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Recent Advances in Optical DNA and RNA-Based Biosensors: Principles, Mechanisms, and Applications

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Abstract

Optical gene sensors are a significant advancement in biosensor technology that utilize light-based detection methods to detect specific nucleic acid sequences. These sensors utilize the principles of optics and biochemistry to achieve high accuracy and precision in the detection of genetic components, making them valuable tools in medical diagnostics, environmental monitoring, and food safety.The main goal of this technology is to improve the accuracy and speed of gene sequence detection and use optical methods for specific detection of DNA or RNA. Optical gene sensors typically use techniques such as fluorescence, surface plasmon resonance (SPR), and absorption measurements to detect target DNA or RNA sequences. The combination of target nucleic acids and the sensor surface produces a measurable optical spot that correlates with the target. There are designs available for optical gene sensors, including fiber optic sensors, microarray platforms, and lab-on-a-chip systems. Each design offers a variety of design considerations, capabilities, and ease of use.These sensors have specific applications ranging from clinical diagnostics to the detection of genetic diseases in food products and environmental samples. Their ability to provide rapid results increases their utility in point-of-care testing scenarios.

Keywords
Biosensor, Luminescence, DNA, Fluorescent, Plasmon resonance.

1. INTRODUCTION

Biosensors are a prominent topic within the current scientific community. By definition, a biosensor is an analytical device that integrates the specificity of a biological sensing element with a transducer to produce a signal proportional to the concentration of a target analyte [1, 2]. A brief recent review contextualizing optical nucleic-acid sensing approaches and trends is provided in the literature. This review highlights advances in amplification strategies, plasmonic- and fluorescence-based transduction mechanisms, and integration with microfluidics for point-of-care use. The following sections summarize the main principles, recent mechanisms, and applications of DNA- and RNA-based optical biosensors [3].

Simply, biosensors are analytical tools designed to detect various analytes. Emerging biosensor technology aims to achieve rapid, specific, and sensitive detection.

A biosensor can be viewed as a sensing device or measurement system tailored for the detection of a specific analyte, utilizing biological interactions to generate a signal that is then transformed into a readable form through a transducer. The essential components of a typical biosensor include biological receptors and transducers. Depending on the types of transducers

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employed, biosensors can be classified as electrochemical, piezoelectric, calorimetric, and optical biosensors. Generally, biosensors provide quantitative information regarding the presence of analytes [4, 5].

From electrochemical to optical biosensors, significant advancements have been made in medical and environmental fields, benefiting from developments in molecular biology, nanomaterials science, and notably, computer science and optoelectronics. These technologies have proven invaluable in the food industry, environmental pollution monitoring, and medical treatment, particularly with optical sensors utilizing optical detection and FRET technology [6-10].

Although research on DNA biosensors remains limited, DNA itself is undoubtedly one of the most vital biomolecules. The distinct complementary pairing between adenine and thymine, as well as cytosine and guanine, has served as the foundation for genetic analysis over many years. The ability of single-stranded DNA (ssDNA) to bind specifically with its complementary strand within a sample is the fundamental concept behind DNA-based detection technologies [11, 12].

It is widely accepted that DNA and its assembled structures can be utilized to identify specific targets, including nucleic acids, proteins, metal ions, and small biological molecules. With advancements in DNA nanotechnology, dynamic networks based on DNA hybridization play a vital role in amplifying the signals generated by biosensors [13, 14].

Screening processes reveal that DNA probes, such as aptamers, demonstrate superior thermal stability, tunable biological affinity, and enhanced resistance to degradation by nucleases. Importantly, biosensors that utilize functional DNA strands, such as those based on DNA hybridization and DNA templates, effectively employ these strands to detect particular targets, including aptamers. In recent years, considerable research efforts have focused on the design and application of DNA-based biosensors capable of performing various biomedical functions, including targeting, sensing, imaging, and therapy. Furthermore, various DNA-based biosensors have been integrated with logic functions to facilitate rapid yes/no responses [15, 16].

In this article, we will examine optical biosensors that utilize DNA and RNA. We will first classify the optical biosensors, which are primarily discussed in the literature.

2. Methodology of the Review

Since this manuscript is a narrative review, no laboratory experiments were performed. Instead, a structured literature- search strategy was applied to collect, screen, and analyze scientific publications related to optical DNA/RNA-based biosensors. A comprehensive literature search was conducted in major scientific databases, including PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar. Studies published between 2000 and 2025, in line with the publication years of the sources already used in this review.

The initial search retrieved over 70 articles. Titles and abstracts were screened manually by the authors to assess relevance. Full-text screening was performed for eligible articles. Finally, approximately 42 high-quality studies were selected. The extracted information was categorized and synthesized into thematic sections PCS, SPR, fluorescence-based biosensors, luminescent platforms, microfluidic-integrated sensors forming the basis of the Results & Discussion section.

3. RESULTS AND DISCUSSION

An Overview of Optical Biosensors: Types, Principles, and Applications

Optical biosensors convert specific biological interactions into measurable changes in light (e.g. via refractive index, fluorescence or plasmonic resonance), enabling real-time and label-free detection [17]. These devices including SPR, waveguide, fiber-optic and resonator-based sensors offer high sensitivity and have been widely applied in clinical diagnostics, environmental monitoring, food safety, and drug discovery [18].

3.1. Photon Correlation Spectroscopy

PCS is a powerful technique used in various fields, including optical biosensors, to analyze the properties of light and the dynamics of particles. This method leverages the temporal correlations of photons emitted from a sample to extract information about its characteristics, such as size, concentration, and dynamics. PCS is primarily employed to study the size and behavior of nanoparticles in suspension through dynamic light scattering (DLS). By analyzing fluctuations in scattered light intensity over time, PCS can determine particle size distributions and monitor changes in particle dynamics, which are crucial for applications in biosensing and diagnostics [19]. Optical biosensors benefit significantly from photon correlation techniques due to their high sensitivity and specificity. These sensors can

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detect biological interactions by measuring changes in light patterns resulting from binding events at the sensor surface.

PCS can be used for the real-time monitoring of biomolecular interactions. By utilizing this capability, it significantly enhances the detection capabilities of biosensors for various critical analytes, including proteins and nucleic acids [20]. A specialized application, Photon-Emission-Correlation Spectroscopy (PECS), is employed in solid-state systems to assess single-photon emission properties. This is an essential function for the development of quantum technology applications. PECS provides crucial information regarding the electronic configuration and optical behavior of quantum emitters, insights that conventional spectroscopy techniques typically cannot provide.

The integration of PCS with microfluidic systems leads to the development of highly effective integrated optical biosensors. This combination enhances the overall performance of these biosensors by enabling the simultaneous monitoring of multiple parameters, which ultimately leads to an improvement in both the accuracy and sensitivity of the detection system.

Recent advancements in detector technologies have propelled the evolution of photon correlation techniques. Innovations such as single-photon detectors (SPDs) allow for more complex measurements, including multidimensional photon correlations. This capability enables researchers to extract additional spectroscopic information by correlating photons based on multiple properties, such as energy and polarization [21].

Despite its advantages, implementing PCS in optical biosensors involves challenges the efficiency of photon detectors is a critical factor that significantly impacts the reliability and accuracy of the measurement outcomes. To perform accurate correlation measurements, it is essential to achieve high detection rates. Low efficiency can result in insufficient data for robust Variability in timing, known as timing jitter, is a challenge that can directly affect the resolution of measurements. To improve the temporal resolution of the PCS system, advanced detector technologies are continuously being developed with the specific aim of minimizing timing jitter.

Another challenge is the complexity of the experimental setup. Implementing more sophisticated PCS-based biosensors often requires a higher level of technical expertise and specialized resources. This inherent complexity can sometimes limit the accessibility of the technology, particularly for certain research applications or smaller laboratories.

Photon-emission-correlation spectroscopy (PECS) serves as a crucial yet frequently underexploited method for investigating the optical and spin behaviors of quantum emitters. This technique focuses on the examination of temporal correlations between photons emitted from a fluorescent source, illustrated in Figure 1. PECS is predominantly utilized to confirm the emission of single photons linked to quantum emitters via the detection of photon antibunching phenomena. As a steady-state measurement that requires only continuous excitation, single-photon detectors, and appropriate timing electronics, PECS is relatively straightforward to execute. Despite its simplicity, it offers extensive insights into the optical behavior of emitters, encompassing excited-state lifetimes, radiative and nonradiative relaxation processes, along with spin and charge dynamics. This document outlines how PECS can be employed as a versatile characterization instrument for solid-state quantum emitters. We provide specific guidelines tailored for effective data collection, analysis, and interpretation. Notably, we illustrate how PECS can reliably validate single-photon emission and facilitate the development of models regarding the electronic states and optical dynamics of the emitter, thus assessing its potential for applications in quantum technology [22]. 3.2. Surface Plasmon Resonance in Optical Biosensors

3.2. Surface Plasmon Resonance in Optical Biosensors

Surface Plasmon Resonance (SPR) is a powerful optical technique used for real-time, label-free detection of biomolecular interactions. This technology has become increasingly popular in biosensing, especially for use in medical diagnostics, environmental surveillance, and drug development research. SPR occurs when incident light interacts with free electrons at the interface between a metal (commonly gold) and a dielectric material. At a particular angle of incidence, referred to as the resonance angle, there is a noticeable decrease in the intensity of reflected light. This effect is highly sensitive to variations in the refractive index close to the metal interface, which happens when biomolecules attach to the probe molecules fixed on the sensor surface [23, 24].

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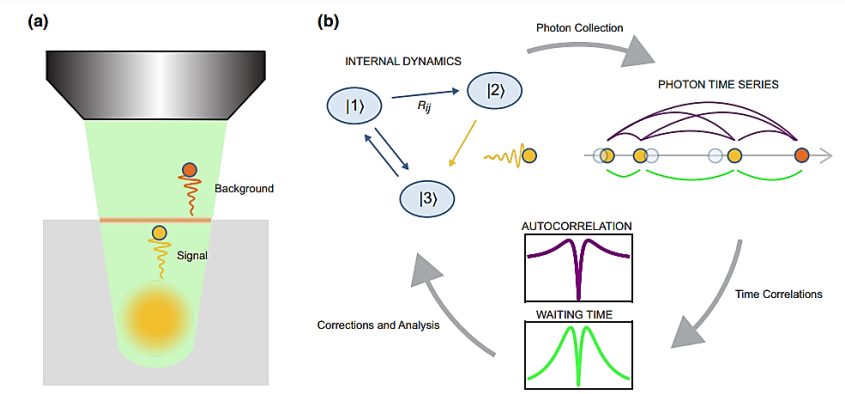

Fig. 1. This figure summarizes the PECS workflow. Laser excitation of a quantum defect in a solid-state crystal produces fluorescence that is collected along with background photons. Time-resolved photon detections are then analyzed through autocorrelation or waiting-time statistics to reveal the emitter's internal dynamics [22].

3.2.1 SPR components

Recent advancements in Surface Plasmon Resonance (SPR) biosensors have been driven by the need to enhance both sensitivity and specificity in detecting biomolecular interactions. Researchers have introduced innovative SPR designs that incorporate advanced materials such as titanium, silver, and graphene. These materials are chosen for their unique optical and electronic properties, which can significantly improve the biosensor's ability to detect specific targets, including cancer cells and hemoglobin, with greater accuracy [25].

In addition to material innovations, considerable progress has been made in surface functionalization techniques. Methods like the creation of self-assembled monolayers are now commonly employed to minimize nonspecific binding, thereby reducing background noise and increasing the reliability of the sensor[26]. These techniques also facilitate the stable and efficient attachment of probe molecules, further enhancing the performance of SPR biosensors.

Overall, Surface Plasmon Resonance represents a transformative approach in the field of optical biosensing. It offers distinct advantages such as high sensitivity, real-time analysis, and the ability to perform label-free detection. The versatility of SPR has led to its widespread application across diverse fields, from medical diagnostics to environmental monitoring, underscoring its significance as both a diagnostic tool and a research instrument. As technological advancements continue, the potential of SPR to unravel complex biomolecular interactions is expected to grow, paving the way for even more sophisticated and effective biosensing solutions in the future [27].

The relationship between RU (Resonance Unit) change and the amount of Fusarium culmorum genomic DNA in PCR, emphasizing the use of different concentrations of durum wheat DNA. This analysis was performed under optimized experimental conditions, which included specific buffer components such as 20 mM Na_2 HPO_4 (pH 7.4), 0.1 mM EDTA, 300 mM NaCl, and 10% formamide. This study has shown how different amounts of Fusarium culmorum DNA template affected the change in RU during PCR amplification. This correlation is crucial for understanding the dynamics of fungal DNA quantity in agricultural samples.

The experiment was conducted under carefully controlled conditions, which are critical for achieving reliable and reproducible results in PCR assays. The selection of buffer components ensured that enzymatic reactions were carried out efficiently and minimized potential inhibitory effects of sample matrices. This fungal species is known for its role in producing mycotoxins that can contaminate cereals, leading to significant agricultural losses and health risks. Understanding the quantity of DNA has helped develop effective monitoring and control strategies in crop management.

The findings of this study have been used to enhance methods for the detection and quantification of Fusarium culmorum in various plant materials. By optimizing PCR conditions and accurately measuring the correlation between DNA concentration and amplification efficiency, researchers have been able to improve diagnostic assays that are essential for food safety and agricultural health. In summary, this research provides valuable insights into the methods used for the detection of Fusarium culmorum and highlights the importance of precise experimental conditions and their impact on PCR results (Fig 2) [28].

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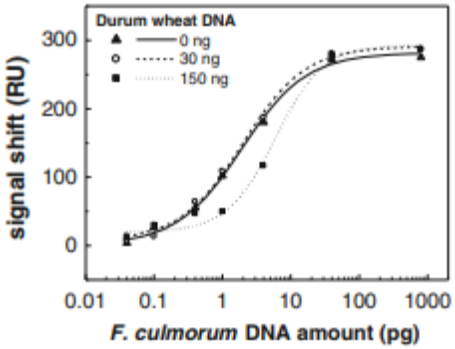

Fig. 2. This study demonstrates the relationship between the RU shift and the amount of initial genomic DNA template from Fusarium culmorum (ITEM 627) utilized in PCR, incorporating various concentrations of durum wheat DNA. All measurements were taken under ideal experimental parameters, which comprised 20 mM Na_2 HPO_4 (pH 7.4), 0.1 mM EDTA, 300 mM NaCl, and 10% formamide [28].

The surface plasmon resonance (SPR) biosensor technology employed for the detection of Fusarium culmorum utilizes a real-time baseline correction method. This method consists of deducting the reference measurement obtained from flow cell 2 (FC2) from the signal captured in flow cell 1 (FC1). The analytical output is indicated by the change in resonance units (RU) observed throughout the injection of the sample [24]. The SPR biosensor system designed for detecting Fusarium culmorum utilizes a real-time baseline correction technique. This approach involves subtracting the reference signal acquired from flow cell 2 (FC2) from the target signal recorded in flow cell 1 (FC1). The analytical output is represented by the change in resonance units (RU) observed throughout the sample injection process. For each measurement, baseline correction is automatically performed by deducting the FC2 reference signal from the FC1 target signal. The analytical response is quantified as the RU shift measured during the sample introduction phase. More specifically, it is calculated by taking the difference between the RU reading obtained after washing the sensor surface following the hybridization event and the initial baseline RU measurement.

To assess the influence of the sample matrix on SPR-based detection of F. culmorum in wheat specimens, PCR amplification was conducted using different genomic DNA amounts of F. culmorum (ranging from 0 to 800 pg). These tests were carried out in the presence of varying concentrations (0, 30, or 150 ng) of durum wheat DNA extracted from sterile-grown wheat buds. The resulting PCR products were then analyzed using the SPR biosensor (Fig. 2)[28]. The findings showed that reliable results, consistent with those obtained from pure F. culmorum cultures, were achieved only in samples containing 30 ng of durum wheat DNA. When 150 ng of durum wheat DNA was included, the observed RU shifts were significantly lower than those seen with pure F. culmorum DNA, indicating that the presence of wheat DNA likely hindered the efficiency of the PCR amplification [29]. The detection thresholds were determined to be 0.05 pg (which corresponds to a signal change of 10.1 RU) and 0.06 pg (associated with a signal change of 17.4 RU without applying blank correction) for F. culmorum DNA, measured in the absence and presence of 30 ng of durum wheat DNA, respectively [23].

In this study, the measurement of *E. coli* DNA was performed as an indicator for assessing maize DNA; the detection limit for measuring *E. coli* DNA was 0.1 pg, which produced a signal shift of 12.5 RU in the presence of 20 ng of maize DNA[30]. In another study, the measurement of *Salmonella* DNA using PCR amplification enabled the detection of as little as 0.03 pg of target DNA, which resulted in a signal shift of 8.2 RU after blank correction[31]. In a separate report, the measurement of *Listeria monocytogenes* DNA in the absence of background plant DNA yielded a detection threshold of 0.04 pg, corresponding to a signal shift of 11.0 RU [32]. Another report demonstrated that the measurement of *Aspergillus flavus* DNA remained feasible even in the presence of 50 ng of rice DNA; the minimum measurable amount was 0.07 pg, producing a signal shift of 15.8 RU[10]. In a related study, the measurement of target DNA with a highly sensitive PCR assay allowed for the detection of 0.02 pg of DNA, generating a signal shift of 9.3 RU even when 10 ng of wheat DNA was added to the reaction mixture [33].

These examples specify the detection limit, the amount of DNA, the signal shift in RU, and the presence or absence of background DNA.

3.3. luminescence in optical genosensor

Luminescence plays a crucial role in the development of optical genosensors, which are devices designed to detect specific nucleic acid sequences. These sensors utilize luminescent materials to provide a detectable signal when the target DNA or RNA is present. The following sections explore the principles, types, and applications of luminescence in optical genosensors [34].

Luminescence can be categorized into two main types: fluorescence and phosphorescence. Fluorescence occurs when a material absorbs light

and re-emits it almost instantaneously (in the range of picoseconds to nanoseconds), while phosphorescence involves longer emission times (milliseconds to hours) due to energy trapping mechanisms.

In optical genosensors, fluorescence is predominantly utilized because of its rapid response and sensitivity.The basic working principle involves the binding of a luminescent probe to a target nucleic acid sequence. When the target is present, it induces a change in the luminescent properties of the probe, such as an increase or decrease in fluorescence intensity. This change can be quantitatively measured, allowing for the detection of specific DNA or RNA sequences.

3.3.1. Types of Luminescent Materials Used

Carbon Dots (CDs) are defined as nanometer-sized carbon particles that exhibit strong photoluminescence. Due to their inherent high stability and biocompatibility, they have been successfully employed in genosensors. For instance, a notable application involved a genosensor that used carbon dots; it demonstrated a decrease in fluorescence when the target DNA was initially present. The fluorescence signal was subsequently restored after hybridization with matching sequences, showcasing a clear mechanism for target detection [35].

Nanomaterials have been widely incorporated into luminescent sensing devices to enhance their performance. Different types of nanomaterials, such as gold nanoparticles and magnesium oxide (MgO), are used for this purpose. These materials can enhance luminescent signals through various mechanisms, including resonance energy transfer, which ultimately leads to an improvement in the overall sensitivity of the sensing device.

Luminophores are defined as chemical compounds that emit light upon excitation. A key application of these compounds is their use in sensing devices, where they can be encapsulated within sensor matrices to create highly stable luminescent probes specifically designed for detecting biomolecules [36].

3.3.2. Applications of Luminescent Optical Genosensors

Optical genosensors that employ luminescence have found important applications across various fields. In Medical Diagnostics, they are utilized to identify pathogens, such as viruses and bacteria, by recognizing particular genetic sequences linked to these microorganisms. For example, a label-free photoluminescent genosensor was shown to have high sensitivity for detecting cholera-related DNA. Additionally, they are used in Environmental Monitoring, where luminescent sensors can detect pollutants or toxic agents by measuring changes in luminescence associated with specific contaminants. Finally, these sensors contribute to Food Safety by helping to identify harmful microorganisms in food products through the targeting of their genetic material.

Despite their advantages, the development of luminescent optical genosensors still faces several challenges. Achieving high sensitivity while minimizing non-specific signals remains crucial for reliable detection, and it is equally important to ensure that these sensors maintain stable and repeatable performance over time for practical applications. Future research in this field may focus on enhancing the design of luminescent probes, improving signal amplification techniques, and integrating advanced nanotechnology to develop more effective genosensing platforms. In conclusion, luminescence provides a powerful mechanism for creating sensitive and selective optical genosensors capable of detecting specific nucleic acids, offering broad applications in medical diagnostics, environmental monitoring, and food safety [37].

3.3.3. Fluorescence genosensors

Fluorescence genosensors are advanced biosensing devices that utilize fluorescence-based detection methods to identify specific nucleic acid sequences. These sensors are particularly valuable in medical diagnostics, environmental monitoring, and food safety due to their sensitivity, specificity, and rapid response times.

Fluorescence genosensors typically operate on the principle of fluorescence resonance energy transfer (FRET) or photoluminescence quenching. In these systems, a fluorescent probe (often a quantum dot or a carbon dot) is designed to emit light when excited. When a target DNA sequence is present, it can induce changes in the fluorescence emission, which can then be measured and quantified. This quantification process enables the precise determination of the target sequence's concentration, providing valuable insights into its presence and abundance.

Fluorescence genosensors have been successfully developed for detecting various viruses, such as the human T-lymphotropic virus (HTLV-I) and Hepatitis B virus. For example, a genosensor using carbon dots demonstrated a limit of detection as low as 10 nM for HTLV-I [38].

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Aptamer-based fluorescence biosensors have shown promise in detecting biomarkers like vascular endothelial growth factor (VEGF) in liver cancer patients. These sensors achieved detection limits around 3.01 ng/mL and demonstrated high accuracy in clinical sample analysis [39]. Fluorescence genosensors are being explored for real-time monitoring of pathogenic bacteria and viruses, providing an alternative to traditional diagnostic methods like PCR and ELISA [40].

Fluorescent biosensors offer several notable advantages that make them highly valuable in various scientific and medical fields. One of the main strengths of these sensors is their high sensitivity, as the use of fluorescent materials enables the detection of even very low concentrations of target nucleic acids. This heightened sensitivity is particularly important in applications where early detection is crucial, such as in disease diagnostics or monitoring environmental contaminants. Additionally, fluorescent biosensors are known for their rapid response times, often delivering results much faster than conventional analytical methods. This quick turnaround is beneficial in clinical settings where timely decision-making can significantly impact patient outcomes. Another important advantage is their versatility; these sensors can be tailored for a wide range of uses, from clinical diagnostics to environmental monitoring, making them adaptable tools for diverse applications.

Despite these strengths, fluorescent biosensors also face some limitations that can hinder their broader adoption. One significant challenge is the stability of the biological components used in these sensors. Over time, these components may degrade, leading to reduced performance and reliability, which is a concern for long-term or repeated use. Furthermore, the design and synthesis of certain fluorescent materials can be quite complex and expensive. This complexity not only increases production costs but can also limit the scalability and accessibility of these sensors, particularly in resource-limited settings. As a result, while fluorescent biosensors hold great promise, addressing these limitations is essential for their continued development and widespread application.

Fluorescence genosensors have found applications across multiple domains, including medical diagnostics, where they aid in identifying diseases, and environmental monitoring, where they help detect pollutants. Despite their potential, fluorescence genosensors face challenges related to stability and operational complexity, which necessitate further research and development to enhance their performance and reliability [41].

Fundamentally, genosensors, often referred to as DNA biosensors, operate based on a recognition reaction involving DNA hybridization between a target and a probe, which serves as the recognition elemen [42]. The technique of DNA hybridization is commonly applied for the examination of particular gene sequences because it is simple and highly specific. These biosensors convert the binding event between the probe and the target into a measurable output by employing a reporter molecule, such as a fluorescent label. Additionally, fluorescent probes can be designed to identify hybridization events, providing remarkable sensitivity and specificity as they generate signals exclusively when hybridized nucleic acids are present. Figure 3a depicts one of the fluorescence genosensor methods used to detect allergenic food substances. A fluorescently tagged single-stranded DNA probe can identify the target DNA by pairing with its complementary strand, resulting in the formation of a double-stranded hybrid [41].

Graphene oxide (GO) is a distinctive and flexible material that demonstrates properties characteristic of polymers, colloids, thin films, and amphoteric substances. It has been extensively employed as a fluorescence quencher in the fabrication of various biosensors utilizing FRET (Förster Resonance Energy Transfer) technology. The mechanism behind fluorescent aptamer-based GO sensors for detecting food allergens is clearly depicted in Figure 3b. In research by Zhang, Wu, Wei, Zhang, and Mo, specific aptamers were utilized as recognition elements targeting the shellfish allergen tropomyosin (TM), resulting in a label-free fluorescent sensing platform based on GO. By optimizing the concentrations of GO and single-stranded DNA (ssDNA) aptamers across different TM levels, they constructed a calibration curve linking fluorescence enhancement to TM concentration. This work highlighted the capability of aptamer-based sensors to deliver high specificity and sensitivity for TM detection in food matrices through an innovative signal amplification strategy [43].

In another study, fluorescein-tagged anti-TM aptamers were initially attached to the GO surface. Upon addition of TM, these aptamers detached from GO and formed aptamer-TM complexes, causing fluorescence to be restored. TM concentrations were quantified by measuring fluorescence intensity, achieving a detection limit of 2 nM within half an hour. Furthermore, engineered a microfluidic device incorporating a quantum dot (QD)-aptamer-functionalized GO nanobiosensor to detect major peanut allergens in just 10 minutes. The QD-aptamer-GO assemblies functioned as probes that interacted with allergens,

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inducing fluorescence changes due to GO's strong quenching ability via FRET. Fluorescence signals were tracked by monitoring the adsorption and release of aptamer-linked QDs, demonstrating excellent sensitivity and selectivity. This method shows promising potential for the detection of various allergens by employing specific aptamer recognition elements [41].

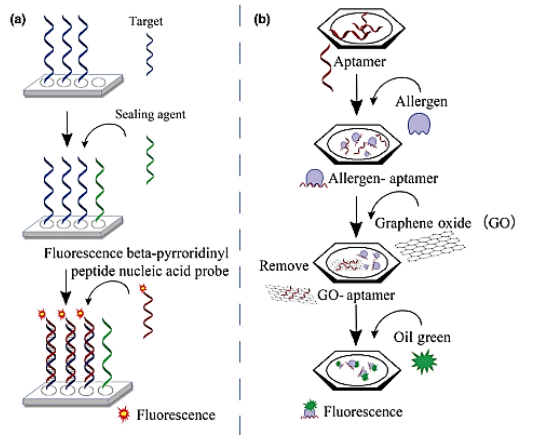

Fig. 3. (a) Diagram depicting the fluorescence genosensor, where a fluorescent beta-pyridinyl peptide nucleic acid probe specifically binds to allergenic gene sequences. This selective interaction enables the measurement of allergen levels by monitoring changes in fluorescence intensity.

(b) Schematic representation of a graphene oxide (GO)-based sensor utilizing fluorescent aptamers. The detection of food allergens is achieved by measuring the fluorescence emitted from the dye-labeled aptamer that targets the allergen, while unbound aptamers are removed through adsorption by GO [41] .

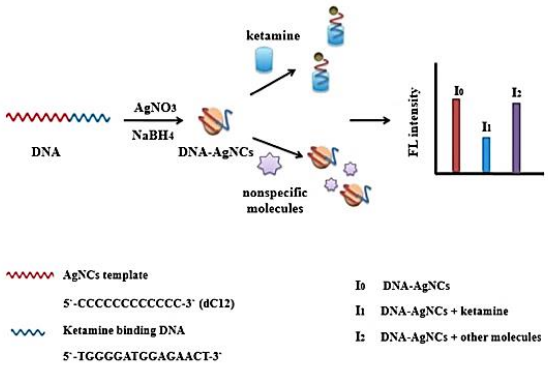

Fig. 4. Schematic illustration of the fluorescence genosensor for determination of ketamine based on DNA-AgNCs [44].

A fluorescence turn-off strategy for ketamine detection was developed using DNA-AgNCs probes, as shown in Figure 4. The probes were synthesized with a DNA template (5′-TGGGGATGGAGAACTCCCCCCCCCCCC-3′), where the dC_{12} segment (5′-CCCCCCCCCCCC-3′) served as the scaffold for AgNC formation and the 5′-TGGGGATGGAGAACT-3′ region functioned as the ketamine-binding domain, consistent with Asghary's report. Upon ketamine addition, structural rearrangements in the DNA-AgNCs induced rapid fluorescence quenching, with the extent of signal reduction proportional to ketamine concentration. Notably, the probes showed minimal response to other compounds, enabling selective ketamine detection through fluorescence intensity analysis [44].

A fluorescent genosensor based on FRET has been developed for the detection of Staphylococcus aureus (S. aureus). This sensor utilizes the interaction between graphene oxide quantum dots (GOQDs) and specific single-stranded DNA (ssDNA) sequences attached to upconversion nanoparticles (UCNPs). When GOQDs assemble onto the fluorescent probes, they quench the upconversion fluorescence signal. However, in the presence of the target S. aureus nuc gene, the ssDNA hybridizes to form double-stranded DNA (dsDNA), which inhibits the binding of GOQDs to the probes, thereby restoring and enhancing the fluorescence intensity. This novel method enables highly sensitive detection of the S. aureus nuc gene and can be adapted for identifying other pathogens by simply modifying the recognition probes and primers within the system. The constructed fluorescent genosensor for Staphylococcus aureus detection includes three key elements (see Fig. 5).

UCNPs functioning as fluorescent reporters, GOQDs acting as nanoquenchers, and tailored ssDNA serving as the recognition element. The specific ssDNA is covalently linked to the UCNPs via carboxyl-to-amino group coupling, creating UCNPs-ssDNA conjugates that act as fluorescent probes. Optical biosensors integrated within microfluidic channels represent a prevalent method in biosensing applications. These sensors utilize microfluidic channels embedded in their substrate to enhance detection capabilities, allowing for rapid and precise identification of pathogens like S. aureus [45].

Recent progress in techniques such as FRET, Fluorescence Lifetime Imaging Microscopy (FLIM), Fluorescence Correlation Spectroscopy (FCS), and fluorescence Imaging (FI) has significantly advanced biological research by enhancing the resolution capabilities of optical microscopy. These methods offer non-invasive ways to observe biological samples in detail. When combined with microfluidic technology, they

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enable highly controlled manipulation of cells and proteins. In this context, we review some of the

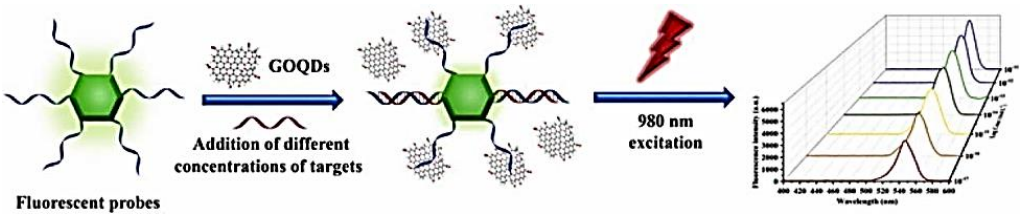

Fig. 5. Graphene oxide quantum dots (GOQDs) are promising carbon-based nanomaterials for sensing applications due to their high surface area, biocompatibility, and strong ability to adsorb ssDNA through π–π interactions. They efficiently quench the fluorescence of probes such as 6-FAM-, JOE-, and Cy3-labeled ssDNA, aided by hydroxyl groups that enhance adsorption. However, FRET-based biosensing using UCNPs–ssDNA and GOQDs for Staphylococcus aureus detection remains largely unexplored [45] .(Reproduced with permission from Subbiah Alwarappan* et al).

latest innovations in these combined approaches. FRET relies on the transfer of excitation energy of a donor fluorophore to a nearby acceptor fluorophore through long-range dipole–dipole interactions. This occurs when the separating distance is within several nanometers. This technique has been widely used in the study of protein and DNA molecules. More recently, Srisa-Art et al. used a microfluidic platform to perform binding assays and kinetics between streptavidin and biotin via FRET. They developed a microfluidic platform to investigate the binding kinetics between streptavidin and biotin using FRET as a readout. In their approach, the microfluidic system enabled precise control over reaction conditions and rapid mixing, allowing real-time monitoring of the binding process with high temporal resolution. By labeling streptavidin and biotin with suitable fluorescent dyes, they could detect binding events through FRET signals, which provided direct kinetic measurements. This method allowed the determination of association rate constants in the range of 3.0×106 to 4.5×107 M-1 S-1 demonstrating the exceptionally fast and strong interaction between streptavidin and biotin. The microfluidic-FRET platform thus offers a powerful tool for high-sensitivity, real-time analysis of biomolecular interactions under controlled conditions [46].

FLIM is a fluorescence imaging technique that relies on differences in the decay rates of excited states in fluorescent samples to create contrast. This method offers robustness against factors such as concentration and photobleaching, making it ideal for functional imaging. Recent studies have demonstrated its utility in advanced applications. For example, Robinson et al. Fluorescence lifetime imaging microscopy (FLIM) has been utilized to perform three-dimensional molecular imaging within microfluidic systems by leveraging the effects of fluorescent quenching (see Figure 6). Moreover, FLIM techniques have been applied for tracking proteins and for an in-depth analysis of the photoconversion behavior of enhanced green fluorescent protein (EGFP).

Our review of DNA and RNA based optical biosensors revealed their strong potential for sensitive and rapid detection of nucleic acids. surface plasmon resonance (SPR) platforms demonstrated real-time, label-free detection with sub-picogram sensitivity, successfully identifying targets such as Fusarium culmorum and Salmonella DNA even in complex backgrounds.

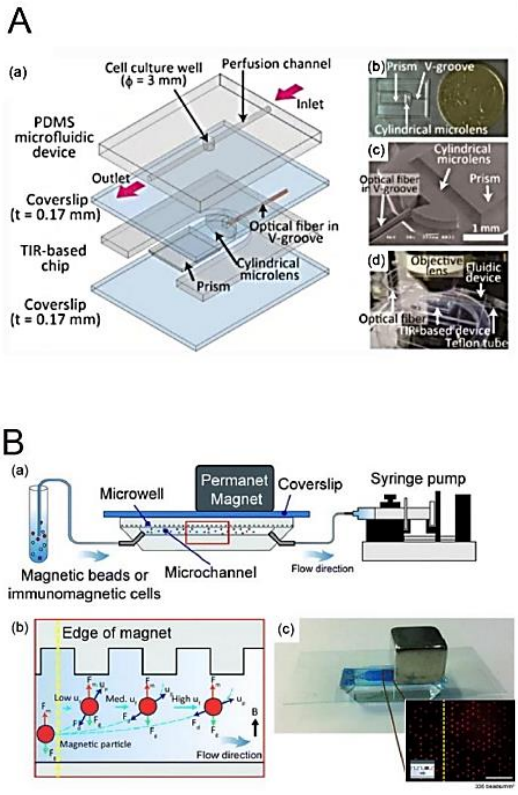

Fig. 6. (A) The microfluidic device with on-chip total internal reflection fluorescence microscopy (TIRFM). (B) A microfluidic microwell device for trapping fluorescent labeled single cells [47].

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Fluorescence-based genosensors, especially those using graphene oxide quantum dots and carbon dots, showed enhanced signal intensity and could detect targets at very low concentrations. These systems also proved effective for detecting viruses like HTLV-I and Hepatitis B virus, as well as allergenic proteins in food samples. Additionally, Photon Correlation Spectroscopy (PCS) enabled detailed analysis of nanoparticle behavior and binding dynamics, improving the precision of optical biosensing. Integration with microfluidic platforms further reduced assay time and sample volume while enhancing reproducibility. Despite these advances, challenges such as probe stability and fabrication complexity remain. Overall, combining advanced optical techniques with innovative nanomaterials has made DNA/RNA-based optical biosensors a promising tool for future diagnostic and environmental applications.

4. CONCLUSIONS

The advancements in optical sensor technology have significantly enhanced their performance metrics, including sensitivity, accuracy, and response time. These improvements enable optical sensors to operate effectively in diverse environments, including harsh conditions where traditional sensors might fail. Moreover, their noninvasive nature makes them ideal for applications involving delicate substances or structures. As research continues to evolve, the integration of optical sensors with advanced materials and techniques promises to expand their functionality further. This includes developments in areas such as smart infrastructure monitoring using fiber optic sensors and innovative medical diagnostics through MIP-based optical sensing systems. The future of optical sensing technology looks promising, with ongoing innovations likely to lead to even broader applications and enhanced capabilities in sensing technologies.

Declaration of interest

There are no conflicts to declare. The authors report no conflicts of interest. Also, the authors are responsible for the writing and content of this article.

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پیشرفت‌های اخیر در حسگرهای زیستی نوری مبتنی بر DNA و RNA: اصول، مکانیسم‌ها و کاربردها

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چکیده

حسگرهای ژنی نوری، پیشرفت قابل توجهی در زمینه فناوری حسگرهای زیستی هستند که از روش‌های تشخیص مبتنی بر نور برای شناسایی توالی‌های خاص اسید نوکلئیک استفاده می‌کنند. این حسگرها از اصول اپتیک و بیوشیمی برای دستیابی به دقت و صحت بالا در شناسایی اجزای ژنتیکی استفاده می‌کنند و آنها را به ابزارهای ارزشمندی در تشخیص پزشکی، نظارت بر محیط زیست و ایمنی مواد غذایی تبدیل می‌کنند. هدف اصلی این فناوری، بهبود دقت، صحت و سرعت شناسایی توالی‌های ژنی و استفاده از روش‌های نوری برای تشخیص اختصاصی DNA یا RNA است .حسگرهای ژنی نوری معمولاً از تکنیک‌هایی مانند فلورسانس، رزونانس پلاسمون سطحی (SPR) و اندازه‌گیری‌های جذب برای تشخیص توالی‌های DNA یا RNA هدف استفاده می‌کنند. تعامل بین اسیدهای نوکلئیک هدف و سطح حسگر، یک سیگنال نوری قابل اندازه‌گیری تولید می‌کند که با غلظت هدف همبستگی دارد. طرح‌های مختلفی برای حسگرهای ژنی نوری وجود دارد، از جمله حسگرهای فیبر نوری، پلتفرم‌های ریزآرایه و سیستم‌های آزمایشگاه روی تراشه. هر طرح از نظر حساسیت، قابلیت‌های چندگانه‌سازی و سهولت استفاده، مزایای منحصر به فردی را ارائه می‌دهد. این حسگرها کاربردهای گسترده‌ای از تشخیص بالینی برای اختلالات ژنتیکی گرفته تا تشخیص پاتوژن در محصولات غذایی و نمونه‌های محیطی دارند. توانایی آنها در ارائه نتایج سریع، کاربرد آن‌ها را در سناریوهای آزمایش در محل مراقبت افزایش می‌دهد.

کلید واژه ها

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