Aptamer-based biosensors: carbon nanomaterial integration for rapid *E. coli* detection in food safety - mini review

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ABSTRACT

Foodborne infections are a major global concern because of the astoundingly high number of diseases they cause each year. A greater number of highly precise and dependable biosensors have been developed in recent decades to bridge the gap between monitoring requirements and the conventional detection techniques now in use. Among these, aptamer-based biosensors have gained significant attention for their high specificity, stability, and adaptability to diverse targets. Aptamers, single-stranded DNA / RNA molecules with intricate three-dimensional structures, exhibit exceptional molecular recognition capabilities. Their chemical synthesis allows precise sequence design and functional modifications, making them ideal candidates for biosensing applications. Detecting Escherichia coli (E. coli), a common foodborne pathogen is critical for ensuring food safety and public health. While traditional methods such as culture techniques and PCR are reliable, they are often time-consuming and unsuitable for real-time, on-site applications. Aptamer-based biosensors offer rapid, sensitive, and portable alternatives, especially when conjugated with nanomaterials like graphene oxide (GO), gold nanoparticles (AuNPs), and carbon nanotubes. These nanomaterials amplify the signals from aptamer-target interactions through their unique electronic and optical properties, significantly enhancing detection performance. However, achieving optimal functionality necessitates the careful optimization of aptamer nucleotide sequences. Sequence attributes such as length, secondary structure, and nucleotide composition play a crucial role in determining binding affinity, stability, and specificity. Tailored sequence modifications enhance the conjugate's performance, enabling advanced biosensors with superior accuracy and reliability. This integration of aptamers and nanomaterials demonstrates immense potential in developing low-cost, efficient, and scalable detection systems, addressing the urgent need for effective tools in foodborne pathogen monitoring.

Keywords: **Foodborne pathogens, aptamer-based biosensors, graphene oxide, nanomaterials, detection**

1. *INTRODUCTION*

Foodborne pathogens, including *Escherichia coli (E. coli)*, are a serious concern to world health because they can cause serious ailments such as systemic infections and gastroenteritis. The prevalent zoonotic pathogen *E. coli* is a rod-shaped, gram-negative bacteria that is often linked to tainted food products. Maintaining food safety and protecting the public's health depends on its prompt and precise identification. Although well-established, traditional detection techniques such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assays (ELISA), and culture techniques have several disadvantages, including being labor-intensive, time-consuming, and unsuitable for real-time, on-site monitoring [1].

Moreover, label-free, real-time detection in complicated matrices has shown promise using field-effect transistor (FET) -based sensors functionalized with GO and gold nanoparticles (AuNPs) [2]. These include synthetic single-stranded DNA or RNA molecules called aptamers, which have shown great promise as recognition elements because of their high specificity, stability, and capacity to adapt to a variety of targets. Aptamers are perfect for identifying *E. coli* in intricate food matrices because they have benefits over antibodies, including in vitro production, sequence modifiability, and improved repeatability [3].

Because of these characteristics, aptamers are ideal for identifying E. coli in intricate food matrices where prompt and accurate detection is essential [4]. Carbon nanomaterials, such as graphene oxide (GO), carbon nanotubes (CNTs), and carbon quantum dots (CQDs), are used in recent developments in biosensor technology to enhance aptamer-target interaction signals. The performance of biosensors is greatly improved by these nanomaterials' enormous surface areas, excellent electron transport capabilities, and adjustable optical characteristics. For example, FRET-based biosensors that use carbon quantum dots have been able to detect E. coli in food samples with detection limits as low as 77 CFU/mL [5]. Furthermore, even in complex matrices like blood serum, FET-based sensors functionalized with GO and AuNPs have demonstrated potential for label-free, real-time *E. coli* detection [2]. However, exact aptamer nucleotide sequence optimization is necessary to get the best performance of aptamer-nanomaterial conjugates. Important determinants of binding affinity, specificity, and conjugate stability include length, secondary structure, and nucleotide composition. The performance of these biosensors has been further improved by developments in sequence engineering, such as the addition of functional modifications and better immobilization techniques, which have made it possible to use them in portable, inexpensive devices for real-time pathogen monitoring. Despite these developments, good specificity, stability, and binding affinity still depend on tailoring aptamer sequences. The performance of aptamer-nanomaterial conjugates has been significantly improved by advancements in sequence engineering and functional changes. This study explores current developments in the integration of aptamers with carbon

nanomaterials for the detection of *E. coli*, emphasizing significant obstacles, creative fixes, and the revolutionary potential of these biosensors in tackling. Fig. 1 illustrates the process of detecting foodborne pathogen *E. coli* using an aptasensor, highlighting its key components and mechanism of signal transduction.

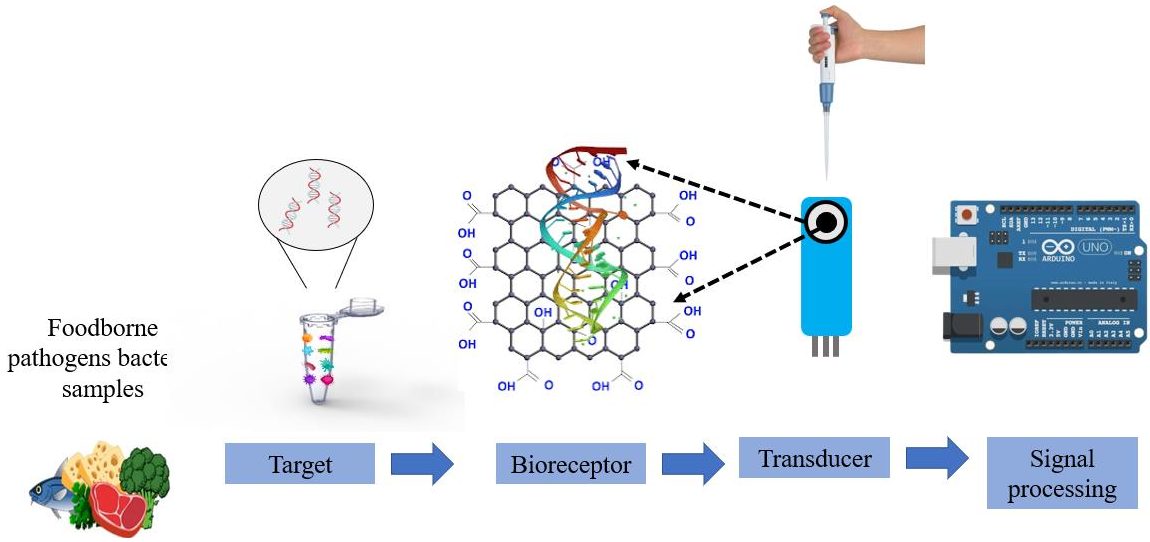

Fig.1. Foodborne pathogen *E.coli* detection by aptasensor [6].

2. Aptamer-nanomaterial conjugation for *E. coli* detection

Nanomaterials are now crucial in biosensing applications due to their unique properties that significantly enhance the performance of aptamer-based sensors. GO, AuNPs, and CNTs are often used nanomaterials in this area, and each offers special advantages [7]. Strong π-π stacking interactions with DNA aptamers are made possible by graphene oxide's large surface area and profusion of oxygen-containing functional groups, which allow for secure attachment and effective signal amplification [8]. Known for their superior optical and electrical characteristics and great biocompatibility, gold nanoparticles are frequently used in colorimetric tests, in which the presence of a target is visually indicated by particle aggregation [9].

Because of their exceptional electrical conductivity and mechanical robustness, carbon nanotubes facilitate electron transfer, which makes them perfect for electrochemical sensors that can identify tiny concentrations of pathogens like *E. coli* [10].

Researchers use a variety of conjugation strategies to guarantee stable attachment and effective target identification when combining these nanomaterials with aptamers for *E. coli* detection [11]. A popular method for achieving strong binding with modified aptamers is covalent bonding, which involves functionalizing the surfaces of nanomaterials with reactive groups like carboxyl or amine groups [12]. For applications needing

long-term stability, this technique guarantees a robust, long-lasting conjugation. Particularly with graphene oxide, non-covalent interactions like electrostatic forces and π-π stacking are especially common. Aptamers can adsorb onto the surfaces of nanomaterials thanks to these reversible interactions, which preserve aptamer functioning and increase sensitivity [13].

The range of aptamer-nanomaterial conjugation has been further expanded by new methods such as click chemistry and biotin-streptavidin interactions [14]. While biotin-streptavidin interactions produce high-affinity linkages that are perfect for applications needing strong and stable binding, click chemistry provides a quick and effective way to create robust conjugates[15]. The particular detection needs, including sensitivity, stability, and the intended signal transduction mechanism, determine which conjugation approach is best. For example, AuNP-based conjugates are advantageous for visual detection in optical biosensors, while CNTs are excellent in electrochemical sensors that need improved electron transfer [16].

The detection of *E. coli* has shown impressive results when nanomaterials and aptamers are combined. For instance, graphene oxide and carbon quantum dots-based biosensors that use fluorescence resonance energy transfer (FRET) have demonstrated detection limits as low as 77 CFU/mL [17]. Similar to this, sensors based on FETs functionalized with GO and AuNPs have demonstrated outstanding performance in identifying *E. coli* in intricate matrices including food extracts. These illustrations show how aptamer-nanomaterial conjugates have the potential to transform food safety monitoring by offering portable, sensitive, and quick detection platforms. Still, there are obstacles in the way of reaching peak performance [18]. Careful optimization is required for elements including aptamer nucleotide sequence design, binding efficiency, and conjugate stability in a range of environmental circumstances. The performance of these biosensors has been further improved by developments in sequence engineering, such as the addition of functional changes and better immobilization techniques, opening the door for their implementation into scalable, reasonably priced devices [19].

3. Aptamer design and application for *e. coli* detection in food safety

scientific investigations, Aptamer IDs are used to uniquely identify Aptamer sequences intended to target *E. coli*. These sequences, which have been carefully selected from validated experiments to guarantee dependable binding performance, are provided in the 5' to 3' orientation [20]. Aptamers are frequently chemically altered to improve their functioning. For instance, biotin alterations allow conjugation with streptavidin-coated nanomaterials, while fluorescent dyes like FAM enable fluorescence-based detection [12]. The particular nanomaterial that is employed for conjugation with the aptamer is referred to as the Linked Nanomaterial [21]. To improve detection sensitivity, nanomaterials with unique qualities—such as a high surface area, excellent conductivity, or optical properties. The dissociation constant (*Kd*), which is a measure of binding affinity, indicates how strongly the aptamer and *E. coli* interact. Stronger binding affinity, which is necessary for identifying low *E. coli* concentrations, is indicated by a lower Kd value. The precise area of the E. coli cell that the aptamer binds to—such as lipopolysaccharides, flagellin proteins, outer membrane proteins, or other cell wall antigens essential for pathogen identification—is referred to as the Target Binding Site [22]. The aptamer-

nanomaterial system's minimal detectable E. coli concentration, usually expressed in CFU/mL, is known as the Detection Limit. Particularly useful for detecting traces of *E. coli* in food safety applications are systems with lower detection limits [23].

4. Food safety challenges and the role of aptasensors

Food safety issues have increased due to the globalization of the food industry, as contamination poses serious health hazards including decreased protein synthesis, DNA damage, and damage to the nervous system [24]. Unhygienic production methods, inappropriate storage, and excessive use of veterinary medications and pesticides are contributing issues. Public health is greatly impacted by foodborne disease outbreaks that are made worse by poor food cleanliness [25]. Conventional detection techniques, such as mass spectrometry, chromatography, and real-time PCR, are very accurate but costly and need skilled personnel. To identify food pollutants, biosensors have been created, including enzyme-based, immune-based, thermal, and piezoelectric systems [26]. High specificity and sensitivity are difficult to achieve with these techniques, though, especially when using single or composite chemical compounds. As functional biosensors, aptamers present a viable substitute. Aptamers have the ability to particularly detect food contaminants due to their three-dimensional conformational changes. Their exceptional purity and economical synthesis increase the repeatability of detection techniques. The lack of certain aptamers and the difficulties presented by complex food matrices, which can disrupt aptamer-target binding, limit the use of aptasensors in food safety despite their potential [27]. Aptamers' recognition stability may be increased by screening them in environments that resemble intricate food matrices to overcome these difficulties. This strategy would improve the usefulness of aptamers in actual food safety monitoring, offering a powerful weapon against foodborne pathogens such as *E. coli* [28].

5. Sequence Optimization Techniques

Enhancing the binding affinity, specificity, and stability of aptamer sequences is essential, especially when coupled to nanomaterials for disease detection like *E. coli*. Methods like as secondary structure analysis, truncation, and mutation have been successful in improving aptamer performance. These methods increase the sensitivity and dependability of *E. coli* detection systems while facilitating stronger interactions with nanomaterials [29]. A popular method for streamlining aptamer sequences is truncation, which eliminates unnecessary parts while leaving the crucial binding domain that interacts with *E. coli* intact. The stability and binding kinetics of the aptamer are improved, and the effectiveness of aptamer-nanomaterial conjugation is increased as a result of this sequence length reduction. Because of these characteristics, truncated aptamers are especially well-suited for real-time biosensing applications, where strong and quick reactions are crucial

[30]. To improve aptamer performance, mutation-based optimization entails making precise modifications to the nucleotide sequence, such as point mutations, insertions, or deletions. By adjusting the way the aptamer interacts with *E. coli* target sites, including lipopolysaccharides or flagellin proteins, these alterations can increase binding affinity and specificity [31]. In nanomaterial-based detection systems, strategic mutations can also improve the aptamer's stability and efficacy by making it more resistant to environmental influences. The performance of aptamers can be significantly improved by using sequence optimization approaches such as truncation, mutation, and secondary structure analysis. Truncation ensures effective conjugation with nanomaterials by enhancing the aptamers' binding kinetics and structural stability. For biosensors functioning in actual food matrices, mutation-based alterations improve robustness in complicated contexts and refine target specificity. The design of aptamers with stable conformations is made possible by secondary structure analysis, which is aided by computational methods. This enhances the aptamers' binding efficiency and compatibility with nanomaterial platforms [19]. The success of these biosensors also depends on the choice of nanomaterials, including carbon nanotubes, Go, and gold nanoparticles. Gold nanoparticles improve visual signal transduction, carbon nanotubes boost electron transport for electrochemical sensing, and graphene oxide gives a wide surface area and π-π stacking interactions. These characteristics produce extremely sensitive and reliable detection platforms when paired with aptamers that have been tuned [32]. Even with these developments, several obstacles still exist. Aptamers that target certain strains of *E. coli* are not widely available, and environmental factors like pH, temperature, and the complexity of food matrices can affect how well they work. To increase detection sensitivity, conjugation techniques also need to balance stability and reversibility. It will take sustained innovation in aptamer screening, reliable conjugation methods, and the creation of biosensors that can operate in a variety of environmental settings to overcome these obstacles. For automated and real-time monitoring, future work should also concentrate on increasing the production of aptamer-nanomaterial biosensors and integrating them with digital platforms. These developments have the potential to completely transform pathogen detection and provide a more practical, dependable, and effective way to address the problems with food safety around the world.

6. Conclusion and future directions

Aptamer-nanomaterial integration has revolutionized biosensing by providing a quick, accurate, and economical way to identify *E. coli* in food safety applications. Researchers have created platforms that surpass the sensitivity, affordability, and ease of use of conventional techniques like PCR by fusing the exceptional qualities of nanomaterials like graphene oxide, gold nanoparticles, and carbon nanotubes with the high specificity and versatility of aptamers. The binding affinity, stability, and resilience of aptamers have been further improved by advancements in sequence optimization, such as truncation, mutation, and secondary structure analysis. Furthermore, sophisticated conjugation techniques have improved signal transduction and overall biosensor performance while guaranteeing stable aptamer-nanomaterial attachment.

Notwithstanding these developments, there are nonetheless issues, especially with guaranteeing aptamer performance in intricate food matrices and honing conjugation methods for the best detection. To overcome these obstacles, interdisciplinary cooperation will be needed to build hybrid biosensors that can combine optical, electrochemical, and pH-based detection, enhance nanomaterial functionalization procedures, and increase aptamer libraries targeting various biomarkers. Future studies should concentrate on optimizing production techniques for scalability and broad adoption. Aptamer-nanomaterial biosensors have the potential to be extremely important in contemporary food safety procedures if these obstacles are removed. They are essential instruments for reducing hazards to the public's health and raising international food safety standards because of their capacity to guarantee the quick, accurate, and early detection of pollutants like *E. coli*.

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