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EDITED BY
Hamed Golmohammadi,
Chemistry and Chemical Engineering Research Center, Iran

REVIEWED BY
Zeinab Bagheri,
Shahid Beheshti University, Iran
Forough Ghasemi,
Agricultural Biotechnology Research Institute of Iran, Iran

*CORRESPONDENCE
Charles S. Henry,
chuck.henry@colostate.edu

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Advancements and challenges in microfluidic paper-based analytical devices: design, manufacturing, sustainability, and field applications

Prakash Aryal[1] and Charles S. Henry[1,2,3,4]*

[1]Department of Chemistry, Colorado State University, Fort Collins, CO, United States, [2]Department of Chemical and Biological Engineering, Colorado State University, Fort Collins, CO, United States, [3]School of Biomedical Engineering, Colorado State University, Fort Collins, CO, United States, [4]Metallurgy and Materials Science Research Institute, Chulalongkorn University, Bangkok, Thailand

Microfluidic paper-based analytical devices (μPADs) have gained significant attention in analytical science and technology due to their numerous advantages over traditional analytical techniques, including cost-effectiveness, miniaturization, and sustainability. μPADs are widely used in point-of-care diagnostics, health monitoring, environmental detection, food safety, forensics, and security. Since the first report in 2007, there have been substantial advancements in their fabrication techniques, detection methods, and applications. Over the years, significant efforts and advancements have been made to improve the cost-effectiveness, manufacturing scalability, user-friendliness, and sustainability of μPADs. In this review, we explore the general trends and advancements in the fabrication, sensing methods, and applications of μPADs, highlighting key improvements and innovations. We also examine the eco-friendliness of μPADs and present real-world success stories from field studies and citizen science initiatives. Additionally, we address the challenges associated with transitioning μPADs from the lab to the field. Finally, we examine the outlook for μPADs and propose strategies to improve their usefulness in various fields.

KEYWORDS

paper-based microfluidics, fabrication methods, detection techniques, applications, point-of-care, point-of-need

1 Introduction

For centuries, paper has been a workhorse in the world of analysis, serving primarily as a writing surface. However, its unique properties extend far beyond this familiar role. Recent advancements have revealed paper as a powerful tool for scientific detection due to its versatility and accessibility (Ozer et al., 2020). Paper is inexpensive and available all over the world, and transports aqueous fluids by capillary forces, offering a passive means of fluid transport without the need for active pumping mechanisms. Paper's composition allows for a wide range of thicknesses, from the thin and lightweight sheets to the thicker and more robust cardboard. This versatility, combined with its stackability, storability, and transportability, makes it ideal for various applications. Due to its cellulose or cellulose-polymer composition, the paper has a natural advantage in working with biological samples,

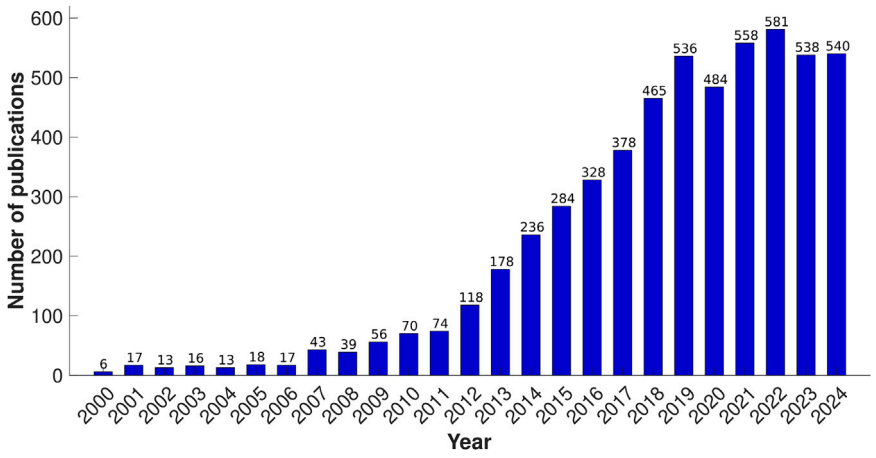

FIGURE 1
Number of publications involving paper-based microfluidics since 2000, derived from Web of Science search results using search criteria: "paper-based*" OR "patterned paper" OR "paper device*" AND "microfluidic*" (Title), as of 2 December 2024.

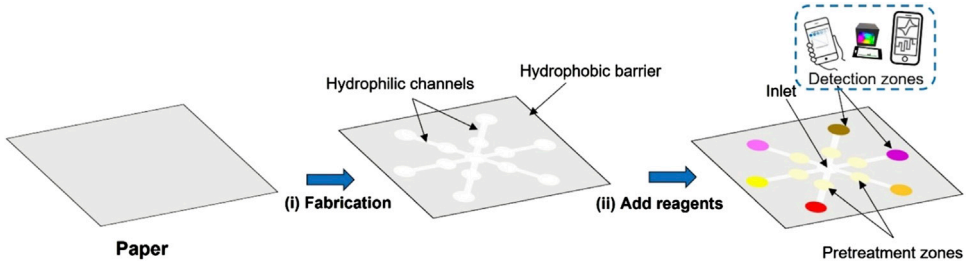

FIGURE 2
General fabrication technique for μPADs.

offering excellent absorbency and biocompatibility. Moreover, its chemical modifiability allows for versatile functionalization, facilitating covalent binding to proteins, nucleic acids, or small molecules. This adaptability broadens the scope of paper-based analytical systems (PADS) in diagnostics, environmental monitoring, and beyond (Noviana et al., 2021; Martinez et al., 2010). As shown in Figure 1, the recent decade has seen a surge in the popularity of paper-based analytical systems following the introduction of the first μPAD in 2007 (Martinez et al., 2007). This simple innovation showcased the potential of paper as a platform for low-cost and portable analysis, sparking its application across various fields.

Many researchers have previously reviewed advancements in microfluidic systems, their commercialization, and their application in various diagnostic, health monitoring, food safety, environmental detection, forensics, etc. (Silva-Neto et al., 2023; Trinh et al., 2022; Han et al., 2020; Hao et al., 2021; Boobphahom et al., 2020; Aryal et al., 2024b; Kummari et al., 2023; Chen et al., 2024; Li and Feng, 2020; Pereira de Oliveira et al., 2018; Qi et al., 2020). Additionally, researchers have explored innovative fabrication techniques for μPADs, extending their use to non-conventional applications (Yamada et al., 2017a; Xia et al., 2016; Noviana et al., 2020a). Building on these recent advancements explored in prior works, this review offers a deeper analysis of overarching trends in microfluidic fabrication techniques, detection methodologies, and their practical applications. We provide a critical assessment of their benefits, drawbacks, and essential implementation considerations. Furthermore, we introduce a novel section exploring the sustainability of μPADs through the lens of the 12 Principles of Green Chemistry. To bridge the gap between research and real-world application, we recognize the challenges and their solutions in transitioning μPADs to practical use, highlighting successful outcomes from field studies and citizen science initiatives. Finally, we provide a future-oriented perspective on μPAD development, proposing methods to enhance their applicability and widespread adoption.

2 Advancements in fabrication techniques

The general principle behind designing μPADs involves creating a hydrophobic barrier to guide aqueous/hydrophilic liquids through capillary-driven cellulose microchannels. These channels contain a cellulose network with numerous -OH groups that interact strongly with aqueous or polar samples to facilitate the flow (Martinez et al.,

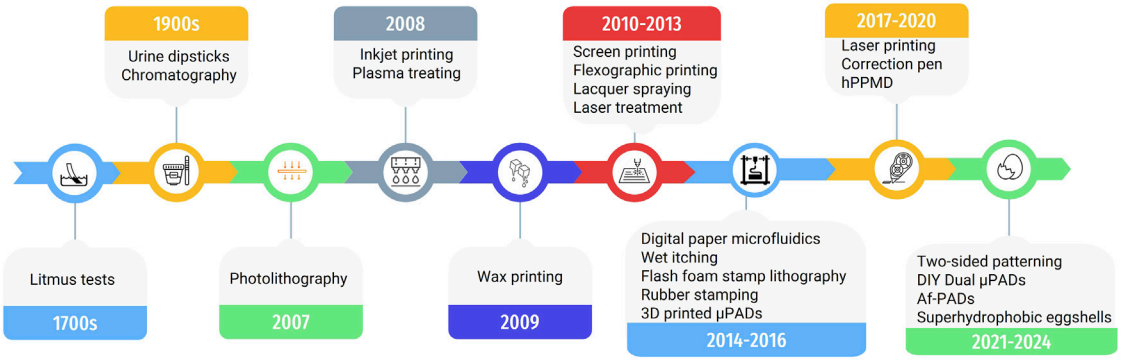

FIGURE 3
Timeline illustrating the development and advancements in fabrication methods for paper-based analytical devices.

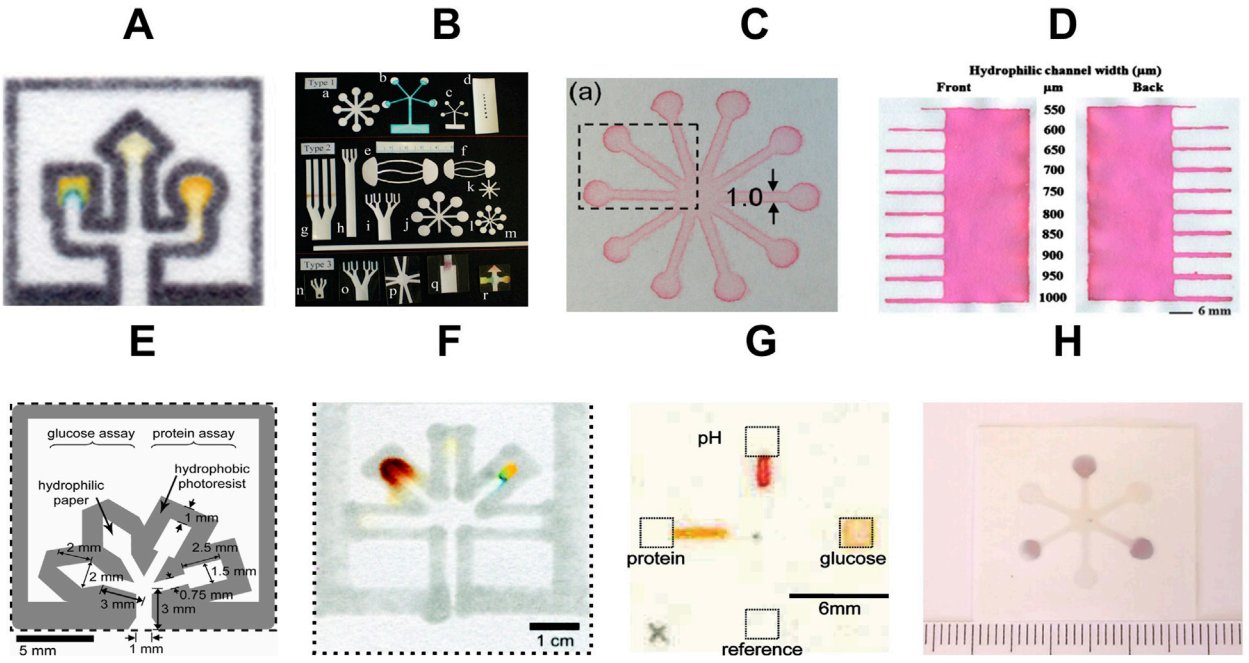

FIGURE 4
Common fabrication methods for paper-based microfluidc devices. **(A)** wax printing (adapted from (Carrilho et al., 2009) with permission, copyright 2009, American Chemical Society). **(B)** knife cutting (adapted from (Fenton et al., 2009) with permission, copyright 2009, American Chemical Society). **(C)** Flexographic printing (adapted from (Olkkonen et al., 2010) with permission, copyright 2009, American Chemical Society). **(D)** wax screen printing (Reproduced from (Dungchai et al., 2011) with permission from the Royal Society of Chemistry). **(E)** photolithography (adapted from (Martinez et al., 2008a) with permission, copyright 2008, American Chemical Society). **(F)** plotting (adapted from (Bruzewicz et al., 2008) with permission, copyright 2008, American Chemical Society). **(G)** inkjet printing (adapted from (Abe et al., 2008) with permission, copyright 2008, American Chemical Society). **(H)** plasma treatment (adapted from (Li et al., 2008) with permission, copyright 2008, American Chemical Society).

2010). The resulting microchannels can be left open or sealed using transparent films, tapes, or thin polymer sheets. The mechanism behind fluid movement in paper-based devices generally adheres to the Washburn equation (Equation 1).

$$l = \sqrt{\frac{\gamma r \cos\theta}{2\eta} t} \quad (1)$$

where l is the distance, γ is the surface tension, θ is the contact angle, η denotes the fluid viscosity, r is the average pore radius, and t is the time (Washburn, 1921). The rate of capillary wicking depends on the channel thickness and the purity of the cellulose. The thickness of the paper substrate determines the height of the microchannels, while the resolution and consistency of the hydrophobic barrier depend on the fabrication method (Martinez et al., 2010). The method chosen for patterning requires the best balance between cost, user-friendliness, and desired resolution.

Once the pattern is created, reagents specific to the assay are applied to the sensing region, and necessary transducers are attached. The sample is then added to the sample inlet, and the

TABLE 1 Summary of fabrication techniques for paper-based microfluidic devices.

Technique	Primary patterning material	Patterning mechanism	Paper types	Benefits	Limitations
Wax Printing/ Patterning	Wax	Physical sealing of paper pores	Cellulose filter papers, chromatography papers	Simple and easy fabrication, environmentally friendly	Expensive printer, requires heating, most commercial wax printers have been discontinued
Photolithography	Photoresist	Physical sealing of paper pores	Thin cellulose, nitrocellulose, glass fiber papers	High-resolution microchannels	Expensive photoresists, costly instruments, extra cleaning step required
Screen Printing	Wax, PDMS, or Carbon	Physical sealing of paper pores	Cellulose papers, polyester films	Easy fabrication, low cost	Low resolution of patterns, requires individual screen for different patterns
Laser Treatment	Any paper	Physical sealing of paper pores	Various cellulose and nitrocellulose papers	Easy to control patterns, good resolution	Requires additional coating step, difficult to achieve lateral flow of fluid
Plasma Treatment	Alkyl Ketene Dimer	Chemical surface modification	Various paper types	Flexible, inexpensive patterning agent	Requires custom masks, potential over-etching problems
Inkjet Printing	Marker ink, AKD, HCAetc.	Chemical surface modification	Smooth papers, photo papers	Relatively inexpensive printers, high-resolution patterns, suitable for mass production	Slow curing, evaporation of volatile reagents
Plotting	PDMS	Physical sealing of paper pores	Filter and chromatography papers	Flexible, inexpensive patterning agent	Not suitable for mass production
Wet Etching	TMOS	Chemical surface modification	Dense, high-quality papers	Inexpensive	Requires manual customization of printing materials
Flexographic Printing	Polystyrene	Physical sealing of paper pores	Coated and uncoated papers	Small microchannels, no heat treatment, roll-to-roll manufacturing	Requires two polystyrene prints (two printing plates needed)

analyte travels to the sensing region through the hydrophilic microchannels. Here, it reacts with reagents to produce signals based on the detection technique. Additionally, the paper can be patterned to include pre-treatment steps such as masking and pH adjustments (Figure 2).

Over the years, significant advancements have occurred in the fabrication of paper-based analytical systems. In 1700s the paper was used as litmus paper tests for pH measurements, marking the early use of paper for analytical purposes (Banks and Watt, 1784). The 1900s saw the introduction of urine dipsticks, spot tests for colorimetric detection, chromatographic methods, and lateral flow assays, further expanding the applications of paper in diagnostics (West, 1945; Comer, 1956; Toennies and Kolb, 1951; Mark et al., 2010; Hawkes et al., 1982). In the late 2000s, groundbreaking fabrication techniques such as photolithography, inkjet printing, wax printing, and plasma treatment (Martinez et al., 2007; Abe et al., 2008; Li et al., 2008; Lu et al., 2009; Carrilho et al., 2009) were developed. These techniques significantly enhanced the precision and capabilities of μPADs.

In the early 2010s, techniques such as screen printing (Dungchai et al., 2011), flexographic printing (Olkkonen et al., 2010), lacquer spraying (Nurak et al., 2013), and laser treatment (Chitnis et al., 2011) advanced the field of paper-based microfluidics, significantly improving the accuracy and speed of these systems. From 2014 to 2016, innovations in digital paper microfluidics (Fobel et al., 2014), wet etching (Cai et al., 2014), flash foam stamp lithography (He et al., 2014), movable-type wax printing (Zhang et al., 2014), rubber stamping (Dornelas et al., 2015), and 3D-printed μPADs (He et al., 2016) marked a transformative shift towards more sophisticated, customizable, and versatile paper-based devices. Between 2017 and 2020, the introduction of laser printed μPADs (LP-μPADs) (Ghosh et al., 2019), correction pens (Mani et al., 2019), and hybrid photo paper-based microfluidic device (hPPMD) (Zargaryan et al., 2020) enabled the creation of more precise and defined patterns on paper substrates, enhancing the complexity of microchannels. More recently (2021-2024), innovations such as two-sided patterning (Monju et al., 2023), DIY dual μPADS (Sousa et al., 2023), acid-free paper-based analytical devices (Af-PADs) (Rawat et al., 2023), and superhydrophobic eggshells (Thangjitsirisin et al., 2024) appear to be promising avenues worth exploring for the development of μPADs.

Among these, particularly noteworthy platforms with significant potential include the incorporation of 3D printing technologies, the development of 3D layered paper-based systems, and the creation of laminate capillary-driven microfluidic systems (Park et al., 2018; Jang et al., 2021; de Oliveira et al., 2017). Additionally, processes like digital light processing have further refined the fabrication and functionality of μPADs (Amini et al., 2023). Looking forward, the field is poised for continued advancements, with an emphasis on even more sensitive, specific, and user-friendly systems. Figure 3 illustrates the timeline of fabrication techniques used for developing paper-based microfluidics. In this section, we will explore the most common fabrication methods in research, prototyping, and commercialization that have been predominantly used in the field.

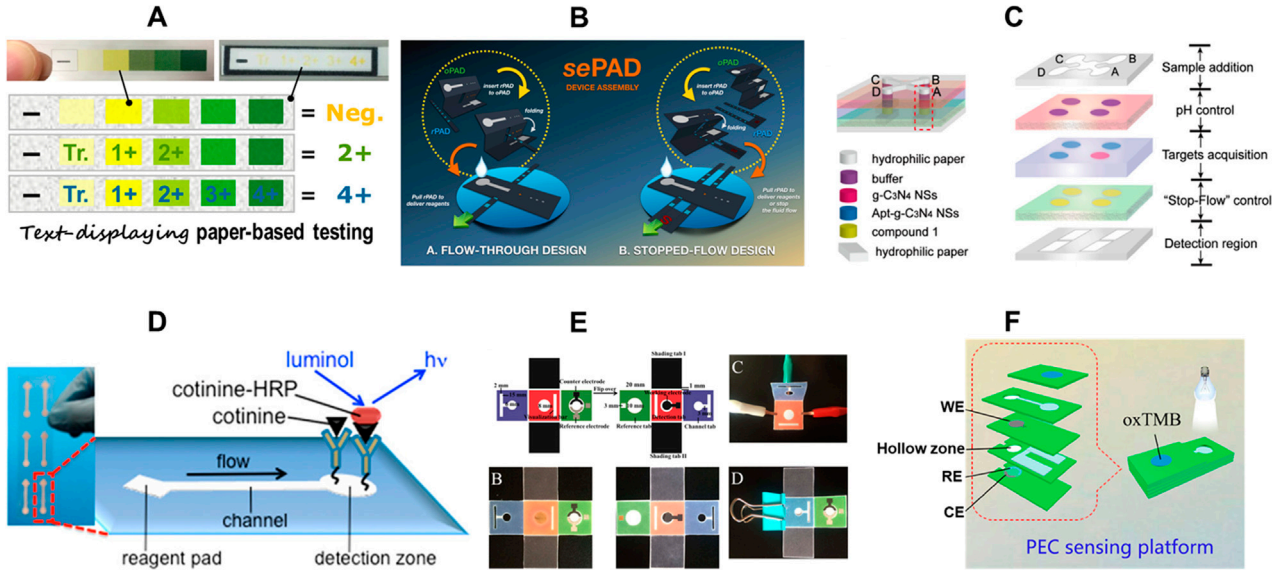

FIGURE 5
Common sensing methods for paper-based microfluidc devices. **(A)** text-displaying colorimetric paper-based sensor (adapted from (Yamada et al., 2017b) with permission, copyright 2017, American Chemical Society). **(B)** 3D Capillary-Driven Paper-Based Sequential Microfluidic Device (adapted from (Yakoh et al., 2019) with permission, copyright 2019, American Chemical Society). **(C)** Ratiometric paper-based device with digital readout via fluorescence detection (adapted from (Wang et al., 2019) with permission, copyright 2019, American Chemical Society). **(D)** Chemiluminescence based μPAD for cotinine detection in mouse serum (adapted from (Liu et al., 2013) with permission, copyright 2013, American Chemical Society). **(E)** Colorimetric and electrochemiluminescence dual-mode sensing of lead ion on μPAD (adapted from (Xu et al., 2018) with permission, copyright 2018, American Chemical Society). **(F)** Photoelectrochemical/Visual Lab-on-Paper Sensing via Signal Amplification of CdS Quantum Dots@Leaf-Shape ZnO (adapted from (Kong et al., 2018) with permission, copyright 2018, American Chemical Society).

2.1 Photolithography

Photolithography is a complex technique that works by transferring structural geometry from a photomask and was the first method used for patterning paper-based microfluidic systems (Martinez et al., 2007). This process begins by coating the substrate with photoresist material. Next, a pattern is designed on the substrate (Figure 4E). The substrate is then cured, and the uncured photoresist is removed using a solvent, leaving behind a cured photoresist barrier (He et al., 2015c).

This technique can achieve the resolution with features as tiny as 100 μm or smaller (Gallatin and Liddle, 2024). This makes it excellent for designing complex devices. However, the process is time-consuming, involving several steps that can take hours to complete. Moreover, the requirement for extra instruments like a UV lamp, photomasks, spin coater, mask aligners, cleanroom facilities, hot plate, etc. can be problematic in terms of cost-effectiveness. While modern tools can speed up production for large-scale manufacturing, the cost is very high (up to millions) (Gallatin and Liddle, 2024). Furthermore, residue from the photoresist can cause colorimetric reactions and unwanted background (Dungchai et al., 2011). These expenses make photolithography less practical for small-scale projects or research with limited budgets. Despite these challenges, its precision and ability to handle large-scale production make it valuable for industries like semiconductor manufacturing. For μPADs, the high cost and complexity often outweigh its benefits in smaller projects.

Over the years, this technology has evolved from manual UV-lithography to faster alternatives such as FLASH (fast lithographic activation of sheets) (Martinez et al., 2008b), DMPC (dynamic mask photocuring) (He et al., 2015b), laser-based direct writing approaches (Songok et al., 2014), and utilizing the photocatalytic properties of TiO_2 to fabricate μPADs (He et al., 2015a). These advancements have simplified the technique further while saving time and money, making it more suitable for high-frequency prototyping and manufacturing.

Despite the advancements in photolithography for μPADs, some key considerations remain crucial for successful μPAD development using this method. The first step is selecting a photoresist compatible with both the paper substrate and the intended chemical or biological assays. Next, it is essential to ensure the photomask design accurately reflects the desired microfluidic patterns, as high-resolution masks are vital for precise patterning. Finally, choosing solvents that effectively remove uncured photoresists without compromising the paper or the cured patterns is critical for achieving reliable and high-quality μPADs.

2.2 Plasma treatment

Plasma treatment is another commonly used technique for creating microchannel patterns on paper substrates. Initially, paper is hydrophobicized using materials such as octadecyl trichlorosilane (OTS), hexamethyldisilane, or fluorosilanes (Li et al., 2021; Lim et al., 2019). Following this, plasma treatment is

performed under vacuum conditions. During plasma treatment, specific regions of the paper become hydrophilic, making channels for fluid flow (Figure 4H). The most common plasma-treated μPADs are created using a sizing agent that makes the paper more water-resistant (like AKD-heptane) (Li et al., 2008).

This technique achieves moderate to high resolution in creating hydrophobic barriers and hydrophilic channels on paper substrates, enabling the production of well-defined microfluidic patterns. One of its key strengths lies in the rapid treatment times, typically requiring only a few minutes to complete the process, which enhances efficiency in device fabrication. However, the adoption of plasma treatment faces significant barriers due to its high initial equipment costs. The specialized plasma generators and vacuum chambers necessary for this process often come with a substantial price tag, ranging from several thousand to tens of thousands of dollars (Ozer et al., 2020). This high upfront investment poses a considerable challenge, particularly for smaller laboratories or startups with limited resources. Additionally, these systems are not compatible with organic solvents. Since many biological and chemical assays require reagents that involve organic solvents, this limitation might pose an issue (He et al., 2015c). Consequently, while plasma treatment offers excellent control over surface properties and produces thermally and chemically stable barriers, its commercial potential remains limited. The requirement for expensive, specialized equipment and the need for vacuum conditions during processing makes it less accessible for widespread adoption, especially in resource-constrained settings or mass production scenarios. As a result, plasma treatment is more likely to find its niche in specialized research environments or high-end applications where the precision and quality of surface modification justify the high equipment costs. Nevertheless, efforts have been underway to develop portable systems that can create microplasmas, offering cheaper and more flexible alternatives that can be used under ambient conditions (Kao and Hsu, 2014).

When patterning paper with plasma treatment, several critical factors must be considered to ensure optimal device performance. The intensity and duration of plasma exposure influence the depth and degree of surface change, while the presence of sizing agents or other chemicals in the paper can affect treatment efficiency. Manufacturing challenges may arise from excessively intricate channel designs, potentially leading to issues like clogging or uneven flow. It's essential to use mask materials resistant to plasma and compatible with clean patterning to maintain channel clarity. Moreover, the surface chemistry post-plasma treatment should be compatible with subsequent treatments, such as biomolecule immobilization for additional device functionalities.

2.3 Inkjet printing

The fusion of paper-based microfluidics with printing technologies represents an important step in μPADs fabrication (Li et al., 2012b; Xia et al., 2016; Tortorich et al., 2018). In the inkjet printing method, hydrophobic barriers are applied onto paper using specialized hydrophobic inks such as Alkyl Ketene Dimer (AKD), Methylsilsesquioxane (MSQ), and Hexadecenyl succinic anhydride (HSA) (Akyazi et al., 2018). Following printing, these inks are cured either through UV exposure or heat treatment, creating high-resolution hydrophobic barriers (Yamada et al., 2015) as shown in Figure 4G. Inkjet printing has numerous advantages beyond channel creation. It serves as a versatile tool for depositing a range of substances, including colorimetric indicators, metal precursors, enzymes, ionophores, pH buffers, masking reagents, and blocking reagents, into both sensing and pretreatment regions (Tenda et al., 2016). This method ensures better accuracy in reagent volumes than manual pipetting techniques. Moreover, it facilitates accurate positioning and spatial confinement of deposited reagents, which is crucial for optimizing device performance. The continuous evolution of inkjet systems, such as Piezoelectric inkjet printing (e.g., Epson) and Thermal inkjet printing (e.g., Canon, Hewlett Packard), has further enhanced the capabilities and efficiency of this fabrication approach (Maejima et al., 2013; Yamada et al., 2015).

Inkjet printing is one of the most effective methods for developing μPADs, offering high resolution in the range of 50–200 μm. This level of precision enables the creation of detailed channel designs and intricate patterns, making it ideal for a variety of analytical applications. In terms of speed, inkjet printing is particularly efficient for small-scale production. Budget inkjet printers can achieve output rates of up to 15–25 pages per minute, making them well-suited for rapid prototyping and small-batch manufacturing. The technique is also cost-effective, striking a balance between affordability and functionality. Entry-level inkjet printers suitable for μPAD fabrication are available for as little as $30 to $100, while more advanced models for specialized tasks can cost up to $250. Operational expenses are reasonable as well, with replacement ink cartridges typically priced between $10 to $35. This combination of precision, speed, and cost-efficiency makes inkjet printing a versatile and accessible option for μPAD production.

When fabricating μPADs via inkjet printing, some factors must be considered. One concern is the potential formation of crystals or solid deposits at the printer nozzle, which can restrict the range of reagent concentrations feasible for use. Additionally, volatile reagents may evaporate too quickly, necessitating the use of less volatile alternatives (e.g., substituting cyclohexane for tetrahydrofuran). Another issue is the slow curing process, which can lead to low pattern resolution due to the spreading of reagents or hydrophobic ink. A potential solution for μPADs that require UV curing after printing is to immediately freeze them post-printing.

2.4 Wax printing/patterning

Since 2009, wax-patterned μPADs have been a popular choice. In these devices, the paper substrate is first patterned with wax to create channels that guide fluids. This initial patterning is followed by a heating step (using an oven, hot plate, or other hot surface) that melts the wax and allows it to penetrate the paper fibers, creating hydrophobic barriers that define hydrophilic channels (Dungchai et al., 2011). After cooling, the solidified wax creates the final design as shown in Figures 4A,D.

A key advantage of this method is the elimination of organic solvents during fabrication. Furthermore, wax patterning offers versatility, with techniques ranging from simple wax pens to commercial wax printers, screen printing, wax dipping, and even 3D printing of wax onto the paper surface (He et al., 2015c). The resolution achievable with wax printing ranges from hundreds of

TABLE 2 Comparison of analytical methods for detection on μPADs. (Adapted from (Noviana et al., 2021) with permissions, copyright 2021, American Chemical Society).

Method	Upsides	Downsides	Ref
Colorimetry	Tests are simple, easy to interpret, no external reader required, quantification can be done using smartphones	Limited information, limited sensitivity and detection limits, color saturation	Li et al. (2015), Lewis et al. (2012), Mahmud et al. (2020), Jeong et al. (2015)
Electrochemistry	Better sensitivity and wider analytical range than colorimetric methods, relatively simple instrumentation, suitable for continuous monitoring	Requires external instrumentation, challenging for untrained user, relatively complex data analysis	Colozza et al. (2019), Adkins et al. (2016), Martínez-García et al. (2017)
Fluorescence	High sensitivity, wide analytical range, requires relatively simple reader	Requires excitation light, autofluorescence from paper, higher detection limit than other luminescence techniques	Qi et al. (2018), Qi et al. (2017), Zong et al. (2019)
Chemiluminescence	Low detection limit, wide analytical range, relatively simple instrumentation	Relatively complex reagents, expensive reader may be necessary to detect very low luminescence signal	Yu et al. (2011a), Li et al. (2019), Yang et al. (2019)
Electrochemiluminescence	High sensitivity, low detection limit, wide analytical range, excellent temporal and spatial resolution	Relatively complex reagents, more complicated instrumentation	Delaney et al. (2011), Ge et al. (2012), Sun et al. (2018)
Surface-Enhanced Raman Scattering (SERS)	Nondestructive, high sensitivity, low detection limit	Requires Raman instrument, diminished SERS efficiency in porous substrate	Yu and White (2013), Lee et al. (2018), Zeng et al. (2019)

micrometers to centimeters, making it suitable for many analytical applications. This technique is highly efficient, offering fast production speeds ideal for mass manufacturing. Wax printing requires relatively low-cost wax materials, making it a cheaper alternative for resource-limited settings. Its simplicity and scalability provide high commercial potential, particularly for producing disposable μPADs for environmental or healthcare monitoring. However, commercial wax printers have been discontinued, so researchers are encouraged to explore alternative options such as thermal transfer printing, laser cutting, screen printing, photolithography, and laminate capillary-driven microfluidics (Ruiz et al., 2022; Le et al., 2017; Chiang et al., 2019).

2.5 Laser treatment

Many researchers use laser treatment in the making of μPADs (He et al., 2015c). The technique uses a CO_2 laser or such laser to create or remove material from the paper substrate. Using a laser's power, speed, and focus, channel depth and width can be controlled. The laser creates these channels by vaporizing the material off the paper. As a result, the unetched areas stay hydrophilic, allowing liquids to flow through and giving the device its microfluidic properties (Ghosh et al., 2019; Sousa et al., 2024). In 2011, laser treatment was first adapted for fabricating μPADs by using a CO_2 laser cutter to treat the surface of parchment paper (Chitnis et al., 2011).

Laser treatment technique achieves high resolution, capable of producing features smaller than 100 μm, which is particularly advantageous for applications requiring intricate channel designs or fine control over fluid flow. The process is relatively fast, especially for small-scale production, allowing for rapid prototyping and iteration of device designs. While the initial investment in laser equipment can be moderate to high, the operational costs are generally low, and the method offers good repeatability. The commercial potential of laser treatment is particularly notable for specialized applications that demand high-resolution features or complex geometries. Its non-contact nature and ability to process various paper substrates make it adaptable to different μPAD designs.

Recent advances in fabricating microfluidic paper-based analytical devices using a laser printer (LP-μPAD) have harnessed the precision of xerography-based commercial laser printers to create high-resolution microfluidic devices on paper (Ghosh et al., 2019). This direct printing procedure automates the deposition of hydrophobic toner ink, eliminating human interference and fabrication irregularities (Ghosh et al., 2019). Moreover, utilizing silver nanowire (AgNW) ink patterned with a solvent-free transfer method is also a promising alternative (Huang et al., 2016).

2.6 Other methods

In addition to these popular methods, various techniques have evolved over the years for the fabrication of μPADs. One commonly used technique is flexographic printing (Figure 4C), where hydrophobic barriers or inks are printed on paper using volatile organic compounds or polystyrene ink (Olkkonen et al., 2010). This is one of the fastest μPAD fabrication techniques but struggles with design flexibility. It also requires multiple printing optimization methods to achieve higher resolution, making it less suitable for lab prototyping but holding potential for commercial manufacturing.

Cutting and shaping to create 2D and 3D μPADs have also been adopted to develop instrument-free fabrications (Figure 4B). This technique involves using handheld blades, biopsy punches, knife cutting, laser cutting, etc. (Zhong et al., 2024). It is economical and advantageous because it does not require any chemical-laden instrumentation. Since no wax, organic solvents, or inks are used, there is a better chance of achieving higher resolution. However, the technique is limited to specific designs because boundaries cannot be defined as precisely as with wax or solvent lining.

Wet etching is another method, where hydrophobic paper is etched with a specific pattern (Cai et al., 2014). It starts with preparing the substrate, which includes cleaning and putting a protective mask or pattern (e.g., trimethyloctadecylsilane (TMOS)) on it. This mask defines the areas that will be unaffected by etching. The substrate is then immersed in an etchant solution (e.g., NaOH), which selectively reacts with the exposed portions based on the mask design. The etchant dissolves or changes the substance in these locations while leaving the masked sections unaffected, creating microfluidic channels. After the desired etching time, the mask is normally removed, and the substrate is cleaned to eliminate any remaining chemicals.

Stacking and origami techniques are also useful methods for developing μPADs (Kaewarsa et al., 2021; Das et al., 2019). These techniques enable multi-step chemical and biological assays. Folding, sliding, and rotating can be used to automate multiple steps in these systems (Carrell et al., 2019; Yakoh et al., 2022; Verma et al., 2018). Furthermore, chemical vapor-phase deposition, corona treatment, chemical modifications, stamping, plotting (Figure 4F), and lacquer spraying methods have also been employed to develop μPADs (Demirel and Babur, 2014; Jiang et al., 2016; Curto et al., 2013; Liu et al., 2017; Shakeri et al., 2019). Figure 4 shows the common fabrication methods for creating patterns on paper-based microfluidic devices.

Table 1 shows the summary of fabrication methods for μPADs. This table compares various techniques based on their suitability for mass production, ease of use, and resource requirements. Among these methods, wax printing, inkjet printing, and flexographic printing show the most promise for commercial applications due to their balance of resolution, speed, and cost-effectiveness. Laser treatment is emerging as a versatile method for high-resolution fabrication. Photolithography, while offering high resolution, faces challenges in large-scale adoption due to its complexity and cost. Screen printing remains relevant for certain applications due to its simplicity and moderate resolution. Plasma treatment, plotting, and wet etching have more limited commercial potential due to various constraints in scalability or process complexity. Therefore, for large-scale production, techniques such as inkjet and flexographic printing are recommended, while more accessible methods like wax printing and plotting are ideal for beginners with limited resources.

In addition to the aforementioned factors, the choice of recognition elements for μPADs is crucial, as their compatibility with the fabrication method directly affects the device's sensitivity and specificity. For wax printing, thermally stable and polar recognition elements are ideal. Reagents requiring organic solvents like DMSO or chloroform should be avoided, as they can dissolve the wax barrier. Photolithography benefits from UV-resistant and stable materials. For screen printing, recognition elements that integrate well with conductive inks are ideal. Recognition elements that are heat-tolerant and stable under high-energy laser processing are essential for laser-treated μPADs. For inkjet printing, recognition elements compatible with low-viscosity ink formulations are essential. Additionally, the elements should be stable in liquid form and maintain their functional properties after deposition. For plotting, elements that can interact effectively with hydrophilic surfaces are ideal. For wet etching, recognition elements must be compatible with chemical solvents and porous substrates, remaining stable in the presence of acids or bases and retaining their functionality throughout the etching process. Most of these physical properties of elements are considered if they are pretreated on substrates before the fabrication process. On the other hand, chemical properties such as solvent compatibility, stability, and potential interactions with the hydrophobic barrier are crucial factors that need to be carefully studied before using a fabrication technique, regardless of pre- or post-treatment.

3 Advancements in sensing methods

In addition to the fabrication technique, when building paper-based microfluidic devices, it is critical to choose the suitable detection technique for the intended application. Colorimetry, fluorescence, electrochemical techniques, and chemiluminescence are some of the most used detection methods. Each method differs in terms of sensitivity and the complexity of the required detection system, which affects its applicability for diverse applications (Yamada et al., 2017a). In this section, we will explore the most frequently used detection techniques, and their common practices, and delve into their distinct advantages and limitations.

3.1 Colorimetry

Colorimetry is a commonly used method to generate signals in μPADs because the analyte concentration can be directly or indirectly correlated with the color intensity. Additionally, the color can be quantified using various portable platforms. There are numerous established colorimetric chemistries, including metal-ligand complexation, enzyme-inhibition assays, acid-base indicators, and redox indicators (Yamada et al., 2017a). Often, these signals are distinguishable enough to allow for semi-quantitative and qualitative observations, as seen in pregnancy tests, pH strips, and home water test kits (Morbioli et al., 2017).

Advancements in colorimetric detection have led to innovations ranging from simple spot tests to distance-based detection, where the distance of the colored signal is proportional to the analyte concentration, making it a valuable tool for semi-quantitative analysis (Cate et al., 2015; Cate et al., 2013). Furthermore, other methods such as time-based quantitative color readouts, counting-based readouts, barcode-like readouts, text-displaying readouts (Figure 5A), and direct result readouts have been developed over the years (Karita and Kaneta, 2014; Guan et al., 2014; Li and Macdonald, 2016; Lewis et al., 2012; Lewis et al., 2013; Zhang et al., 2015; Li et al., 2012a). These innovative techniques enable calibration-free assays, enhancing the utility and accuracy of colorimetric detection in paper-based microfluidics.

While colorimetric systems are simple and effective, they do have drawbacks. A significant issue in quantitative colorimetric systems is the non-homogeneity of color (Morbioli et al., 2017; Yan-Qi and Liang, 2020). In μPADs, colored indicators used for detection can migrate unevenly, leading to patchy color development. This happens because highly soluble signaling molecules spread outwards during the sample flow, accumulating at the edges of the designated detection zone. This issue can be tackled by employing attachments, either

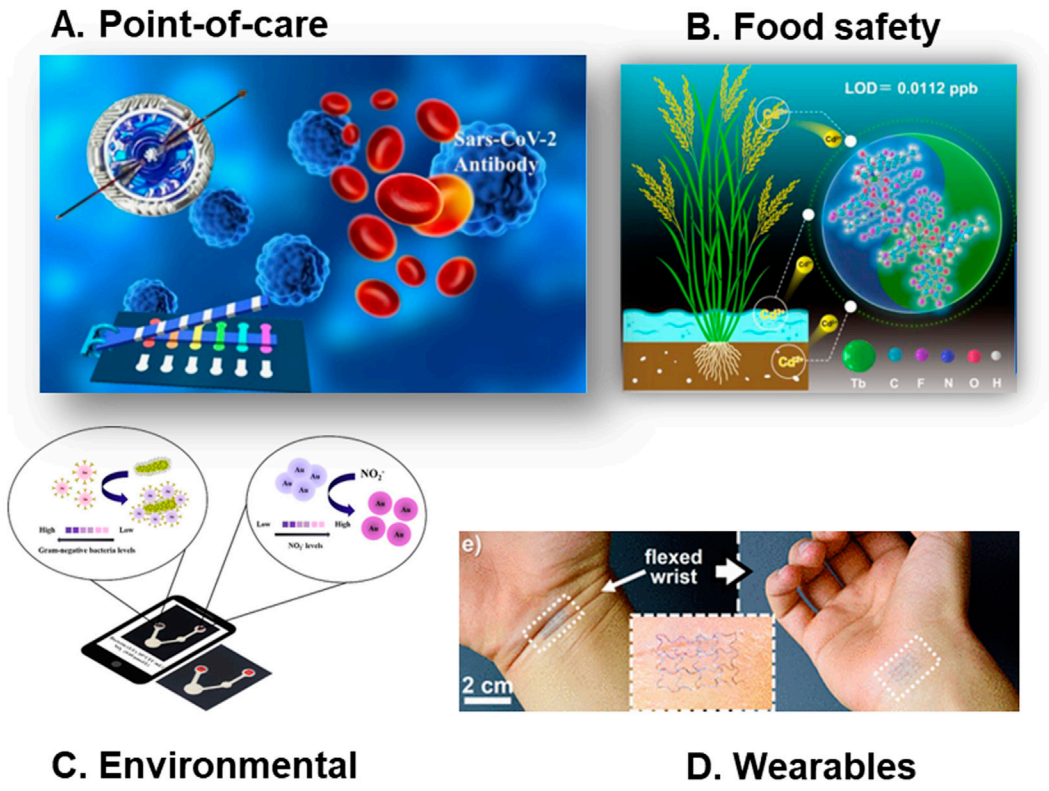

FIGURE 6
Common applications of paper-based microfluidc devices. **(A)** A pulling-force spinning top used for serum separation, integrated with paper-based microfluidic devices for COVID-19 diagnosis (adapted from (Gong et al., 2021) with permission, copyright 2021, American Chemical Society). **(B)** Visual and real-time monitoring of Cd^{2+} in rice using [2+2] lanthanide clusters (adapted from (Yu et al., 2023) with permission, copyright 2023, American Chemical Society). **(C)** A microfluidic paper analytical device utilizing gold nanomaterials for the simultaneous quantification of Gram-negative bacteria and nitrite ions in water samples (adapted from (Khachornsakkul et al., 2023) with permission, copyright 2023, American Chemical Society). **(D)** Wearable and Implantable Epidermal Paper-Based Electronics (adapted from (Sadri et al., 2018) with permission, copyright 2018, American Chemical Society).

covalent or non-covalent, directly onto the paper itself (Tan et al., 2022). Another major hurdle is the inconsistency of lighting. Initially, colorimetric systems were imaged using scanners, which provided higher resolution and consistent lighting. However, for Point-of-Need (PoN) or Point-of-Care (PoC) devices, it is ideal to have systems that are instrument-free or can be quantified using easily accessible tools like smartphones. Constructing a lightbox for imaging is one solution to this issue, allowing the use of a smartphone camera. Introducing a background region of interest (ROI) is another way to mitigate this problem, as it helps normalize intensities under varying lighting conditions (Tan et al., 2022; Aryal et al., 2024d; Richardson et al., 2021). Recently, third-party apps for color quantification have become available in app stores that have an onsite calibration feature built into them. Another challenge arises if the analyte itself is colored. In such cases, using a color control can help mitigate this issue. While distance-based semi-quantitative signal readouts allow for calibration-free assays, they are susceptible to observer-dependent readout errors. Counting-based semi-quantitative signal readouts are less prone to observer-dependent errors and can achieve calibration-free assays, but they have relatively limited resolution and are often time-dependent. Similarly, timing-based signal readout systems face the same limitations. Text-based detection platforms are often restricted by the target analytes that can be used for detection, as they may not be applicable for every assay (Yamada et al., 2017a).

3.2 Electrochemical detection

Electrochemical paper-based analytical devices (ePADs) are among the most used μPADs after colorimetric μPADs. This technology offers several advantages. Firstly, ePADs leverage external detectors like potentiostats, enabling them to achieve significantly lower detection limits, reaching the picomolar range (Aryal et al., 2024b; Adkins et al., 2015; Oh and Chow, 2015; Mettakoonpitak et al., 2016). This surpasses the capabilities of techniques like fluorescence and colorimetry (Pohanka and Skládal, 2008; Dungchai et al., 2009; Liu et al., 2014a). Additionally, ePAD results are unaffected by color variations within the sample itself, unlike colorimetric assays which can be prone to misinterpretations due to background color (Dortez et al., 2024). ePADs exhibit minimal impact from variations in sample volume as long as the designated electrode area is adequately covered (Yamada et al., 2017a). This provides greater flexibility compared to colorimetric methods where precise volume control is

often critical. Moreover, electrochemical measurements can be performed relatively quickly, taking only seconds to minutes. This speed is especially advantageous when handling many samples (Noviana et al., 2020b). The conventional configuration for an electrochemical system consists of three electrodes: a working electrode, a counter electrode, and a reference electrode (Adkins et al., 2015; Dungchai et al., 2009). Since the development of the first ePAD in 2009 by screen printing carbon ink on paper (Dungchai et al., 2009), many other methods have been developed over the years. These methods include stencil printing, inkjet printing, microwire placement, laser scribing, using carbon tape, pencil drawing, spray and spin coating, laser scribing, and sputtering (Noviana et al., 2020b; Ataide et al., 2020; de Araujo et al., 2017). Figure 5B shows an example of innovative electrochemical sensing on paper using 3D capillary-driven sequential microfluidics.

While electrochemical μPADs outperform colorimetric paper devices in terms of analytical performance owing to the usage of external detectors (e.g., potentiostats), due to their high cost (usually > $1000) and operating complexity, laboratory-type electrochemistry stations are inaccessible to end consumers. The most important problem for the real-world deployment of ePADs is to build user-friendly signal detection interfaces. User-friendly signal detection interfaces allow for real-time data analysis and easy-to-understand data displays, helping users make quick decisions. To bridge the gap, many handheld potentiostats have been developed that allow for onsite/remote sensing. Some notable examples of portable potentiostats integrated with μPADs include inkjet-printed paper-based potentiostats (Bezuidenhout et al., 2018), USB-controlled potentiostats (Chhorn and Teeramongkonrasmee, 2018), battery-less NFC potentiostats (Chaiyo et al., 2024), and Wi-Fi cloud-based portable potentiostats (Bianchi et al., 2020). Among these portable systems, the wireless NFC potentiostats hold great promise. These potentiostats utilize near-field communication (NFC) for wireless power and data exchange with a user-friendly smartphone app (Krorakai et al., 2021). These portable and cost-effective systems offer analytical capabilities on par with traditional benchtop potentiostats (Beck et al., 2023). These potentiostats have been applied in μPADs-based electrochemical sensing of analytes like cholesterol and glucose and in performing various electrochemical lateral flow assays (Chaiyo et al., 2024; Gonzalez-Macia et al., 2024; Promsuwan et al., 2023; Lazaro et al., 2023). However, compared to benchtop potentiostats, portable potentiostats usually have higher noise levels, a smaller voltage range, and lower accuracy. Because of these drawbacks, portable potentiostats may not be as ideal for uses needing a broad voltage range or high precision and require further research and development (Aryal et al., 2024b).

Researchers using ePADs may face challenges such as restricted mass transfer, electrode fouling, a limited dynamic range, and inconsistent performance with disposable electrodes. To address these issues, smaller channels and increased flow rates can be employed to enhance mass transfer and allow analyte molecules more time to interact with electrode surfaces. To mitigate electrode fouling and improve electron transfer in electrochemical sensors, various strategies can be explored. These include employing nanoengineered surfaces like nanoporous metals and nanocarbons, applying antifouling layers such as PEG, zwitterionic polymers, and biopolymers, utilizing nanoporous membranes, and integrating hydrogels (Russo et al., 2021). In certain instances, the limited dynamic range issue can be resolved by expanding the electrode surface area (Arroyo-Currás et al., 2017). The use of ePADs coupled with wireless technology has been increasing and holds promising potential for the future (Solhi et al., 2020; Ruecha et al., 2017; Deroco et al., 2023; Fan et al., 2017). This method enables the wireless acquisition of data, which can be utilized for continuous monitoring in various fields such as sports, healthcare, agriculture, and defense. It allows users to receive alerts in case of abnormal situations and to be warned before these situations worsen. However, one challenge that wireless ePADs face in terms of sensing is the lifespan of the signals (Solhi et al., 2020). Researchers are encouraged to work on integrating wireless and remote sensing technologies to make this system more robust and reliable.

3.3 Chemiluminescence

Chemiluminescence (CL)-based paper microfluidics (μ-PCLDs) have also gained popularity, particularly in CL-immunoassays. CL works on the principle of generating photons through a chemical reaction, and these photons are typically quantified in the dark to avoid non-specific and background signals (Calabretta et al., 2021). Figure 5D shows an example of a chemiluminescence based detection of cotinine in serum using a μPAD. With advancements in noise control modifications, this method has become more sensitive and specific, allowing for more accurate routine biomedical and health monitoring applications (Ge et al., 2014). μ-PCLDs can be tailored to achieve a broad dynamic range, depending on the detection target. They can detect analytes like uric acid and glucose at millimolar concentrations while also being capable of detecting DNA at molar levels (Wang et al., 2013c; Yu et al., 2011b; Yu et al., 2011a). CL on paper is more efficient than typical CL tests because it consumes fewer samples and reagents, shortens analysis time, and uses automated, compact, and integrated detection techniques (Aryal et al., 2024b). Additionally, the development of antibody-based probes has enabled these systems to detect antigens at trace levels, even in complex matrices (Calabretta et al., 2021). Over time, CL-based microfluidics have been employed for detecting tumor markers, DNA, antibodies, and other biomolecules (Yamada et al., 2017a).

However, there remains a significant gap in translating μ-PCLDs from lab to practical use. This gap is primarily due to the need for expensive instruments, such as luminescence readers. While μ-PCLDs offer incremental gains in sensitivity compared to colorimetric μPADs, these gains are often overshadowed by the exponential increase in operational costs. Despite this, the development of CMOS sensors, which are less complex and less expensive than conventional instruments, offers a promising solution (Punjiya et al., 2014; Hu et al., 2020; Khan et al., 2018).

3.4 Fluorescence

Fluorescence based methods are also used with μPADs. The unique properties of fluorescent materials enable the creation of these assays. Tiny fluorescent particles, such as rhodamine, pyrene,

or anthracenes, are deposited onto the paper (Nawaz et al., 2021). These particles then interact with the target molecule of interest. When ultraviolet light of a specific wavelength shines on the paper, any bound analyte triggers the fluorescent particles to emit light (Patel et al., 2021; Lian et al., 2020). The intensity of this emitted light, captured with a smartphone or scanner, can be analyzed using software like ImageJ to quantify the amount of target analyte present (Patel et al., 2021). Figure 5C shows an example of ratiometric sensing with fluorescence detection and digital readout.

μPADs using fluorescence measurements face more limitations compared to other detection methods. First, the paper itself can introduce noise by reflecting the excitation light, leading to a lower signal-to-noise ratio compared to techniques like electrochemiluminescence (Yamada et al., 2017a). Additionally, when using non-pure cellulose paper for convenience, the presence of optical brightening agents can further disrupt the assay by causing unwanted background fluorescence (Kwon et al., 2012). Some fluorescent molecules are unstable on paper across time and varying assay conditions, especially in fluctuating temperature and humidity environments. Use of stabilizing buffers or chelating agents can be used to enhance their stability.

3.5 Electrochemiluminescence

Electrochemiluminescence (ECL) is a light emission process that occurs when chemical species generated through oxidation-reduction reactions undergo exothermic reactions, emitting light energy. Depending on the source of radicals, there are two main ways electrochemiluminescence (ECL) can occur. In one method, light is produced when a single molecule undergoes a reaction that creates energetic particles. In the other method, light is generated by the interaction between a special molecule and another molecule in the solution (Chinnadayyala et al., 2019).

ECL-μPADs have gained popularity in healthcare applications, particularly for PoC diagnosis and disease monitoring. However, due to the electrochemical nature of the process, most luminophores in ECL are less soluble in water, making it challenging to create binding sites without affecting their chemical properties. This presents a significant hurdle in designing μPADs with ECL readouts, often resulting in compromised sensitivity compared to electrochemical methods. To address these challenges, various signal amplification strategies have been developed over the years. One promising advancement is the incorporation of nanomaterials, which can act as electrocatalysts in ECL reactions. These nanomaterials promote electron transfer at the electrode interface and increase the loading capacity of ECL labels. Common labels include carbon dots, inorganic complexes, semiconductor nanocrystals, graphene quantum dots, and photoluminescent carbon nanocrystals (Chinnadayyala et al., 2019). While paper-based ECL biosensors have seen progress in research labs, their commercialization also remains a challenge. A crucial step towards practical application involves a comprehensive understanding of key design aspects for ECL paper devices. This includes factors like detection limits, specificity, assay speed, analysis format, compatibility with various sample types, packaging considerations, labeling requirements, shelf life needs, and target production costs (Chinnadayyala et al., 2019).

3.6 Other sensing methods

Other methods for signal detection in μPADs include photoelectrochemical detection (Figure 5F), surface-enhanced Raman scattering (SERS), digital microfluidics on paper, calorimetry, dual mode detection (Figure 5E), and light-reflectance (Davaji and Lee, 2014; Abbas et al., 2013; Liu et al., 2014b; Fang et al., 2014; Fobel et al., 2014). In photoelectrochemical (PEC) detection, the process is reversed compared to electrochemiluminescence. Instead of light triggering a chemical reaction, chemical light emission from an internal system, such as luminol-hydrogen peroxide, is converted into an electrical current (Zhao et al., 2016). This current is then detected by a semiconductor material, such as quantum dots. Researchers have used PEC as a labeling technique for various immunoassays, including those targeting tumor marker proteins, ATP (adenosine triphosphate), and DNA (Wang et al., 2013a; Wang et al., 2013b; Ge et al., 2013). Digital microfluidics (DMF) technology offers a powerful tool for handling tiny liquid droplets, ranging from nanoliters to microliters in volume (Choi et al., 2012). This technique uses an array of electrodes and electrical fields to manipulate these droplets. By controlling the electrical fields applied to the electrodes, functionalities such as merging separate droplets, splitting a single droplet into smaller ones, mixing different droplets, and directing the flow of droplets across the surface can be achieved (Choi et al., 2012). This technique is an exciting addition to sensing on μPADs. Table 2 compares analytical methods for detection on μPADs, showcasing their strengths and weaknesses. Figure 5 depicts common sensing methods for μPADs.

The integration of smartphones with microfluidic paper-based analytical devices (μPADs) has significantly advanced their capabilities, transforming them into portable, user-friendly diagnostic tools ideal for point-of-care applications and resource-limited settings. Lately, colorimetric paper-based platforms have seen a significant advancement. A few notable examples include the following: a novel smartphone-based solution with a color correction algorithm to achieve accurate and absolute color measurements (Zhang et al., 2023), the development of smartphone applications for color quantification (Shen et al., 2012; Wu et al., 2024), and the development of a custom app to analyze specific μPAD regions to provide accurate concentration data (Liu et al., 2024). For electrochemical μPADs, miniaturized potentiostats connect to smartphones via audio jacks or data ports, enabling real-time control, data processing, and signal analysis directly on the smartphone screen (Beck et al., 2023). Fluorescence-based μPADs have also seen notable advancements, A notable advancement is the smartphone-integrated colorimetric sensor array and fluorometric detection system, which uses image processing algorithms to quantify fluorescence intensity (Chellasamy et al., 2021). Together, these innovations make μPADs increasingly sophisticated and accessible for on-site, real-time diagnostics without the need for complex laboratory equipment.

4 Applications

One of the primary goals of μPADs is to offer users easy-to-use, inexpensive, and portable testing/monitoring tools. This translates

to real-world benefits across a wide range of applications where fast results and user-friendliness are critical. The significance of μPADs lies in their ability to empower anyone, regardless of technical expertise, to conduct tests or monitor samples on-site, at the point of care, need, or incident. This opens doors for applications in various fields, including diagnostics, disease monitoring, environmental checks, food safety analysis, and even forensics and defense.

4.1 PoC and disease monitoring

While urbanization is increasing globally, a sizable proportion of the population in emerging countries still lives in rural areas (Desa, 2014). Even though poverty is a difficult concept to define, many people in developing countries rely on subsistence activities for a living, which significantly impacts their health (Yetisen et al., 2013; Sociales, 2009). These resource-limited environments frequently lack the technical skills and staff available in urban areas, making access to healthcare difficult. Affordable technologies are critical for empowering these local populations and improving their healthcare access. This is where μPAD technologies can be particularly beneficial. While these technologies are more urgently needed in developing nations, where even basic illness or disease diagnosis can be challenging, recent pandemics have also underscored the importance of PoC sensors in developed countries as well. This demonstrates the broad applications and usefulness of μPAD technologies across various global contexts. A prime example of the importance of these devices emerged during the COVID-19 pandemic. On 30 January 2020, the World Health Organization (WHO) declared the COVID-19 pandemic a global health crisis (Ravi et al., 2020; World Health Organization, 2020). Since the onset of the pandemic, a significant number of paper-based systems for COVID-19 diagnostics have been developed and published (Pinheiro et al., 2021; Kim and Lee, 2022; de Araujo et al., 2024). Long before and throughout the COVID-19 pandemic, researchers have actively explored using paper-based platforms to create biosensors for diagnosing various diseases and infections (Silva-Neto et al., 2023). These devices offer a promising and versatile approach to healthcare diagnostics. Here are some examples of μPADs for diagnostics and health monitoring:

4.1.1 DNA extraction and detection

μPADs can be used to extract and detect DNA from biological samples. These instruments can be designed to extract DNA from a variety of biological samples by employing integrated buffers to degrade cells and specialized molecules to extract DNA (Shetty and Shetty, 2020; Ye and Lei, 2023; Chong et al., 2021). Moreover, they amplify DNA directly on paper using methods like recombinase polymerase amplification (RPA) (Jauset-Rubio et al., 2016; Lobato and O'Sullivan, 2018; Bai et al., 2022) and loop-mediated isothermal amplification (LAMP) (Seok et al., 2017; Das et al., 2023; Atceken et al., 2023), which enable accurate identification of certain DNA targets.

4.1.2 RNA extraction and detection

μPADs are also useful for RNA extraction and detection by employing techniques like solid-phase or magnetic bead-based capture (He et al., 2017; Liu et al., 2009; Malpartida-Cardenas et al., 2023; Bender et al., 2021). These methods use RNA-specific binding agents, enabling direct capture and purification of RNA on the paper itself, promoting selectivity and simplifying the process. Following extraction, the RNA can be utilized for downstream processes like sequencing or reverse transcription-polymerase chain reaction (RT-PCR), which can help with RNA-based assays, including gene expression analysis and RNA virus identification and characterization (Oruganti et al., 2023; Huber et al., 2004; Basiri et al., 2021).

4.1.3 Antigen detection

These paper-based sensors can identify antigens through immunoassays. Antibodies specific to the target antigen are usually immobilized onto the paper substrate in immunoassays conducted on paper-based sensors. If the antigen is present in the sample, it attaches to the immobilized antibodies and forms a complex. Then, using signal amplification techniques such as colorimetric changes, fluorescence, or electrochemical signals, this interaction is observed (Liang et al., 2012; Jaewjaroenwattana et al., 2023; Hristov et al., 2021; Baldo et al., 2024). This offers a rapid and user-friendly method for diagnosing active infections.

4.1.4 Antibody detection

These devices can detect antibodies produced by the immune system in response to a specific infection, such as SARS-CoV-2 (Kasetsirikul et al., 2021; Bao et al., 2022; Antiochia, 2021). First viral antigens or specific recombinant proteins are immobilized onto the paper substrate. When a sample like blood, serum, or plasma is applied, any antibodies present in the sample that recognize the immobilized antigens bind strongly. This binding is visualized and/or quantified using various detection methods (Tenda et al., 2018; Kasetsirikul et al., 2020; Zhao and Liu, 2016). This helps determine past exposure and an individual's immune status (Silva-Neto et al., 2023; Solhi et al., 2020). Figure 6A shows a pulling-force spinning top used for serum separation, integrated with paper-based microfluidic devices for COVID-19 diagnosis. the paper-based microfluidic ELISA detects antibodies targeting the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, confirming it is designed to assess the body's immune response

4.1.5 Wearables

The field of non-invasive health monitoring is witnessing a surge in the use of wearable sensors that incorporate μPADs. Most of these tools analyze sweat, a readily accessible biofluid, to detect a range of biomarkers like glucose, lactate, pH, and chloride (Noviana et al., 2021; Deroco et al., 2023; Baldo et al., 2020). This enables real-time monitoring of factors like physical activity and cardiovascular health. Furthermore, μPADs are being integrated into systems designed for continuous screening of biochemical markers in other body fluids like tears and saliva as well (Noviana et al., 2021). This eliminates the need for complex procedures like pre-treatment or sample manipulation, significantly improving user experience. Additionally, the integration of Internet of Things (IoT) technology with these wearable sensors is gaining traction (Deroco et al., 2023). This marriage of technologies allows for real-time data collection and management, even in remote or resource-limited settings, making personalized healthcare more accessible.

Figure 6D shows a very creative wearable and implantable epidermal paper-based electronics.

There have been numerous works in μPAD-based sensing techniques, particularly in the PoC field. These innovations have significantly enhanced diagnostic capabilities and disease monitoring. Blood typing (Jarujamrus et al., 2012), coagulation monitoring (Hegener et al., 2017), inflammatory biomarkers testing (Kamakoti et al., 2023), glucose monitoring (Liu et al., 2016), cancer biomarkers testing (Kaewarsa et al., 2021), cardiac biomarker testing (Boonkaew et al., 2021), salivary iron testing (Prakobdi et al., 2024), urinary tract infection (UTI) diagnosis, (Hasandka et al., 2022), and sexually transmitted infection (STI) Screening (Gaydos and Hardick, 2014) are some examples of promising advancements by μPADs for PoC diagnosis.

4.2 Environmental monitoring

Pollution is a major issue worldwide, affecting the health of people in developed and developing countries. Pollution can be of greater concern in developing countries, where large populations suffer from industrial waste, poor hygiene, unsafe drinking water, and unclean indoor air caused by burning organic matter due to the lack of established and enforced regulatory policies. The problem worsens in these areas because they lack affordable and fast ways to test for pollution/toxins. The progress of industry in developed countries has also created new types of pollution that can spread quickly and overwhelm the environment's natural ability to clean itself. While traditional methods for monitoring environmental contaminants are reliable, they suffer from being time-consuming, expensive, and require specialized personnel. These limitations render them unsuitable for on-site testing. Therefore, researchers have increasingly focused on μPADs as a promising alternative for the detection of a wide range of contaminants, including heavy metals, pesticides, nutrients, microorganisms, and emerging threats like PFAS (per- and poly-fluoroalkyl substances) and particulate matter. We recently published a critical review on microfluidics for environmental monitoring, encompassing all the latest advances in the field (Aryal et al., 2024b). In this section, we will only focus on the general trends and gaps observed in μPADs for environmental monitoring.

The use of μPADs in environmental analysis spans various matrices, such as water, soil, air, agriculture products, etc. The most targeted contaminants are heavy metals, pesticides, nutrients, and per- and polyfluoroalkyl substances (PFAs) (Aryal et al., 2024b). Among the detection techniques employed, colorimetric methods are the most prevalent due to their simplicity and cost-effectiveness as well as their ability to be used onsite (Patel et al., 2021; Aryal et al., 2024a). Most applications have also focused on water samples. This is because water's natural fluidic properties, such as low viscosity and surface tension, enable consistent flow and efficient mixing within the microchannels, ensuring precise sample processing (Saez et al., 2021; Atencia and Beebe, 2005). In contrast, for soil or air matrices, the samples need to be pretreated to get them into fluids, or complicated detection methods must be used to accommodate the differences in sample properties. In soil matrices, μPADs are primarily used for detecting nutrients, microorganisms, and other inorganic contaminants. The detection techniques used in soil analysis are more varied compared to those in water-based systems where colorimetric detection is very common. This diversity arises because soil samples require pretreatment and clarification before colorimetric analysis, as the natural color of the soil can interfere with the results. In addition to detecting contaminants, μPADs in soil are also used to monitor the behaviors of microorganisms, including their growth patterns, metabolic activities, and interactions with soil components. For air matrices, the primary focus is on detecting particulate metals and airborne pathogens. The most common techniques in air analysis are electrochemical and colorimetric methods. Figure 6C shows an innovative use of μPAD utilizing gold nanomaterials for the simultaneous quantification of Gram-negative bacteria and nitrite ions in water samples.

Detecting contaminants using μPADs in complex matrices like soil, seawater, and air remains challenging. Factors such as topography, temperature, and humidity can further complicate the measurement in these matrices. Therefore, when designing μPADs for complex environmental matrices, it is not possible to create a perfect miniaturized copy of a real soil or air environment within a microfluidic chip. Instead, these devices should focus on mimicking specific aspects of soil or air under controlled conditions. Moreover, when designing these tools, scientists need to clearly define which soil or air features they aim to replicate, and their findings should reflect the specific conditions they mimicked. μPAD-based PoN sensors are a promising development for environmental monitoring; however, many of these quick tests often rely on qualitative colorimetric methods to indicate results. Such methods can be subjective to the reader/user. Alternatively, techniques like EChem or fluorescence require external power and additional equipment. Therefore, there's a critical need to develop quantitative/semi-quantitative colorimetric methods specifically for PoN applications. This is important because the majority of contamination occurs in resource-limited settings where sophisticated quantification resources may not be readily available.

4.3 Food safety

Ensuring the safety of food is a top priority for both the food industry and those involved in agriculture. This means in the food industry, thorough checking for biological hazards, chemical contaminants, and allergic substances is essential to ensure food safety. Similarly in agriculture, regular monitoring of pesticide content, nutritional content, and potential health risks from cultivation to harvest is crucial for producing safe and healthy food. μPADs are increasingly being used to identify various contaminants in foods and beverages due to their portability and simplicity (Nilghaz et al., 2021). These devices provide a quick and easy solution for food safety and quality control in a variety of situations, including food processing plants and residences (Mitrogiannopoulou et al., 2023). They are used to identify foodborne pathogens like *E. coli* and *Salmonella* and pollutants like pesticides and heavy metals (Ji et al., 2020). They are also used to detect food spoilage, evaluate food quality indicators, and even analyze nutritional content (Hua et al., 2018; Soman et al., 2024; Wang et al., 2022). Figure 6B shows an innovative μPAD-based method of visual and real-time monitoring of Cd^{2+} in rice using [2+2] lanthanide clusters.

Detection of contaminants and microorganisms in food and beverages using μPADs involves several sensing methodologies depending upon the detection limit required and complexity of sample pretreatment. For bacterial detection, enzymatic activity or immunoassays are frequently employed (Nadar et al., 2021). These methods rely on colorimetric detection based on enzyme reactions with chromogenic substrates or electrochemical detection based on redox reactions involving the transfer of electrons between the bacterial metabolites or immunological markers and the electrode. To detect heavy metal contaminants like lead (Pb) and cadmium (Cd), electrochemical methods are commonly used (Busa et al., 2016). The most commonly used method for pesticide detection involves enzymatic inhibition by the pesticide, with color or an electrochemical signal used as the readout (Nadar et al., 2021; Hefner et al., 2024). Additionally, for detecting some other pesticide contaminants, methods like electrochemiluminescence (ECL) and chemiluminescence are employed (Busa et al., 2016). Even after years of advances, the promise of μPADs for food quality and safety analysis remains largely unfulfilled due to limitations in user-friendliness and sensitivity. The majority of food samples need to be pretreated before using them in μPADs. Therefore, more research on integrating on-chip sample pretreatment is needed.

4.4 Security and forensics

In critical security concerns such as terrorist attacks, chemical spills, biological warfare incidents, radiological emergencies, hazardous material accidents, fire, and explosion accidents, swift detection of contaminants is crucial. In the aftermath of an incident, it's essential to quickly screen numerous people and surfaces for harmful substances before sending samples to a lab for confirmation. Similarly, at high-traffic checkpoints, rapid tests are necessary to detect harmful chemicals and prohibited drugs that might be smuggled. This is where μPADs can provide prompt results that save time and enable security officials to make timely decisions. Paper-based microfluidics have the potential to be utilized in international security systems for the detection of various prohibited chemicals, warfare agents, and explosives. They are employed in screening luggage, packages, and suspicious materials at checkpoints. Common targeted chemicals include peroxide explosives like triacetone triperoxide (TATP) and diacetone diperoxide (DADP), as well as nitroaromatic explosives such as trinitrotoluene (TNT), picric acid (PA), and dinitrotoluene (DNT) (Gökdere et al., 2019; Tian et al., 2017; Pramanik et al., 2019). μPADs are also used to detect illegal drugs in the field, thereby supporting law enforcement efforts (Noviana et al., 2020a).

In forensic analysis, presumptive tests serve as rapid initial screenings that help investigators detect potential substances. However, these tests have significant drawbacks: they yield a lot of false positives or negatives, potentially damage valuable DNA evidence, are not label-free, and can contaminate samples with chemical reagents (Bazyar, 2023). To overcome these challenges, μPADs can offer a compact size, portability, reduced risk of contamination, and safe sample storage for subsequent analysis (Bazyar, 2023; Harshey et al., 2023). Forensic investigations involve a wide array of diagnostic tasks, ranging from identifying and analyzing body fluids to detecting drugs of abuse and explosive residues. Common toxins detected include cyanide, ethanol, nitrite, and arsenic (Musile et al., 2023). They are also used to identify drugs and drugs of abuse, such as cathinones, 3,4-methylenedioxymethamphetamine (MDMA), xylazine, morphine, synthetic cannabinoids, benzodiazepines, cocaine, fentanyl, tetrahydrocannabinol, and ketamine (Musile et al., 2023). Moreover, μPADs are employed to detect psychoactive substances commonly used in drug-facilitated crimes, such as gamma-hydroxybutyric acid (GHB), metamizole, midazolam, flunitrazepam, and scopolamine (Musile et al., 2023). Additionally, μPADs can be employed for the preliminary analysis of gunshot residue, counterfeit pharmaceuticals, and evidence at crime scenes (Bazyar, 2023; Harshey et al., 2023; de Oliveira et al., 2017). While μPADs hold promise for security and forensic analysis, additional research is necessary to integrate them with portable and cost-effective platforms. This integration is crucial for ensuring that the developed sensors are both portable and quantifiable at the point of incident. Figure 6 shows the common applications of paper-based microfluidics in different sectors.

5 Sustainability and eco-friendliness

Many existing analytical methods consume a disproportionate amount of energy to detect small amounts of analytes. The trade-off between incremental gains in detection sensitivity and substantial energy consumption raises questions about the overall sustainability and cost-effectiveness of such approaches. Fundamentally, the environmental cost of employing these energy-intensive methods may outweigh the benefits gained from the increased precision, especially when detecting small contaminations. This highlights the need for a more balanced and sustainable approach to sensing technologies. In this context, μPADs emerge as a convincing alternative, offering effective detection capabilities without the excessive energy demands of traditional methods. μPADs ability to operate with low to negligible energy consumption challenges the assumption that advanced functionality must be achieved at the cost of sustainability. In this prospect, paper is an easily available, renewable resource compared to plastics and silicon, which are normally used in microfluidic devices. This reduces dependence on non-renewable resources and promotes the use of sustainable materials. Manufacturing μPADs typically involves simpler techniques that use less energy and fewer harsh chemicals compared to making conventional microfluidic chips.

Given the variability in assay components within μPADs, it's challenging to make sweeping generalizations and assign an average greenness score. However, herein we analyze the μPADs' compliance with the twelve main principles of green chemistry by Anastas and Warner (Anastas and Warner, 2000; Gałuszka et al., 2013). Using the criteria for each principle by the AGREE Greenness matrix (Pena-Pereira et al., 2020), these characteristics are evaluated to assess the environmental friendliness of the μPADs (Table 2).

Figure 7 depicts the spectrum of greenness comparing paper-based microfluidics to traditional heavy instrumentation-based measurements. Traditional analytical methods, situated at the lower (red) spectrum of greenness and sustainability, are

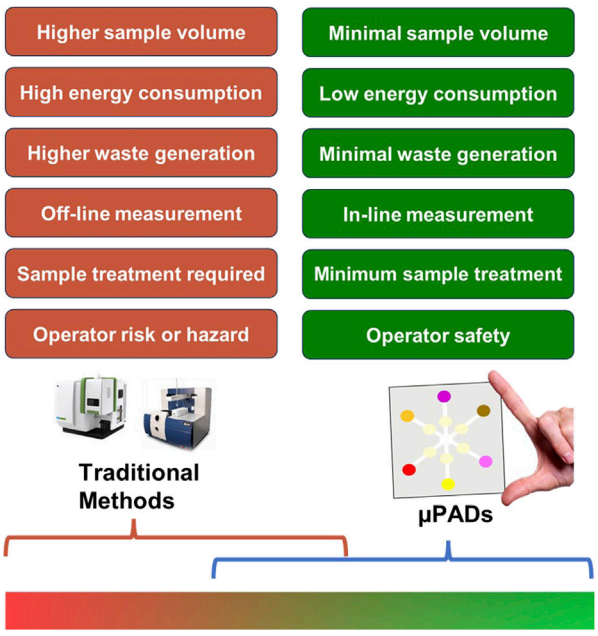

FIGURE 7
Spectrum of greenness analysis in µPADs compared with traditional analytical methods. The color gradient indicates the greenness spanning from low (red) to high (green) sustainability levels.

characterized by high energy consumption, greater sample requirements, longer analysis times, and numerous procedural steps. In contrast, the majority of microfluidic µPADs adhere to the principles of green chemistry, placing them at the higher (green) end of the spectrum. Within µPADs themselves, systems at the lower end often require off-chip pretreatment steps and may involve the use of toxic reagents applied on paper. Furthermore, these systems depend on energy-consuming instruments for analysis. Conversely, at the higher end of the spectrum, there are qualitative or semi-quantitative green sensors. These sensors utilize natural reagents and offer the advantage of not needing any external readout instruments. Table 3 presents the assessment of µPADs in relation to the 12 principles of green chemistry, comparing their alignment with these principles.

6 Bench-to-field challenges

In academia, there's often a prevailing notion that researchers should focus solely on developing methods or making discoveries, leaving the task of implementation to industry (Kumar et al., 2015). While there is some truth to this perspective, it can result in a plethora of research findings languishing in academic journals, merely serving as academic achievements rather than making tangible impacts. This phenomenon isn't confined to theoretical research alone; even practical innovations, like µPADs and sensors, can suffer a similar fate. Many of µPADs inventions, including point-of-care (PoC) or point-of-need (PoN) sensors, are thoroughly crafted in academic settings but fail to transition into practical use outside the lab.

While academia is increasingly investigating the potential of µPADs for PoC diagnostics and PoN testing, putting them into practice proves more challenging than expected. Initial field evaluations of µPADs for PoC diagnostics and PoN testing encounter several challenges. These include sample preparation, limited accuracy, batch variability, short shelf life, reliance on specific readout systems, usability issues, significant production costs, and regulatory approval. While addressing these challenges can yield considerable benefits, such as enhancing technology, cutting development costs, and attracting additional funding, a central obstacle remains. The skillset necessary for successful field testing often diverges from the expertise typical within academic departments like chemistry or biology. As indicated by Kumar et al., for every 60 papers published on laboratory tests aimed at PoC diagnostics, only one paper focuses on field testing or clinical evaluation (Kumar et al., 2015). Public health professionals or scientists skilled in fieldwork pose a distinct advantage here. Conversely, the academics may lack relevant experience. To bridge this gap, increased collaboration with public health experts and greater integration of field evaluation training into relevant academic programs are essential.

PoC testing refers to medical diagnostic tests conducted directly where a patient receives care. While many of these paper-based tests have made strides in transitioning from proof-of-concept to PoC, researchers continue to develop tools to further facilitate this shift. Whiteside's group used µPADs to design a liver function test, which they then trialed in a hospital setting in Vietnam (Vella et al., 2012; Mace et al., 2012; Kumar et al., 2014). Additionally, they pioneered self-forming step-gradients in density to create a test for sickle cell disease, which underwent evaluation in a clinical setting in Zambia (Kumar et al., 2015). Diagnostics for All (DFA) has successfully commercialized paper-based liver function tests, which are now used in resource-limited settings (Pollock et al., 2013). Fio Corporation's Deki Reader, a mobile device-based platform for reading and interpreting paper-based rapid diagnostic tests, has been deployed in multiple countries for malaria and HIV testing (Noble et al., 2020). Biosense Technologies' uChek, a smartphone-based urinalysis system using paper test strips, has gained traction in India and other developing countries (Flaucher et al., 2022). Abbott's BinaxNOW COVID-19 Ag Card, a paper-based rapid antigen test, received FDA Emergency Use Authorization and has been widely used for point-of-care COVID-19 testing (Pollreis et al., 2021; Genomeweb, 2020; Valera et al., 2021). Nima Sensor, a portable gluten and peanut detector using paper-based microfluidics, has been commercialized and is helping individuals with food allergies (Taylor et al., 2018; Burt et al., 2018). Spot-On Sciences has commercialized HemaSpot, a paper-based blood collection device that simplifies remote sampling for various diagnostic applications (Prosperi et al., 2021). Paperfuge, a low-cost paper-based centrifuge developed by researchers at Stanford University, has been successfully deployed for malaria diagnosis in Uganda (Bhamla et al., 2017; Bhamla et al., 2016). The SARS-CoV-2 Rapid Antigen Test developed by SD Biosensor and Roche has been widely adopted globally, demonstrating the scalability of paper-based diagnostics (Flinck et al., 2022). Foldscope, an ultra-low-cost paper microscope, has been distributed to over 150 countries, enabling field diagnostics and science education (Moreno-Roman and Bobick, 2022). These works showcase the potential of µPADs in collaborating with communities in various regions. These examples illustrate the

TABLE 3 Assessment of μPADs in relation to green chemistry principles.

Principle	μPADs compliance
1. Direct analytical techniques should be used, and sample treatment should be avoided	Most μPADs support remote sensing without damaging the sample, remote sensing with minimal physical damage, or noninvasive analysis prioritizing preservation of sample integrity
2. Minimizing the sample size and the number of samples	μPAD systems are miniaturized and focus on small volume analysis (mostly <1 g or mL of sample volume per analysis) ensuring efficient use of resources and reducing environmental impact
3. Performing *in-situ* measurements	Portable devices designed for field use and compact analytical systems such as μPADs demonstrate the potential for facilitating real-time measurements at the point of sampling
4. Integrating analytical processes and minimize reagent usage	μPADs have fewer procedural steps than traditional techniques promoting efficiency and sustainability in analytical workflows
5. Selecting automated and miniaturized methods	μPADs typically fit into the category of automatic and miniaturized or semi-automatic and miniaturized systems
6. Avoiding derivatization	Although some μPADs assays may still require derivatization, the majority of μPADs use detection techniques such as electrochemical sensing, fluorescence detection, and colorimetric assays. These methods can directly interact with analytes without needing chemical modification
7. Minimizing analytical waste generation	Waste generation is significantly reduced compared to traditional methods. This reduction is primarily due to μPADs requiring small amounts of samples and reagents
8. Preference for multianalyte or multiparameter methods over single-analyte analyses	Many μPADs are designed with multiplexing capabilities and integrated sample pretreatment within the chip, which allows for in-line measurement. This significantly reduces analysis time compared to methods relying on heavy instrumentation
9. Minimizing energy consumption	With the majority of μPAD systems featuring portable sensing devices that consume less than 0.1 kWh per sample, they demonstrate strong adherence to this principle. Furthermore, many μPAD-based systems operate without external readouts, further reducing power consumption
10. Preference for reagents obtained from renewable sources	In the case of μPADs, while there are sensors utilizing natural green dyes as sensing elements, there's also considerable use of synthetic reagents, depending on the extent of utilization of bio-based reagents in specific applications
11. Minimum use of toxic reagents	The amount of reagent or solvent employed is typically very low, often ≤0.1 g or mL per analysis. As a result, μPADs demonstrate a reduced environmental impact from toxic reagents
12. Improving operator safety	The μPADs usually require working with lesser volumes of reagents and samples, reducing the risk of unintended exposure to hazardous compounds. Furthermore, the automated or semi-automated operation of μPAD tests significantly lowers the need for human chemical manipulation, minimizing the risk of operator exposure to hazardous substances

promising transition of μPADs from research to real-world use, demonstrating their commercial viability and impact. Their success, particularly in medical diagnostics, highlights their potential to address global health challenges in resource-limited settings.

In the realm of environmental applications, most developed systems are designed for point-of-need scenarios, where contamination or issues arise. Unfortunately, many μPADs developed for environmental monitoring have yet to bridge the gap from proof-of-concept to user-friendly, non-expert-based monitoring. Affordable and user-friendly devices are key to implementing high-frequency measurements, enabling non-experts and citizens to actively monitor their surroundings. There are some examples of where μPADs have been used by citizens to gather their crucial environmental data without the scientist visits. Pamme's team developed a paper microfluidic device for monitoring phosphate levels in freshwater, leveraging citizen science involvement (Richardson et al., 2021). We recently developed a citizen-led global phosphate monitoring sensor and mapped the phosphate levels of countries like Nepal, Thailand, Germany, Chile, Brazil, and the USA (Aryal et al., 2024c). Sameenoi's team developed an on-site chloride test in tap water, empowering untrained local community staff to conduct the tests (Tasangtong et al., 2024). These achievements and developments are promising examples of translating μPADs into the hands of citizens, though such examples are very few.

7 Conclusion and future perspectives

While advancements have been made in paper-based sensor performance through material selection, design optimization, advanced manufacturing, and functional material integration, achieving ideal performance across all metrics remains a challenge. The following key aspects need to be considered to realize the commercial success of μPADs.

7.1 Enhanced sensitivity and multiplexing

To improve the sensitivity and multiplexing capabilities of μPADs, researchers must focus on developing advanced microfluidic networks while incorporating signal amplification techniques. Interconnected designs with multiple channels and reaction zones enable the simultaneous detection of various targets, enhancing diagnostic efficiency. Incorporating signal amplification techniques, such as CRISPR/Cas biosensing systems

combined with novel nanomaterials, can significantly boost detection sensitivity and signal strength, making μPADs more effective for complex analytical applications.

7.2 Integration with smart technologies

By leveraging artificial intelligence, machine learning, and the Internet of Things, data analysis and interpretation can become more efficient and accurate. Furthermore, the development of universal data processing applications compatible with various smartphone models would simplify result interpretation, making these platforms more user-friendly and widely applicable.

7.3 Improved fabrication and reproducibility

Optimizing patterning techniques can reduce batch-to-batch variations and enable stable mass production. Standardizing fabrication protocols and promoting the complete disclosure of methodologies in published works will enhance reproducibility, fostering greater confidence in μPAD-based technologies among researchers and users alike.

7.4 Automation and portability

Automated test readouts are essential for improving detection reproducibility and minimizing user error. Integrating μPADs with portable detectors can enable real-time, on-site diagnostics and testing, making them invaluable tools in various real-world applications. These systems include smartphone-based detection systems, portable transmittance colorimeters, paired-emitter-detector diode (PEDD) systems, miniaturized electrochemical detectors, and automated lateral flow devices.

7.5 Materials and manufacturing consistency

Achieving scalability in μPADs requires a focus on material consistency, user-centric development, and manufacturing precision. It is essential to use consistent materials for both prototyping and mass production, reducing the risk of costly redesigns. Additionally, prioritizing user needs is crucial by considering safety for non-professional use and designing devices with user-friendly dimensions that cater to the target audience. Finally, manufacturing precision must be assessed by evaluating parameters like volumes, setup costs, and batch sizes to ensure cost-effective and high-quality production of μPADs.

7.6 Real-world testing and validation

The primary scalability issues arise from the lack of field trials within the research. This gap is evident in many μPADs platforms, where researchers often neglect the importance of testing sensors with actual real-world samples. Consequently, there is a significant misuse of the term "proof-of-concept," with published μPADs results often being difficult or impossible to reproduce. Many published works fail to properly mention the full protocols or describe them very poorly. To ensure μPADs perform effectively outside the laboratory, extensive field trials with real-world samples are critical. These trials help validate the performance of μPADs in diverse environmental conditions. Efforts should also focus on integrating sample collection, pretreatment, analysis, and data interpretation into single, user-friendly platforms tailored for resource-limited settings, ensuring practical usability.

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