Chapter 2

Advances in Paper-Based Biosensing Technologies: From Principles To Practical Applications

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Abstract

Paper-based biosensors have become of significant interest in recent years owing to their affordability, mobility, and eco-friendliness. Platforms like dipstick assays, lateral flow assays (LFA), and microfluidic paper-based analyzers (μPADs) offer swift and accessible detection capabilities in healthcare, food safety, and environmental monitoring. This study examines the latest advancements in paper-based biosensors, highlighting the enhanced sensitivity and specificity attained by the incorporation of aptamers, CRISPR/Cas systems, and isothermal nucleic acid amplification methods (RPA, RAA, MIRA). Moreover, the utilization of functional nanomaterials, including gold nanoparticles and upconversion nanoparticles, has markedly improved signal amplification and detection efficacy. Although these sensors were originally intended for qualitative assessments, the shift towards quantitative detection is intensifying due to the demand for precise measurements in clinical and food applications. However, challenges such as variability in paper substrates, environmental conditions, and matrix effects remain critical issues. Future perspectives include advancements including smartphone integration, multi-parameter detection, biodegradable materials, and 3D paper-based biosensors.

1. Introduction

Biosensors are analytical instruments that facilitate the rapid, precise, and sensitive identification of specific analytes by combining biological recognition components (such as enzymes, antibodies, aptamers, DNA, and cells) with a physical or chemical transducer. These technologies

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transmute alterations from a biological interaction into electrical, optical, or mechanical signals, facilitating quantitative or qualitative examination. In recent years, biosensors have made substantial advancements in several domains, including medical diagnostics, environmental monitoring, food safety, and biotechnological applications. Paper-based biosensors (PBBs) signify a substantial transformation in biosensor technologies. Paper microfluidic devices (μPADs), created in 2007 by Martinez and colleagues, have expedited research in this field (1). Paper, being a cost-effective, biodegradable, and readily processable material, has emerged as an optimal substrate for biosensor platforms. Paper-based biosensors were created to enhance healthcare accessibility and facilitate quick diagnostic methods, especially in resource-constrained areas. They are also notable for their user-friendly designs, requiring minimal training, and rapid analysis times. The principal factors contributing to the increasing significance of paper in biosensor design are its low manufacturing cost, high mobility, and environmental sustainability. The cellulose-based structure promotes capillary liquid transfer, allowing analyses to occur without external pumps or energy sources. Moreover, paper may be effortlessly cut, folded, and incorporated into diverse designs. Its biodegradability and recyclability provide substantial benefits, especially for the advancement of eco-friendly technologies (2,3).

Paper-based biosensors have established their utility across several applications. In the healthcare industry, they are extensively utilized for early diagnosis of infectious diseases, chronic disease monitoring, and point-of-care (POC) testing (4). In food safety, they have emerged as a crucial instrument for pathogen identification, toxin assessment, and quality assurance. Moreover, they are regarded as an efficient medium for identifying heavy metals, herbicides, and contaminants in environmental surveillance. These adaptable applications establish paper-based biosensors as prominent technologies for the future in biotechnology and analytical chemistry. This chapter will outline the fundamental categories and overarching attributes of paper-based biosensors and synthesize the existing literature on each biosensor type. The main aim of this review is to thoroughly assess the benefits, existing limits, and possible solutions of these biosensor platforms across diverse application domains (healthcare, food safety, environment, etc.). Furthermore, the potential future research and application opportunities for paper-based biosensors will be discussed.

2. Fundamentals Of Paper-Based Biosensors

Paper is a porous, hydrophilic material composed primarily of cellulose fibers. This porous design facilitates the capillary action characteristic of paper. The fiber network of the paper creates a microfluidic environment by enabling the spontaneous movement of liquids driven by surface tension. This feature establishes an optimal medium for the conveyance and distribution of liquids without need on an external pump or energy source. Moreover, the flexibility, lightweight nature, and biodegradability of paper facilitate the efficient manufacture and transportation of microfluidic systems.

The types of paper utilized in paper-based biosensors different based on the device's performance and intended application. Cellulose-based papers are natural and extensively utilized. They possess elevated capillary activity, facilitating fast absorption and conveyance of liquids. They are both economical and ecologically sustainable. Nitrocellulose membranes are typically favored for lateral flow biosensors. Their elevated protein binding ability renders them useful in immobilizing biomolecules and is typically appropriate for color or optical signals (4). Modified papers are papers that have undergone chemical alteration by the introduction of functional groups or polymer coatings. Performance can be improved by including attributes such as targeted analyte interactions, hydrophobicity regulation, or electrical conductivity.

3. Dipstick Biosensors

Dipstick biosensors represent one of the most straightforward paper-based analytical systems, functioning by immersing a paper strip into a sample solution. The liquid traverses the paper through capillary action, engaging with pre-immobilized biosensing molecules, resulting in a visual reaction, typically a color change. The primary benefits of these sensors encompass inexpensive manufacturing expenses, convenient mobility, straightforward operational principles, and swift responsiveness. Moreover, they necessitate no user training, rendering them appropriate for practical applications. Nonetheless, the constraints of dipstick biosensors encompass inadequate analytical sensitivity, semi-quantitative outcomes, and frequently restricted specificity. Moreover, the characteristics of the sample matrix (pH, ionic strength, etc.) can adversely affect analytical performance.

Belsare et al. have created an economical and accessible paper-based dipstick biosensor for the monitoring of gestational diabetes. This technique emphasizes the quantification of glycosylated albumin, an intermediary biomarker, to address the shortcomings of glucose and HbA1c assessments.

In this study involved the invention of two dipstick colorimetric biosensors utilizing aptamers: one tailored for glycosylated albumin and the other for total serum albumin. Gold nanoparticles served as signal generators, while aptamers were chosen as biological recognition elements. The created paper-based dipstick biosensor system is an advanced platform that provides quantitative analytical capabilities, surpassing a mere qualitative test reliant on visual color change. Subsequent to the testing, the paper strips were digitally scanned utilizing a flatbed scanner, and the resultant images were meticulously evaluated employing ImageJ software. Consequently, quantitative analysis of glycosylated albumin and total serum albumin was conducted with excellent precision within physiological concentration ranges. Additionally, limit of detection (LOD) values were determined, and the method's sensitivity was quantitatively validated. Both glycosylated and non-glycosylated albumin forms were quantified within the pertinent physiological concentration ranges—50 μM–300 μM with a LOD of 6.5 μM for glycosylated albumin, and 500 μM–750 μM with a LOD of 21 μM for non-glycosylated serum albumin (5). This method ensures long-term stability at ambient temperature, great specificity, and an extensive dynamic range, in addition to being cost-effective and portable. This study exemplifies the utility of aptamer-based colorimetric sensors, especially in point-of-care (POC) diagnostics for conditions like gestational diabetes.

Nucleic acid amplification-based biosensors are an effective instrument for the swift and sensitive identification of target pathogens, particularly in low concentrations. Isothermal amplification methods, like as Recombinase-Aided Amplification (RAA), are commonly employed in these systems. The RAA method is a swift and economical isothermal DNA amplification approach functioning at low temperatures (37–42°C). Amplification products are frequently discernible by lateral flow dipstick (LFD) tests. This method provides a straightforward, expedient, and equipment-free solution, rendering it especially appropriate for field applications and accessible due to its visual outcomes. In this regard, the RAA-LFD-based biosensor created by Lin et al. presents a novel method for the swift and visible identification of Plasmodium species, the etiological agent of malaria. Its originality consists of being among the initial methods to integrate RAA with LFD for Plasmodium detection, yielding very sensitive findings in about 20 minutes. The assessment is wholly qualitative and depends on the creation of color bands; outcomes are visually evaluated as "positive" or "negative." Moreover, the sensitivity of the approach was thoroughly examined in the study. The LOD for recombinant plasmid DNA was established at 1 copy/mL; in clinical samples, sensitivities of 0.1 pg/mL were attained for

Plasmodium falciparum, 10–100 pg/mL for P. vivax and P. ovale, and 100 pg/mL for P. malariae. Moreover, sensitivities of 0.5 parasites/mL were attained with cultivated parasites. The results indicate that the procedure is a reliable alternative for applications such as screening blood donors or asymptomatic persons in resource-limited settings.

Zheng et al. integrated the reverse transcription recombinase-assisted amplification (RT-RAA) technique with a dipstick platform for the quick, economical, and field-compatible identification of SARS-CoV-2. This work demonstrated nucleic acid amplification in under 30 minutes at a constant temperature of 39°C, with findings interpretable visually by a straightforward dipstick. The new technique has exceptional analytical sensitivity, with a LOD established at 1 copy/mL, surpassing the sensitivity of most current quick testing methods. The measurement approach, although visually impactful as a qualitative test, is semi-quantitative owing to the established LOD and sensitivity analysis. The study demonstrated complete concordance with RT-qPCR in a comparative examination of 100 clinical samples, with specificity and sensitivity rates reported at 100% (6). This platform, characterized by its quick reaction, minimal equipment needs, and elevated sensitivity, has considerable promise for the early detection of infectious illnesses like COVID-19 in field settings or resource-constrained environments.

CRISPR (clustered regularly ınterspaced short palindromic repeats) technology has facilitated groundbreaking advancements in biotechnology, especially in genome editing, and has recently arisen as a potent instrument in the creation of highly specific nucleic acid-based detection systems. These technologies, particularly when integrated with isothermal amplification techniques, has the capability to develop quick, portable, and user-friendly diagnostic platforms. The ERA (enzymatic recombinase amplification)-CRISPR/Cas12a-based lateral flow dipstick biosensor for porcine parvovirus (PPV), created by Wei et al., is significant in this context. This study is unusual since it integrates CRISPR/Cas12a technology with a lateral flow dipstick for the first time, allowing for the detection of PPV within 30 minutes at a constant temperature as low as 39°C. The devised technology facilitates a clearly discernible hue change. The measurement approach relies on visual observation, rendering it fundamentally qualitative; yet, the study explicitly defined the LOD and exhibited high sensitivity. The LOD was recorded at 3.75×10^2 copies/mL, indicating a notable sensitivity superiority compared to PCR. Additionally, no cross-reactivity was detected in specificity assays involving several porcine viruses. In clinical specimens, test outcomes demonstrated complete agreement with qPCR (7). The

approach presents considerable promise for identifying viral infections, especially in resource-constrained regions, due to its rapidity, portability, affordability, and appropriateness for field use. The combined application of Recombinase Polymerase Amplification (RPA) with the CRISPR/Cas12a system facilitates extremely selective and visually discernible detection due to target-specific cleavage activity following amplification. Furthermore, Multienzyme Isothermal Rapid Amplification (MIRA) and lateral flow dipstick (LFD) techniques provide considerable benefits in field applications due to their affordability and swift colorimetric results. In this context, in the study conducted by Li et al., two distinct visual biosensor systems, RPA-CRISPR/Cas12a and MIRA-LFD-based, were created for the swift and precise detection of the quarantine pest Bactrocera correcta. The study's innovation is in the simultaneous optimization of both methodologies, yielding quick, portable, and cost-effective diagnostic platforms applicable across several domains. Both systems yield entirely qualitative results; the RPA-CRISPR/Cas12a system differentiates positive from negative by producing a green fluorescent signal under UV light, whereas the MIRA-LFD system differentiates positive from negative by creating a colored line on the lateral flow strip. This work thoroughly examined the sensitivity of both approaches, establishing the LOD for each system at 1.0×10^{-1} ng/μL DNA. Moreover, due of the expedited DNA extraction methodology, these techniques have been effectively utilized across larval, pupal, and adult phases, yielding results within 10 to 30 minutes (8). This study exemplifies the advancement of biosensor-based, portable, and user-friendly systems for detecting agricultural and quarantine pests.

4. Microfluidic Paper-Based Biosensors

Microfluidic paper-based devices (μPADs) provide regulated fluid movement across paper through hydrophobic and hydrophilic zones established by precise patterning methods. These devices employ capillary action to convey fluid to various analysis zones, facilitating the concurrent detection of many analytes. Principal advantages encompass multiplexed analysis, minimal sample volume requirements, cost-effectiveness, and the absence of pump operation. Moreover, their modular architecture facilitates the creation of tailored platforms. Nonetheless, the intricacy of the patterning procedures in the manufacturing process and restricted sensitivity are considerable disadvantages. Moreover, the efficacy of quantitative measurements may be constrained in certain applications, and concerns related to long-term stability may emerge (9–11).

Colorimetric analyses, a prevalent technique in paper-based microfluidic biosensors, are particularly advantageous for field applications owing to their simplicity, cost-effectiveness, and minimal equipment needs. In these systems, color alterations due to enzymatic processes are evaluated either visually or via software analysis. Distance-based approaches, specifically, create a direct correlation between analyte concentration and the extent of color change, minimizing user-induced errors and yielding visually accessible quantitative findings. These methodologies have secured a significant role in biosensors, especially for the identification of analytes at minimal concentrations. In this regard, the research by Allamah et al. (12) involved the development of a distance-based μPAD for glucose detection in tear samples, created using laser cutting. This study's innovation is in a device, constructed by a CO_2 laser cutting method, tailored for low glucose levels and offering visual detection through distance-based quantitative assessment. The measurement method is a quantitative system reliant on the length of color change, with the color field length exhibiting a strong correlation with glucose concentration. The study reported the LOD as 0.1 mM. The assay utilizes merely 10 μL of material, with results readily observable to the unaided eye within 3–5 minutes, and necessitates no complicated apparatus. The developed sensor emerges as a formidable alternative, especially in resource-constrained settings and for applications like minimally invasive tear-based diabetes monitoring.

Fluorescence-based biosensor systems are extensively utilized in food safety, environmental monitoring, and biological diagnostics owing to their exceptional sensitivity, rapid reaction times, and minimal detection limits. Platforms utilizing Förster resonance energy transfer (FRET) can produce optical signals directly correlated with analyte concentration via fluorescence quenching and recovery processes. Aptamers in these approaches offer excellent specificity for target molecules, allowing for quantitative measurement of signal changes. In biosensor systems, fluorescence methodologies, along with portable devices and basic imaging systems, have emerged as potent analytical instruments for field applications. Tong et al. (13) incorporated multicolor fluorescent carbon dot (CD) aptamer sensors (mCD-μPAD aptasensors) onto a laser-printed paper-based microfluidic chip, facilitating the concurrent and visual identification of three distinct antibiotic residues (sulfamethazine, oxytetracycline, and chloramphenicol) in seafood. This study's innovation lies in its capacity to visually and concurrently quantify three distinct antibiotics in a single assay utilizing multicolor carbon dots and MoS_2 nanosheets, demonstrating great specificity. The measuring method employed is quantitative; fluorescent

color alterations were captured with a smartphone, and the RGB values were assessed using a color recognition tool to determine the analyte concentration based on the gray value. The determined LOD were 0.47 ng/mL for sulfamethazine, 0.48 ng/mL for oxytetracycline, and 0.34 ng/mL for chloramphenicol. The test lasted about 15 minutes and was intended for field applications utilizing a portable 3D-printed apparatus.

Chemiluminescence-based immunosensors are significant in biosensor technology because to their exceptional sensitivity, extensive dynamic range, and minimal background signal. These approaches often transform the particular binding of a target analyte, usually by enzyme-labeled antibodies, into a chemiluminescent signal, facilitating quantitative assessment. When integrated with μPADs, these methodologies provide swift analysis in the field, characterized by cheap cost, mobility, and user-friendliness. Lazzarini et al. (14) created a novel chemiluminescent immunosensor for detecting ovalbumin (an egg allergy) utilizing magnetic microspheres and an origami-based paper microfluidic substrate. The revolutionary aspect is the automation of the multi-step analytical procedure on paper by an origami folding technique, together with the incorporation of all pre-dried reagents within the device. The sensor functions by an immunoassay method that relies on competition between ovalbumin fixed on magnetic microspheres and ovalbumin present in the sample. Chemiluminescent signals were quantitatively assessed utilizing a portable CCD camera and ImageJ software. The reported LOD in the study is very low, at around 1 ng/mL. This work serves as a notable example for the advancement of portable, sensitive, and user-friendly food allergy detection systems.

5. Lateral flow assay (LFA) biosensors

Lateral flow assay (LFA) biosensors are one of the most widely used paper-based biosensor types today and are particularly well-known for pregnancy tests. These devices employ capillary action to convey fluid to various analysis zones, facilitating the concurrent detection of many analytes. Principal advantages encompass multiplexed analysis, minimal sample volume requirements, cost-effectiveness, and the absence of pump operation. Moreover, their modular architecture facilitates the creation of tailored platforms. Nonetheless, the intricacy of the patterning procedures in the manufacturing process and restricted sensitivity are considerable disadvantages. Moreover, the efficacy of quantitative measurements may be constrained in certain applications, and concerns related to long-term stability may emerge (15–17).

The incorporation of CRISPR-Cas systems, especially with LFA-based biosensors, facilitates the creation of accessible, fast, and visually interpretable diagnostic tools. The Bio-SCAN platform, created by Ali et al. (18), integrates the CRISPR/dCas9-specific binding mechanism with isothermal amplification (RPA) and lateral flow technology, providing a novel method for detecting SARS-CoV-2. This study's innovation is in the visual detection of FAM-tagged target DNA on a lateral flow strip utilizing solely biotin-labeled dCas9, so obviating the necessity for intricate apparatus. This offers a technology with elevated sensitivity akin to PCR, yet appropriate for field applications. The measurement approach is fundamentally qualitative, producing visual "positive/negative" outcomes; yet, the established LOD value of 4 copies/μL demonstrates considerable sensitivity. In clinical samples, a positive concordance of 96% and a negative concordance of 100% were attained, with sgRNAs adjusted to guarantee variant specificity. Su and colleagues (19) developed the OC-MLFA (Orthogonal CRISPR-Mediated Multiplexed Lateral Flow Assay) platform by merging orthogonal CRISPR-Cas12a and Cas13a systems, enabling the simultaneous detection of two distinct SARS-CoV-2 genes (ORF1ab and N) on a single lateral flow strip. This study's innovation is in the simultaneous optical detection of two targets on the same strip without cross-talk, thereby surpassing the single-target constraint commonly observed in CRISPR-Cas systems. This approach is qualitative, producing results through color change in various lines on the test strip. The LOD was documented as 10 copies per test, attaining 100% accuracy in clinical specimens. Moreover, it demonstrated the capacity to differentiate from analogous viruses such as SARS-CoV and MERS-CoV with great specificity, underscoring its relevance in the domain. Both research illustrate that CRISPR-based biosensors may be included into lateral flow platforms, thereby converting them into efficient, portable, and sensitive diagnostic instruments. They assert that CRISPR-based biosensors can significantly contribute to the identification of infectious illnesses.

Nanoparticle-based LFA devices have made considerable advancements in biosensor technology, especially for colorimetric and fluorescence-based investigations. In these techniques, metal or fluorescent nanoparticles function as both signal amplifiers and carriers of specialized recognition elements. Nanoparticles, due to their extensive surface area, tailored functionalization, and enhanced optical characteristics, provide markedly higher sensitivity and quantitative measuring capabilities relative to traditional colored gold nanoparticle (AuNP)-based systems. The incorporation of various nanoparticles onto LFA systems has emerged as a crucial approach for quick, portable, and extremely sensitive diagnostic

applications. A study by Yue and colleagues (20) created a fluorescent lateral flow assay platform based on gold nanoclusters (AuNC) for the detection of melamine. AuNCs stabilized with the 6-Aza-2-thiothymine (ATT) ligand generated a robust fluorescent signal via the aggregation-induced emission (AIE) mechanism due to their interaction with melamine. The measurement method employed was quantitative, and the rise in fluorescence was shown to be directly related to the concentration of melamine. The determined LOD value was 217 nM, and significant linearity was attained within the range of 1–100 μM. This technology is distinguished by its simplicity, speed, and cost-effectiveness, functioning exclusively through chemical recognition without reliance on biomolecules. Jin et al. (21) created a LFA utilizing upconversion nanoparticles (UCNPs) for the differential diagnosis of monkeypox virus (MPXV) clades. This sensor functions in both qualitative and quantitative modes because to the robust anti-Stokes fluorescence characteristics of UCNPs. The dual-T-track construction facilitated the concurrent detection of two distinct MPXV clades. This system, readable with a smartphone camera or a benchtop fluorescence analyzer, attained LOD in the picomolar (pM) range, with data produced in eight minutes. This approach exemplifies an effective strategy for diagnosing viral diseases in field settings. In the LFA system created by Ai et al. (22), a mesoporous silica@upconversion nanoparticle@polydopamine (MSUD)-based fluorescence-colorimetric hybrid nanoparticle system was employed for the detection of methamphetamine (MATM). In this instance, UCNPs incorporated into magnetic mesoporous silica (MS) with a substantial surface area were enveloped in a polydopamine (PDA) layer, yielding robust fluorescence and colorimetric signals. This technology possesses a bi-modal reading capability, facilitating qualitative analysis visually and quantitative analysis via a smartphone. The LOD established for methamphetamine were notably low, measuring 1.047 x 10^4 pg/mL for the colorimetric analysis observable by the naked eye and 47.25 pg/mL for the fluorescence-based analysis utilizing a smartphone. It has been evaluated with excellent precision in authentic urine and hair specimens. Gold nanoclusters improved chemical selectivity and fluorescence intensity, whilst UCNPs offered elevated sensitivity and signal-to-noise ratio due to their robust near-infrared-induced fluorescence. The MSUD architecture incorporated fluorescence and color signals, facilitating dual-mode analysis. These investigations illustrate that nanoparticles serve as crucial elements in LFA-based biosensors, functioning as both signal enhancers and facilitators of particular recognition and diverse readout modalities. Research indicates that gold nanoclusters improve chemical selectivity and fluorescent signals,

whilst UCNPs offer elevated sensitivity and a superior signal-to-noise ratio due to their robust NIR-induced fluorescence. The MSUD architecture incorporates fluorescence and color signals, facilitating dual-mode analysis. These investigations illustrate that nanoparticles serve as pivotal elements in LFA-based biosensors, functioning as both signal enhancers and facilitators of particular recognition and diverse readout modalities.

Antibodies are typically employed as biorecognition components in conventional lateral flow assay techniques. Nonetheless, the constraints of antibodies, including their elevated manufacturing costs, restricted stability, temperature sensitivity, and variability during the production process, are amplifying interest in alternative biorecognition agents. Aptamers are distinguished by their exceptional properties. Synthetic DNA or RNA sequences, such as aptamers, have great specificity and affinity, can be chemically produced, demonstrate significant thermal stability, and are readily modifiable due to their modular architecture. Moreover, their target-specific binding affinities are analogous to those of antibodies, enhancing repeatability in biosensors. The Aptamer Sandwich Lateral Flow Assay (AptaFlow) platform, created by Yang et al. (23), clearly illustrates that aptamers can serve as a formidable substitute for antibodies in lateral flow assays. This study utilized two aptamers (SNAP1 and SNAP4) that exhibit high specificity for the N-terminal domain (NTD) of the SARS-CoV-2 spike protein to construct a fully aptamer-based sandwich structure, eliminating the necessity for antibodies. This dual-aptamer technology facilitated both capture and signal production, while aptamer-functionalized AuNPs delivered visual and quantitative signals through colorimetric changes. This method has garnered interest not only for its total neutralization of antibodies but also for its capacity for swift adaptability to emerging variations due to the chemical versatility of aptamers. Moreover, the AptaFlow platform's minimal production expenses and appropriateness for field applications indicate that aptamer-based LFAs may become more prevalent as a practical, cost-effective, and easily scalable diagnostic instrument in the future. This study convincingly illustrates that the incorporation of aptamers into LFA systems is not merely a technological option but also a potential catalyst for a paradigm shift in bioanalytical diagnostic methodologies.

6. Conclusion and Future Perspectives

Paper-based biosensors have achieved significant prominence in biosensor technologies due to its affordability, portability, straightforward working principles, and eco-friendly design. Their swift proliferation, especially in health, food safety, and environmental monitoring, is significant. In recent

years, sophisticated biotechnological methods, including CRISPR/Cas systems, isothermal nucleic acid amplification, aptamers, and nanoparticles, have been effectively incorporated into diverse paper-based platforms, such as lateral flow assays (LFA), microfluidic paper-based devices (μPADs), and dipstick systems. These devices facilitate swift diagnosis through visual or digital output, while providing the benefits of little user training and compatibility in field conditions. Nonetheless, inadequate sensitivity, restricted shelf life, prolonged durability concerns, and absence of standardization persist as considerable constraints in many applications. Moreover, there exists a possibility of false-positive or false-negative results, as well as a potential loss of quantitative precision, particularly in intricate biological samples.

For many years, qualitative assessments have constituted the principal emphasis of paper-based biosensors. Although visual outcomes derived from color changes in lateral flow assays have benefits of simplicity and rapidity, the demand for quantitative analysis is progressively becoming more prominent. In numerous domains, ranging from clinical diagnosis to food safety, merely identifying the presence is inadequate; precise and reproducible quantification of a given analyte's concentration is essential. Nonetheless, quantitative assessment on paper-based technologies continues to pose considerable obstacles. The precise assessment of color intensity or signal strength may be influenced by factors including lighting circumstances, imaging equipment, user error, and discrepancies in paper composition. Moreover, the intricacy of biological sample matrices might adversely affect quantitative precision. Consequently, the advancement of quantitative paper-based biosensors necessitates extensive enhancements in sensor design, signal amplification techniques, and the incorporation of digital technology for data interpretation. Despite this, interest in paper-based biosensors is rising swiftly. The integration of smartphones is a leading focus in contemporary research trends. This facilitates immediate examination of color alterations or fluorescence signals, digitization of outcomes, and cloud-based data transmission. Moreover, multiparameter detection (multiplexing) capabilities are increasingly prevalent, especially in lateral flow and microfluidic systems, facilitating the concurrent identification of multiple biomarkers with high specificity. The advancement of entirely biodegradable systems is becoming increasingly significant due to the rising need for eco-friendly analytical devices and provides benefits regarding waste management. Moreover, advanced designs, like 3D paper-based biosensors, are garnering significant attention in clinical and environmental

applications, facilitating intricate flow control and multi-stage analysis inside a single device.

The significance of paper-based biosensors will continue to escalate in the future. They are anticipated to proliferate as readily available, cost-effective, and user-friendly platforms in domains such as field diagnostics, personalized health monitoring, food contamination management, and environmental surveillance. Aptamer-based biosensors, CRISPR integrations, and nanoparticle-assisted multiplex readout modalities enable the creation of versatile and sensitive diagnostic instruments that surpass conventional antibody-based assays. With the acceleration of standardization, stability enhancement, and industrial scalability, these technologies are anticipated to secure a prominent position in both academic research and as commercial products within the global health, agriculture, and environmental sectors.

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