized sensing devices designed to detect specific molecules, often referred to as biomarkers. Molecular recognition, also known as biorecognition, plays a crucial role in the detection of these biomarkers within the biosensor. The process commences with the recognition step, during which biomarkers or analytes bind to recognition molecules, resulting in the generation of signals within the sensor device. Biorecognition can be finely tailored for specific analytes, ensuring a high level of specificity in target detection.

The biomolecular recognition elements utilized in electrochemical biosensors for ctDNA can be classified into various categories, including enzymes, nucleic acids (such as aptamers), organelles, immune substances (antibody-antigen reactions), and more. These biomolecular receptors can be immobilized on the biosensor's surface through physical adsorption, covalent bonding, or embedding techniques [12].The recognition or sensing element, which has a strong affinity for the analyte, selectively interacts with the analyte. The sensing component might be made up of many molecular entities called recognition receptors [13].

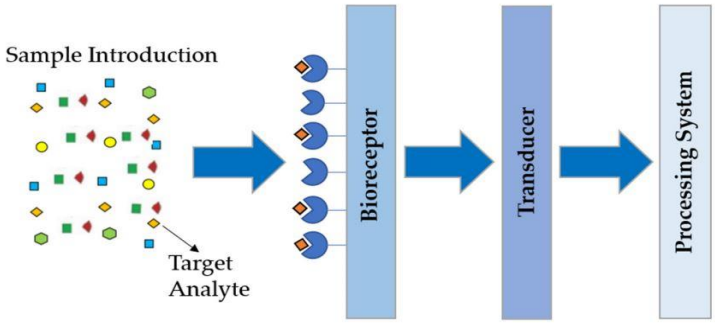

Figure 1. The pivotal role of the biorecognition element on the electrode surface. Various components, including antibodies, enzymes, nucleic acids, microorganisms, and cells, can be immobilized onto the electrode surface. When a target analyte is present, the electrode generates a physical signal, such as an electrochemical or optical signal.

In the following sections of this article, we delve into the biorecognition elements employed for the detection of ctDNA, specifically focusing on nucleic acids and antibodies. These recognition elements are immobilized on the electrode surface and interact with the target analyte to facilitate the detection process.

2.1 Nucleic Acid Probe-Based Detection

Palecek first noticed the electrochemical activity of nucleic acids using a mercury electrode. The electrical response, according to later research, results from the reduction of adenine and cytosine residues [14].

Nucleotide probes, comprising RNA and DNA oligonucleotide sequences, can be designed to target specific DNA or RNA sequences through Watson-Crick base pairing. There are two types of nucleotide probes: RNA probes and DNA probes. These probes are integral to hybridization techniques. In this process, a complementary DNA sequence to the target sequence is obtained and labeled. The label can be radioactive, a fluorescent tag, or an enzyme, referred to as a probe. When this probe is introduced to the analyte sequence, it leads to hybridization. The presence of the label allows for the detection of hybrids. For instance, if the probe is labeled with an enzyme, detection occurs through a color or light-producing reaction catalyzed by the enzyme [15].

Nucleic acid hybridization-based electrochemical biosensors are predominantly employed in clinical and microbial diagnosis due to their high sensitivity, rapid response, ease of use, and cost-effectiveness. After designing the probe, it is immobilized onto an electrode. When the analyte is introduced, hybridization occurs, converting the chemical reaction into an electrical signal.

There are three types of nucleic acid probes: PNA (peptide nucleic acid), DNA probes, and RNA probes, all of which can be utilized for DNA detection. By employing different probes and techniques, highly sensitive detection can be achieved. PNA is a synthetic organic polymer similar to DNA and RNA, but its sugar backbone is replaced by glycine linked through a peptide bond. This unique structure reduces electrostatic repulsion when hybridized with DNA, resulting in superior hybridization capability compared to DNA/DNA interactions. Consequently, using PNA probes in electrochemical methods is important. Through covalent bonding, significant amounts of PNA can be immobilized on a screen-printed electrode (SPE) surface that has been treated with gold nanoparticles. The working electrode, auxiliary electrode, and reference electrode are the three electrodes that make up the SPE. Both serum and peripheral blood can be used as sample matrices, with serum often exhibiting higher sensitivity. When the sample is applied to the working electrode, mutants bind to the complementary PNA probe, leading to a potential change [16].

Alternatively, DNA or RNA probes can be employed for ctDNA detection. For example, graphene-oxide-coated gold nanoparticles can be modified onto a glassy carbon electrode, with probes fixed through affinity interactions. The introduction of the analyte leads to hybridization, resulting in a change in current. Sensitivity can be enhanced by altering the surface material, dimensions, size, and physical transduction methods, such as the type of electrochemical signal utilized [15]

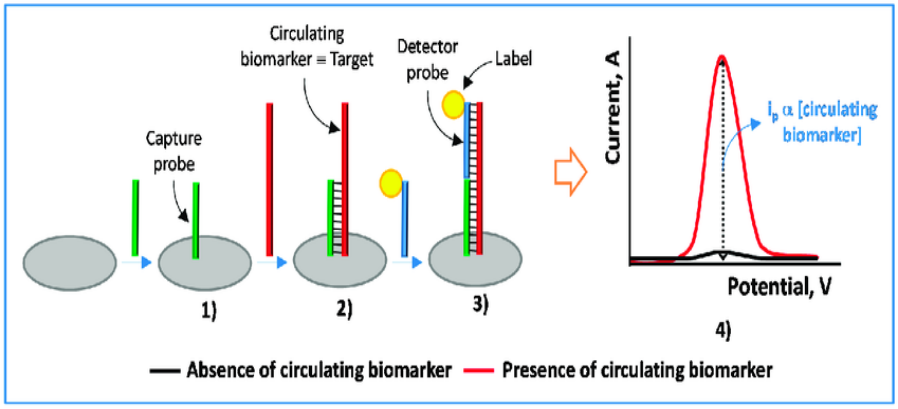

Figure 2: The Immobilized Green Capture Probe on the Electrode, Binding to the Target, resulting in a Current Change on the Electrode, Visualized in the Voltammogram [17]

2.2 Antibody Probe-Based Detection

The antibody-antigen reaction is a widely employed method in both diagnosis and treatment. Antibodies exhibit specific binding capabilities for various antigens, including pathogens, micro-molecules, cells, bacteria, and other molecules. Utilizing this principle for detection offers advantages such as achieving lower limits of detection. In the context of ctDNA detection, specific antibodies can be immobilized on an electrode surface [18]. A binding event between the antibody and antigen takes place when the antigen is added, leading to physical effects like a mass change or the usage of signaling tags, like fluorescent labels affixed to one of the reactants. These complexes can then be detected through signal transduction, either via electrochemical methods or through techniques like ELISA (enzyme-linked immunosorbent assay).

At present, DNA site-specific methylation is becoming increasingly popular as a biomarker [19]. Additionally, ctDNA methylation plays a vital role in regulating tumors, and evaluating the level of ctDNA methylation can effectively gauge the tumor's degree of malignancy [20]. Methods for analyzing ctDNA methylation primarily encompass PCR, sequencing, and microarray techniques, among others. However, it's worth noting that all these methodologies

require prior ctDNA pretreatment [21]. In contrast, 5-methylcytosine (5-mC) monoclonal antibodies can be directly attached to the electrode through covalent coupling. This approach enables the capture of methylated ctDNA without necessitating sample pre-treatment.

3. Electrochemical Methods for ctDNA Biosensors

Electrochemistry, as the name suggests, involves chemical reactions and the exchange of electrons. In this process, chemical reactions yield physical signals such as changes in voltage, current, or impedance. These signals can indicate the occurrence of a chemical reaction and allow for the measurement of molecule concentrations within the reaction. This technique has enabled the development of cost-effective, rapid-response, highly sensitive, and selective biosensors.

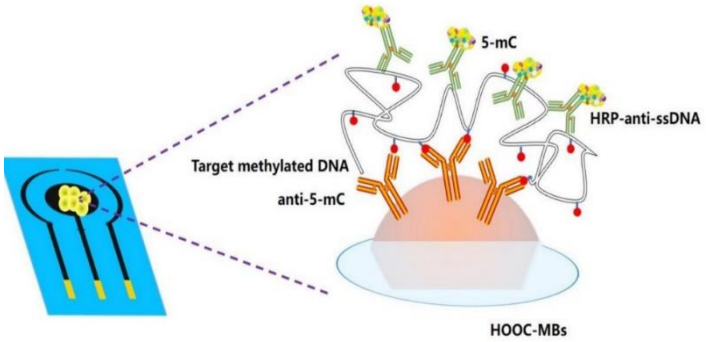

Figure 3. Schematic display of the immunosensor and ctDNA detection. Anti-5-mC immobilized on the electrode as a bioreceptor to capture methylated ctDNA. [19]

The inception of electrochemical biosensors dates back to 1950 when they were first designed to monitor oxygen (O2) levels in industrial settings, with Leland C. Clark leading the way. These early O2 sensors employed a two-electrode system with an O2-permeable membrane that separated the electrode from the electrolytic solution. When O2 diffused through the permeable membrane, it interacted with the electrode, resulting in a change in current. This change in current is directly correlated with alterations in the oxygen levels. Over time, this technique found applications in environmental monitoring, industrial processes, and medical diagnostics [22].

For aqueous solution samples, electrochemical transduction methods are frequently employed to create biosensors for detecting molecules like ctDNA. These biosensors convert biological signals, generated by the binding of an analyte to a biorecognition molecule, into electrical signals for detection. In the context of ctDNA detection, two commonly used electrochemical techniques are Differential Pulse Voltammetry (DPV) and Electrochemical Impedance Spectroscopy (EIS). This review focuses on these two types of electrochemical cells for the detection of ctDNA.

3.1. Differential Pulse Voltammetry

Voltammetry is an analytical method used to measure the current in electrochemical cells after applying a potential. One specific technique within voltammetry is Differential Pulse Voltammetry (DPV), which involves the application of small amplitude potential perturbations that gradually slow down. The current is measured at two points for each pulse, both before the pulse is applied and at the end of the pulse. DPV is particularly effective for measuring short intervals after the current has dropped. In this technique, potential readings are taken after adding each sample, and as subsequent samples are added, the potential decreases. DPV is highly sensitive, allowing for direct analysis at the parts per billion level. It detects the current response of a chemical under an applied potential difference, providing insights into the behavior of ions, molecules, or atoms undergoing oxidation or reduction reactions in a solution.

The voltammetric method family includes differential pulse voltammetry, which is used as an electrochemical detection method that may amplify signals more than 30 times. Longer pulse periods, which allow for the collection of a full charge current and improve the signal-to-noise ratio (SNR), are used to achieve this amplification. DPV excels in measuring trace substances, making it particularly advantageous when detecting low-abundance molecules [24].

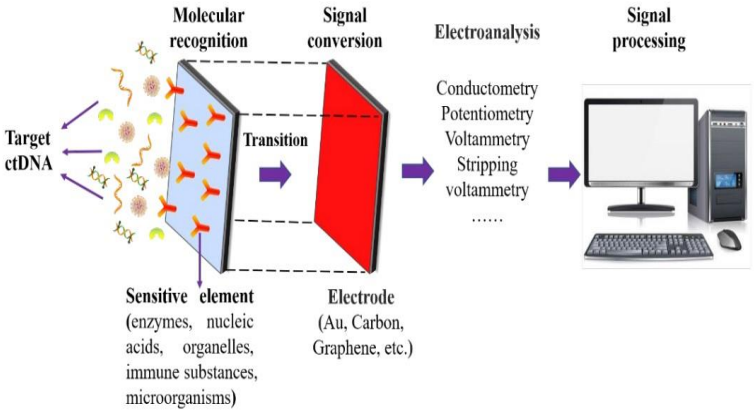

Figure 4. Schematic Representation of a ctDNA Electrochemical Biosensor Detection System. The biosensor components include molecular recognition elements such as enzymes, acids, organelles, and more. Additionally, it features a signal conversion electrode that has been enhanced through the use of various nanomaterials. Various electrochemical techniques, including potentiometric and voltammetric methods, are employed for signal detection [23].

As another example, a carbon electrode is enhanced by modification with nanomaterials to boost its catalytic activity. This modified electrode is then immobilized with single-strand DNA probes designed to specifically recognize mutant ctDNA. Hybridization between the two strands, the probe, and the ctDNA, initiates. By monitoring Differential Pulse Voltammetry (DPV), changes in current are observed, indicating the presence of a mutant analyte [25]

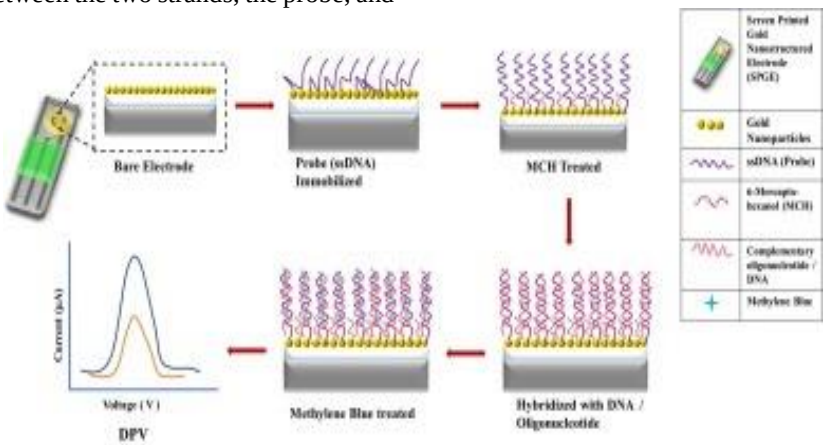

Figure 5. Principle of Differential Pulse Voltammetry (DPV) for Detecting DNA from a Specific Bacterial Strain. The biosensor employs a synthesized probe called sgDNA as the biorecognition element, which is immobilized on gold nanoparticles. Following the introduction of the target DNA, hybridization occurs, and the resulting hybridization is depicted on the plot by observing changes in the DPV, which yield varying current values [24]

3.2. Electrochemical Impedance Spectroscopy (EIS)

Electrical Impedance Spectroscopy is an exceptionally sensitive technique employed in electrochemical biosensors to detect and quantify physical changes resulting from chemical interactions between analytes and biorecognition elements. In this method, an alternating voltage is applied to the working electrode at various frequencies, inducing alternating current responses due to the REDOX (charge transfer) reactions of electrochemical species at the electrode's surface.

Electronic Impedance Spectroscopy can detect single-base variations or mismatched hybridization signals in addition to identifying DNA hybridization signals. The basic idea is based on the fact that DNA molecules have a negative charge, causing a coating of negatively charged material to build on the electrode's surface. Electrostatic repulsion happens when an oxidation-reduction electrode is present, increasing resistance. By measuring changes in resistance, impedance can be quantified following Ohm's law [26]

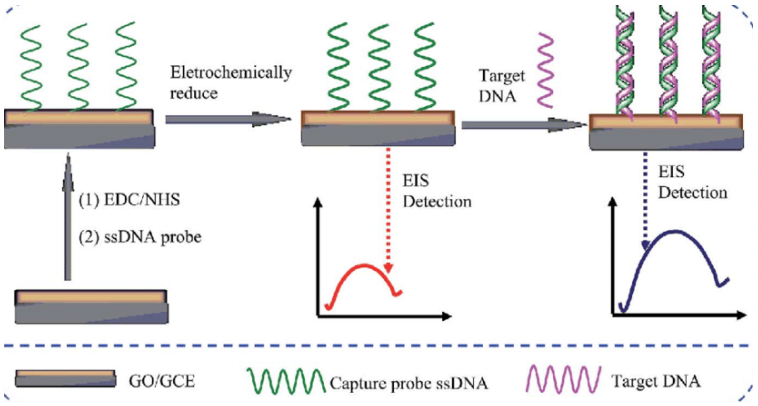

Figure 6. Illustration of the EIS Principle for Detecting Target DNA Hybridization Utilizing an ssDNA Probe Synthesized on the Electrode Surface. The presence of an analyte results in impedance changes on the electrode, which is visualized on the plot through the measurement of peaks [27]

4. Current novel Studies for ctDNA detection

Here, we will elaborate on several innovative techniques that have been explored for the detection of ctDNA, utilizing various biosensors in conjunction with distinct amplification methods.

4.1. Utilization of the CRISPR-dCas9 Enzyme Principle for ctDNA Detection

In this study, a highly sensitive, selective, cost-effective, and rapid-response biosensor was developed. The biosensor design involved the modification of an electrode with graphene oxide (GPHOXE), a carbon nanomaterial known for its high electron mobility, strength, and flexibility, all of which are crucial in biological sensing applications.

To create the biorecognition receptors, deactivated Cas9 (dCas9, endonuclease deficient Cas9) protein and synthetic guide

RNA (sgRNA) was immobilized on the electrode. This modification effectively eliminated the endonuclease activity of dCas9 by mutating its endonuclease domains. As a result, dCas9 was capable of binding to sgRNA and the specific DNA strand targeted by sgRNA. This catalytically inactive Cas9 is only bound to the predetermined region on the DNA.

The PIK3CA exon 9 mutation was chosen as the tumor-related mutation that the biosensor was developed to identify utilizing electrochemical impedance spectroscopy (EIS) as the electrochemical technique. The subjects were women with newly diagnosed breast cancer who had not undergone treatment.

The biosensor's performance was assessed through a series of tests conducted after the modification of the nanomaterial and immobilization of the biorecognition elements. The Limit of Detection (LOD) was determined to be 0.65 nM, and the Limit of Quantification (LOQ) was found to be 1.92 nM. Various concentrations of 120bp ctDNA fragments were generated and used to enhance the GPHOXE-dCas9-sgRNA electrode for chronoimpedimetric detection.

Chronoimpedance measurements were used to keep track of the biosensor surface's ctDNA saturation. Following the initial linear increase in signal, the biosensor's detection time was set at 40 seconds. The detection time was determined, and a linear calibration range was created using 120bp ctDNA standards. The circuit model shown in Figure 13 is the result of plotting the concentration-binding time (the length of time it takes for ctDNA to attach to the electrode) against the EIS spectra [28]

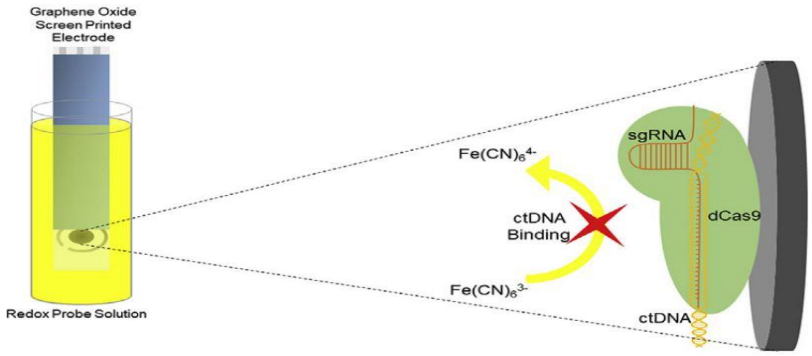

Figure 7. Utilizing a Graphene Oxide Screen Printed Electrode Modified with CRISPR dCas9 and sgRNA for ctDNA Detection. Upon the addition of ctDNA, a conversion of $Fe(CN)63^{-}$ to $Fe(CN)64^{-}$ occurs, resulting in the generation of a detectable signal.

4.2. Investigating the Use of AuPt Alloy Nanoparticles as an Amplification Strategy for the Detection of Circulating Tumor DNA (ctDNA)

The detection of circulating tumor DNA (ctDNA) as a liquid biopsy for cancer diagnosis and treatment monitoring has garnered significant attention. However, designing an effective biosensor for this purpose comes with its challenges. In this article, a solution was developed using improved nanocomposites with high carbon activity, which enhances conductivity. Additionally, Au-Pt (gold-platinum) nanoparticles were synthesized and utilized as a signal amplification strategy to create a sandwich-type biosensor.

Due to the strong electrochemical characteristics of HAC-AuPt (high carbon activity Au-Pt), the current response is considerably increased in this biosensor design when an analyte is present. As biorecognition components, DNA probes, and capture probes were mounted on the electrode surface. To detect ctDNA, the target DNA and DNA probe were incubated on capture probes in a sandwich-like shape.

With an incredibly low detection limit of 3.6 x 10-17 mol/L (S/N 1/4), this biosensor displayed a wide linear detection range from 10-8 mol/L to 10-16 mol/L. A conventional three-electrode system, consisting of the modified working electrode, counter electrode, and reference Ag/AgCl electrode, with a potential of 0.4 V, was used to perform amperometric current-time (i-t) measurements. To learn more about the electrode's interface properties, more cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) tests were made.

H2O2 was added to phosphate-buffered saline (PBS) to start the current response after the sandwich structure was formed by the hybridization of the target DNA and SPs-label. Due to its exceptional catalytic properties, the addition of the label HAC-AuPt considerably enhanced the current signal. As a result, the biosensor created for this work has a high sensitivity for detecting ctDNA [29]

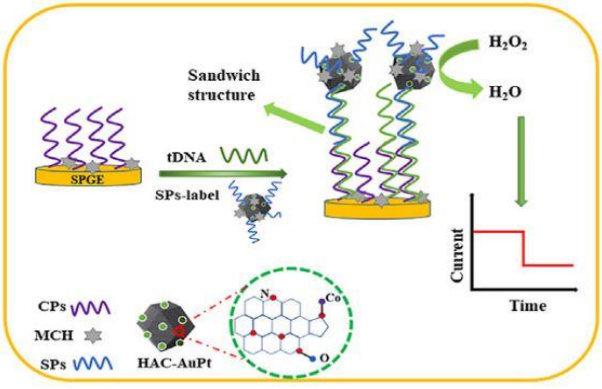

Figure 8. Utilization of a Gold Electrode Immobilized with SPs-Label for ctDNA Detection. In the presence of target DNA (mtDNA), the conversion of H_2O_2 to H_2O occurs, resulting in a signal represented by changes in current over time [29]

4.3. Leveraging Hybridization Chain Reaction for Enhanced Sensitivity in Detecting Circulating Tumor DNA

This study established a novel electrochemical biosensor for the extremely sensitive detection of ctDNA bearing the PIK3CA E545K allele mutation, employing the hybridization chain reaction (HCR) approach as a biorecognition mechanism. The biosensor's construction required the hybridization of two dumbbell-shaped DNA units that were put together using two different classes of painstakingly

created DNA probes. This complex structure started a chain reaction of nested hybridization.

In the presence of the particular allele mutation, the amplification products produced during the hybridization chain reaction were successfully caught by the target ctDNA. This interaction greatly enhanced the electrochemical signal. The main element of the biosensor was a 2mm gold electrode. Three dumbbell-shaped DNA units (U10, U1, and U2), capture probes, and gold electrodes made up the novel HCR sensing apparatus. The system's extraordinary amplification made it possible to use differential pulse voltammetry (DPV) to measure the electrochemical signal. As a result, the detection of an electrochemical signal was made possible by the presence of HCR products, offering a stable foundation for the detection of sensitive ctDNA [30]

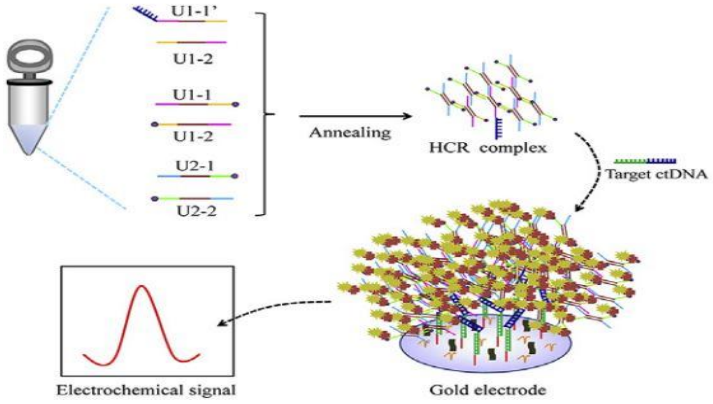

Figure 9. illustrates the underlying principle of the reaction involving target DNA and the utilization of the HCR amplification technique before immobilization onto the gold electrode. Additionally, it presents a differential pulse voltammetry (DPV) graph that discerns the presence or absence of the analyte [30]

Leveraging a Dual Enzyme Multiple Amplification Strategy for the Detection of Circulating Tumor DNA

In this study, electrode biosensors were amplified using a wide range of amplification strategies. A triple-helix molecular switch (THMS) was used in the design of the biosensor as the molecular recognition and signal transduction probe. To enable different amplification pathways, ribonuclease HII (RNase HII) and terminal deoxynucleotidyl transferase (TdT) were added as dual enzymes.

Target ctDNA caused the THMS to open, which started RNase HII-assisted homogeneous target recycling amplification. A significant signal transduction probe (STP) was produced because of this technique. The captured probe was then hybridized with the released STP using an electrode made of gold. To facilitate TdT-mediated cascade extension and ultimately the production of stable DNA dendritic nanostructures, TdT, and an assistance probe were used.

EIS was used in this study to analyze the electrochemical production and amplification processes since it has shown to be a highly effective method for examining the interfacial reaction mechanism of electrode surfaces [31]

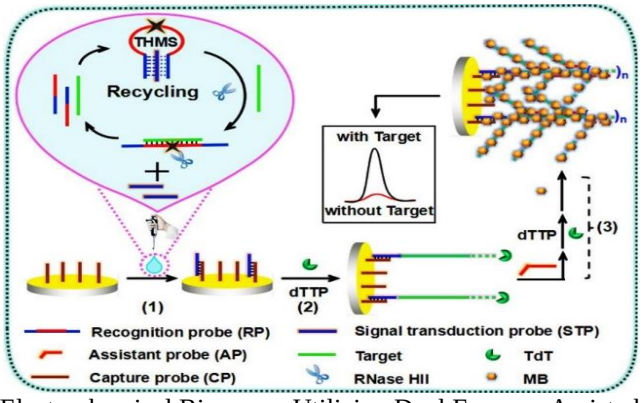

Figure 10. Depiction of the Electrochemical Biosensor Utilizing Dual Enzyme-Assisted Multiple Amplification [31]

5. Future perspective and conclusion

In recent years, advancements in technology and nanoscience have led to the development of portable, sensitive, quick-response, and cost-effective diagnostic tools. Electrochemical-based biosensors have emerged as crucial instruments for detecting circulating tumor DNA (ctDNA), aiding in the diagnosis and treatment monitoring of patient's. However, current electrochemical biosensors can typically only detect a single analyte, whereas clinical diagnosis often requires the detection of multiple analytes. Therefore, further research is needed to enhance ctDNA detection capabilities.

The diverse biosensor methods presented in the table 3 exhibit distinctive characteristics in terms of sensitivity, response time, and linear range, catering to a range of applications. From the ultra-sensitive label-free electrochemical biosensor with a LOD of 2.4 aM to the versatile LAMP electrochemical biosensors designed for cervical precancerous lesions, each method offers unique advantages in sample testing and biorecognition elements. The innovative use of CRISPR-dCas9, AuPt alloy nanoparticles, triple-helix molecular switches (THMS), DNA probes, and SPs-label showcases the evolving landscape of biosensor technologies. Researchers and practitioners can leverage this comprehensive overview to select the most suitable biosensor method for their specific analytical needs, considering factors such as LOD, response time, and linear range in real sample testing scenarios.

CtDNA levels are typically low in the early stages of cancer, and their concentration varies among different types of cancer lesions. As a result, sensors must possess a lower detection limit, as well as high sensitivity and specificity. By harnessing various nanoscale materials, electrode conductivity and surface area can be increased, leading to more sensitive electrodes. Additionally, it is essential to integrate biosensors with other analytical methods to minimize harm to the human body during sample collection, such as employing liquid biopsy techniques instead of invasive tissue biopsies.

This paper provides an overview of novel methods for ctDNA detection using electrochemical biosensors while also discussing traditional approaches like PCR and their associated drawbacks, such as high costs, complex procedures, and frequent false positives. The trend in ctDNA detection is shifting towards portable, cost-effective solutions. Furthermore, the paper explores the various types of electrochemical sensors used for ctDNA detection and highlights recent studies in this field. Introducing these methods into clinical diagnosis and mobile health applications offers both opportunities and challenges, which must be addressed as we move forward in the field of ctDNA detection.

Table 3. Comparing electrochemical biosensors

Biosensor Method	LOD	Response Time	linear Range	Electrochemical method	Sample testing	Biorecognition element
Impedimetric biosensor[28]	0.65 nM	40 Seconds	2-20 nM	EIS	Real Blood Sample (Serum)	CRISPR-dCas9
AuPt alloy nanoparticles electrochemical Biosensor[29]	3.6-10^{17}mol/L	quick response	10^{8}mol/L - 10^{16}mol/L	EIS/CV	Real serum sample	capture probes (CPs)
label-free electrochemical biosensor[32]	2.4 aM	Response in 30 seconds	0.01 fM to 1 pM	EIS	Real plasma sample	triple-helix molecular switch (THMS)
HCR electrochemical biosensor[30]	3 pM	Response time in seconds	5 pM to 0.5 nM	EIS/SWV	Real serum samples	DNA probes
gold-coated magnetic nanoparticles electrochemical biosensor[33]	3.3 aM	20 min response time	200 aM to 20 nM	SWV	whole blood	probe DNA
LAMP electrochemical biosensors[34]	10 pg and 150 ng	40 min	150 ng	amperometry measurement and CV	cervical precancerous lesions	Probe DNA
sandwich-type electrochemical biosensor[35]	5×10^{-16} mol/L	Rapid responce	$1 \times 10^{-15}$to 1 x 10^{-8} mol/L	CV/EIS	Real serum sample	SPs-label

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