ACS MATERIALS LETTERS

www.acsmaterialsletters.org

REVIEW

Paper-Based Materials for Diagnostics

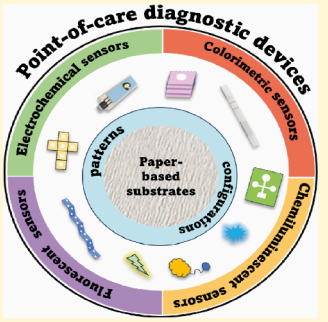
Panagiota M. Kalligosfyri and Stefano Cinti*

Cite This: ACS Materials Lett. 2024, 6, 1184–1198

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: **Nowadays point-of care (POC) devices dominate the field of bioanalysis as they play a pivotal role in improving different aspects of diagnostics including screening, early diagnosis, and disease monitoring. These devices comply with the World Health Organization ASSURED criteria that describe the ideal POC tool. While conventional materials like polymers, silicon, metallic foils, and glass have been exploited in POC device manufacturing, recent innovations relying on paper-based materials have introduced a new era of versatile diagnostic tools. Because of their properties, cellulose and its derivatives have emerged as the most common paper-based substrate in device fabrication. The present review explores recent developments in paper-based diagnostics, covering a wide range of applications including reagent storage and isolation/extraction of the analyte and also serving as a sensing platform. The versatility of paper substrates in various diagnostic devices such as lateral flow assays, electrochemical sensors, microfluidics, etc. is discussed, highlighting their advantages, challenges, and limitations on the context of precision medicine.**



In the era of current cutting-edge diagnostics, point-of-care (POC) devices are at the forefront of bioanalysis. These devices can facilitate and also improve the main aspects of the diagnostic umbrella: screening, diagnosis, and monitoring of the diseases. All the POC devices rely on the same common features: affordability, sensitivity, specificity, user-friendliness, rapidity and robustness, equipment-free, and deliverability known as the ASSURED criteria. These characteristics were introduced from the World Health Organization (WHO) to describe the ideal POC device.[1] Many materials such as polymers, glass, silicon, and metallic foils are used in traditional POC device manufacturing. Each of the materials mentioned provides different features and properties to the diagnostic tools, like a high aspect ratio, optical transparency, flexibility, electrical interconnections of the different main components of the device, the usage of small and controllable sample volumes, etc.[2] However, the exponential growth of POC devices is directly linked to key innovations in their fabrication, like the exploitation of paper-based materials, leading to the development of new, powerful, and versatile diagnostic equipment.

Paper-based diagnostics constitutes a great category of the POC technologies and also complies with the ASSURED criteria leading to the development of an ideal device. The appearance of paper as a substrate in bioanalytical devices was reported many years ago. Beginning in 1949, Müller and Clegg designed a rapid and automatic paper-based chromatography where they controlled the flow of the liquid using a paraffin outline and providing the possibility to exploit small sample volumes.[3] This system has paved the way for the next generation of paper-based diagnostic tests. Several years later, in 1980, Leuvering et al. introduced an immunoassay with a lateral flow assay (LFA) format exploiting, for the first time, colloidal gold and silver nanoparticles for the visual detection of a pregnancy hormone, human chorionic gonadotropin, on a solid phase.[4] As a result of this advancement, during the mid-1980s, the first commercially available pregnancy test for home testing was introduced.[5] Later, in 2007, the concept of paper-based analytical devices (PADs) was first presented by the group of Whitesides. The research group developed the first diagnostic PAD which consisted of millimeter-sized channels that required a small sample volume. This was achieved by exploiting the hydrophilic paper and the hydrophobic polymer interface.[6] The same group in 2008 designed and reported the first three-dimensional (3D) PAD by stacking multiple layers of pattern paper for multiplex analysis and proposed the device for diagnostic applications.[7] This specific paper-based analytical tool soon stimulated the scientific interest worldwide as it was introduced as a new and cost-effective alternative in the diagnostics field and was the first approach of the more advanced 3D-PADs that are based on the origami principle, i.e. paper folding.[8] Based on this approach, in 2011, Liu and Crooks reported a 3D-PAD combined with the origami principle by simple paper folding and a single patterning step, regardless of the layers of the paper used. The analysis could be

Received: November 27, 2023
Revised: February 9, 2024
Accepted: February 13, 2024

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184–1198

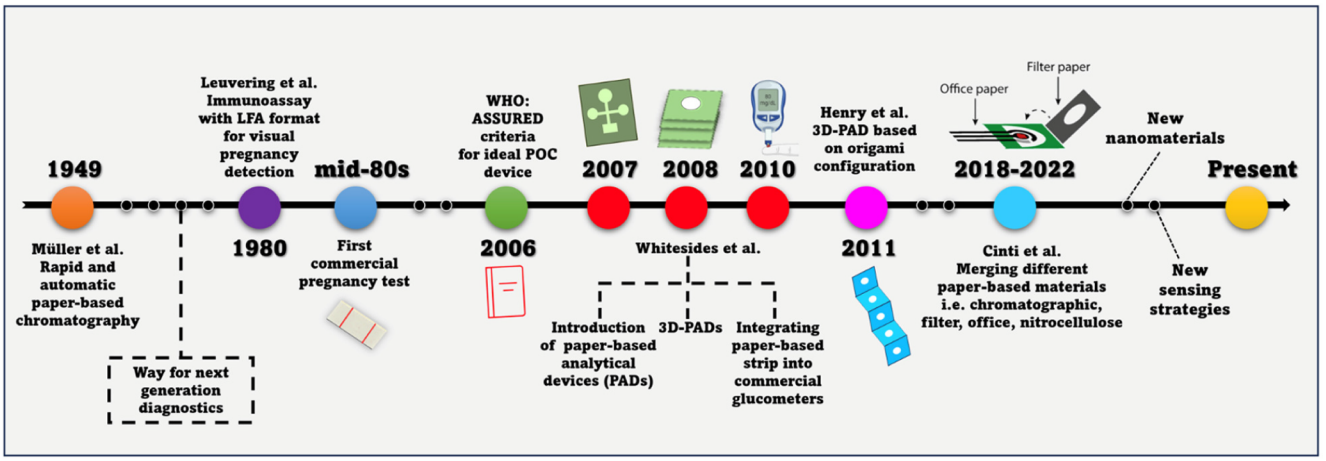


Figure 1. Timeline of paper-based bioanalytical tools development.

performed by the naked eye after unfolding the device. As a result, rapid fabrication time and low cost of the device were achieved.[9]

These former paper-based devices were combined with new nanomaterials, sealing parts, and advanced transducers to overcome the limitations of poor detection limits and mechanical strength.[10] During the timeline (Figure 1) of paper-based bioanalytical tool development, various substrates were used.

For example, cellulose and its derivatives remain widespread materials in traditional industrial papermaking, making them a prevalent choice as substrates in paper-based devices. As will be discussed further in the present paper, paper consisting of cellulose fibers is cost-effective, hydrophilic, biodegradable and biocompatible, flexible, easily engineered, and therefore a very promising substrate in POC devices construction. All these important features and some of the physical properties of a paper substrate, i.e. pore size, fiber structure, capillary action, and density, are mostly defined and affected from the initial pulped material and the whole papermaking process.[11]

In the field of diagnostics, traditional methods, such as enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR), provide biomarker screening for disease and therapy monitoring. These methods require expensive equipment and highly qualified personnel. In contrast, the new POC devices exhibit advantageous characteristics such as portability, versatility, and robustness. Paper-based materials are integrated to POC devices such as LFAs, electrochemical sensors (ECSs), microfluidics, immunosensors, wearable sensors, for the design of diagnostic tools for various biomarker screenings, health monitoring, and finally precision medicine.[12]

In this review, we aim to present recent paper-based approaches in the field of diagnostics. Paper-based materials are used in a plethora of applications such as isolation/extraction, reagent storage or immobilization, and target amplification. They also serve as substrates for the sensing area and consequently as a platform for analytical results communication. Finally, we will discuss the possibilities of paper substrates exploited in diagnostic devices and share our aspect regarding the analytical capabilities of paper-based POC devices presenting their advantages, challenges, and limitations.

Paper has been used for many years as a substrate for manufacturing diagnostic devices. In POC devices, paper-based substrates refer to thin manufactured materials consisting of fibers and pores. This specific paper structure provides foldable and versatile substrates for multiple applications in diagnostic devices. The most important advantages of paper substrates are the following: (i) the manufacturing cost, (ii) the fluid flow that occurs through capillary forces without requiring external forces, (iii) their biocompatibility that enables immobilization of biomolecules, and (iv) the possibility of surface modification.[13]

Paper has been used for many years as a substrate for manufacturing diagnostic devices.

The most important advantages of paper substrates are the following: (i) the manufacturing cost, (ii) the fluid flow that occurs through capillary forces without requiring external forces, (iii) their biocompatibility that enables immobilization of biomolecules, and (iv) the possibility of surface modification.

The material used mostly in PADs is cellulose substrate and especially filter paper, as it maintains more stable basic properties, during manufacturing processing, in comparison to other paper materials. Filter papers consisted of fiber networks distributed in all directions that are formed through the interaction of cellulose hydroxyl groups. The specific filter papers used in diagnostic devices consist of a single type of cellulose fiber obtained from cotton. These fibers define the wettability, the internal surface, and density of the material. In particular, refined fibers, such as nanocellulose crystals, can be used to increase the internal surface area and decrease the pore size of the paper.[14] Furthermore, there are several grades, e.g. 1−6, 595, 597, 598, 602, of commercially available filter paper that provide different flow rates, pore size, and particle retention up to 10 mm, thickness, and weight.[15]

The porous network of the paper-based materials plays a crucial role in the liquid flow. More specifically, with a decrease

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

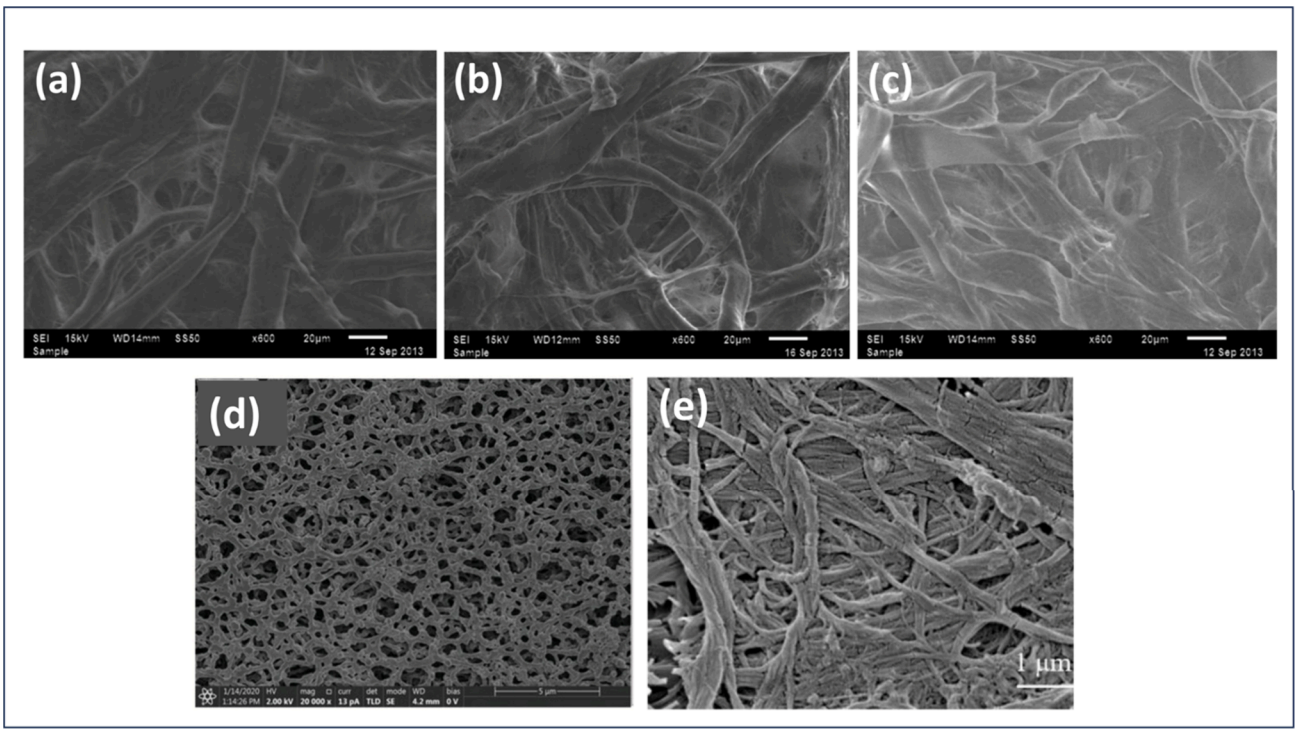


Figure 2. SEM image of A) grade 1 filter paper, B) grade 3 filter paper, and C) grade 4 filter paper. Reprinted with permission from ref. [25]. Copyright 2012, Royal Society of Chemistry. D) NC membrane pore structure and fiber orientation. Reprinted with permission from ref. [26]. Copyright 2021 Wiley Online Library. Licensed under CCBY. E) Office paper structure. Reprinted with permission from ref. [27]. Copyright 2023, MDPI. Licensed under CCBY.

in the pore size, a decrease in permeability follows. This leads to delayed fluid flow, that can increase the interaction of the analyte on the sensing area and therefore enhance the sensitivity of the POC device.[16] The pore size, the geometry of the fibers, and the width of the channels in a paper-based material can also affect the capillary forces as studied previously.[17] Consequently, the wettability of the fibers is also affected. For example, paper fibers with bigger pores should demonstrate a faster wicking rate because of the lower flow resistance.

As mentioned above, capillary-driven paper-based devices offer the advantage of continuous fluid movement without the need for external equipment. In addition to the properties of paper substrates, the viscosity of the fluid plays a role in controlling the capillary action. However, these devices come with certain limitations, including backflow issues and liquid evaporation. These limitations can be addressed by incorporating various microchannels to regulate capillary flow and decrease the drying of reagents.[18]

Another paper-based substrate used in POC devices is a nitrocellulose (NC) membrane. Blotting assays that exploit NC porous substrates were first introduced in the 1960s. Since then, NC membranes have been used in LFAs as substrates for biomolecule immobilization such as antibodies and nucleic acids. Moreover, the NC membrane is a critical component of the lateral flow strip test as both binding and detection of the analyte occur on it. NC membranes, with tunable pore size, are combined with glass fibers and filter paper to enhance the capillary action and ensure the flow of reagents through the LFA strip.[19] In addition to these, another paper-based substrate is represented by commercial office paper, also known as copy paper. Office paper has been recently used as a substrate in paper-based devices, especially in the field of electrochemistry. The usage of simple office paper in ECSs provides an improved diffusion rate and thus higher sensitivity as the sample flows directly toward the electrode's surface. On the contrary, when filter paper is used, the analyte diffuses through the cellulose fibers prior to contact with the electrode and leads to a lower signal response.[20] Currently office paper has been exploited, in a few reported works, as a substrate in ECSs for diagnostic applications. For example, electrodes have been designed by Chagas et al. on office paper via a simple pencil for the development of an electrochemical platform. The authors reported that in comparison to filter paper, office paper demonstrated a higher electrical conductivity and, thus, improved performance. The proposed sensor was applied for the detection of Na^+ and K^+ in tear samples.[21] In another approach, Cinti et al. introduced an office paper-based ECS. The electrodes were screen printed on office paper toward the fabrication of an ECS for the monitoring of Zn(II) in serum and sweat.[22] Screen printed electrodes on office paper were later applied, by Moccia et al., for the fabrication of an ECS for specific pancreatic adenocarcinoma microRNA biomarker detection.[23] The same year Caratelli et al. fabricated an origami paper-based ECS. In this case, the authors combined a plasma separation membrane, filter paper for reagent storage, and sustainable office paper for the development of origami-based and reagent free sensor Alzheimer's therapy monitoring, by detecting specific inhibition reactions.[24]

All of the above advantageous physical properties of the paper-based materials are strongly connected to their structures. As shown in Figure 2, scanning electron microscopy (SEM) images highlight the morphology of filter papers of different grades, NC and commercial office paper, displaying differences in fiber orientation and pore size of the above materials.

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

The functionalization of paper-based substrates, during or after the manufacturing process, significantly affects their surface and structure properties and thus their biocompatibility, even though the chemicals used in the paper-making process consist of only 3% of the total pulp material. These species are used (i) to increase the hydrophobicity of the paper, through the formation of negative charges on the fiber surface, (ii) as retention agents to form homogeneous materials and also (iii) as dry and wet strength agents. When functional groups are added on the paper substrate, a bioactive paper can be obtained. For example, positively charged starch molecules might be added to modify the surface charge of the fibers. Furthermore, various chemical agents that provide wet strength in bioactive paper can be added. When polymers such as melamin-formaldehyde, glyoxalated polyacrylamide, and polyamidoamine-epichlorohydrin are added to the paper substrates, this leads to wet strength improvement. These additives surround the fibers, forming a polymer network and thus leading to a more robust product. However, most of the paper-based assay's configuration consists of a biomolecule-immobilization step on the substrate's surface, and as a consequence, it is crucial to be aware of the above wet strength agents' presence.[28] The surface chemistry of the paper-based substrates should be compatible for the immobilization of biorecognition elements that occurs with physical absorption via electrostatic interactions or covalent bonds utilizing heterobifunctional cross-linkers.[29,30]

The surface chemistry of the paper-based substrates should be compatible for the immobilization of biorecognition elements that occurs with physical absorption via electrostatic interactions or covalent bonds utilizing heterobifunctional cross-linkers.

Paper substrates are versatile and can be handled in different ways. Their surface can be modified with different techniques, creating hydrophobic barriers and providing the ability to control the flow of the sample and the reagents. Paper can also be folded forming different shapes and structures toward the fabrication of one-, two-, and three-dimensional PADs. In these configurations, the flow of the fluid occurs through capillary action causing wicking of the paper substrates. The flow rate as well as the wicking surface depends on (i) the design of the hydrophobic patterns, (ii) the pore sizes, and (iii) the overlapping of the individual paper materials during the PAD assembly. All these features produce a passive flow of the reagents/samples through the structure of paper-based substrates.[31]

LFAs are performed on a strip test, also known as the dipstick test. An LFA strip test consists of four different paper-based materials.[32,33] The first material is a filter paper. This part is called sampling or the immerse pad and is used for sample or assay buffer application, respectively. The second material is the conjugate pad where specific biorecognition molecules are immobilized, usually antibodies functionalized with gold nanoparticles, biotin molecules, oligonucleotides, or enzymes. The conjugate pad is usually made from glass fiber, but also polyester and cellulose fiber might be used for its fabrication. This component of the LFA strip is very important for the long-term storage of the assay reagents. The third material is the NC membrane, and it is available with different pore sizes to facilitate different assay configurations for the analysis of various targets. In most LFAs, there are two lines on the NC membrane: (i) the test zone (TZ) where the analyte detection occurs and (ii) the control zone (CZ) that must be always visible to ensure the functionality of the strip test. Finally, the fourth material, that is, the absorbent pad, is used to absorb the excess of the reagents and to achieve a continuous flow of the sample to the upper level of the LFA test. All of the parts of the LFA strip are attached to a solid substrate by overlapping endings which enables capillary forces to occur.

Paper-based microfluidics represent an important category of PADs. A lot of different methods for PAD manufacturing have been reported and can be categorized in (i) physical patterning and (ii) chemical patterning for the creation of hydrophobic barriers: these barriers lead to hydrophobic channel creation that serve as manipulation tools of the reagent's flow on the PADs. One of the paper patterning techniques is photolithography. In this technique, different patterns are designed using a photoresist. An alternative method involves the wax pattern technique. This technique allows the design and printing of paper patterns surrounded by wax, creating hydrophilic channels. As a result, a low-cost, disposable, and nontoxic platform is achieved. Other methods proposed for the fabrication of cost-effective and robust PADs are polydimethylsiloxane (PDMS) potting, wax screen-printing, and plasma treatment.[34] Also, inkjet printing is an alternative method for patterning paper-based substrates: herein, the basic principle is the contactless printing of hydrophobic materials or chemical compounds toward the creation of microfluidic channels after solvent treatment.[35] Different patterns on paper-based substrates, manufactured with various traditional as well as alternative techniques, such as wax printing, chemical vapor deposition, inkjet and laser-induced printing, 3D printing, etc., are reported in Figure 3.

Paper-based substrate manipulation by designing hydrophobic barriers and folding or stacking can lead to different PADs' configurations. Two-dimensional (2D) PADs (Figure 4A) can be created with the connection of various hydrophilic channels connecting circle, rectangular, or square shaped areas.

The width and length of the channels can be designed to control the speed of reagent flow by changing the interacting areas. By monitoring the configuration of the PADs' components, tasks including the sample preparation, the mixing of reagents, washing steps, and detection can be performed within a unique and integrated device.[45]

Except for the 2D-PADs, 3D formations (Figures 4B, 4C) have also been reported. These formations facilitate reagent flow and sample flow monitoring and can be created by superimposing or folding the paper-based substrates. These approaches are known as origami formation, i.e., 3D configurations with different ways of paper folding. There are several ways of folding configurations, and they can be inward, alternating, T-type, Z-type, and also edge folding.[46] Each one of the above specific paper handlings provides reagent addition or mixing during a reaction, storage, filtration, analyte separation from the matrix, and also preconcentration steps.[47]

The design of immunosensors with more complex configurations has been achieved by folding different layers of paper to realize one origami structure for the simultaneous and selective detection of various diseases. For example, a

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

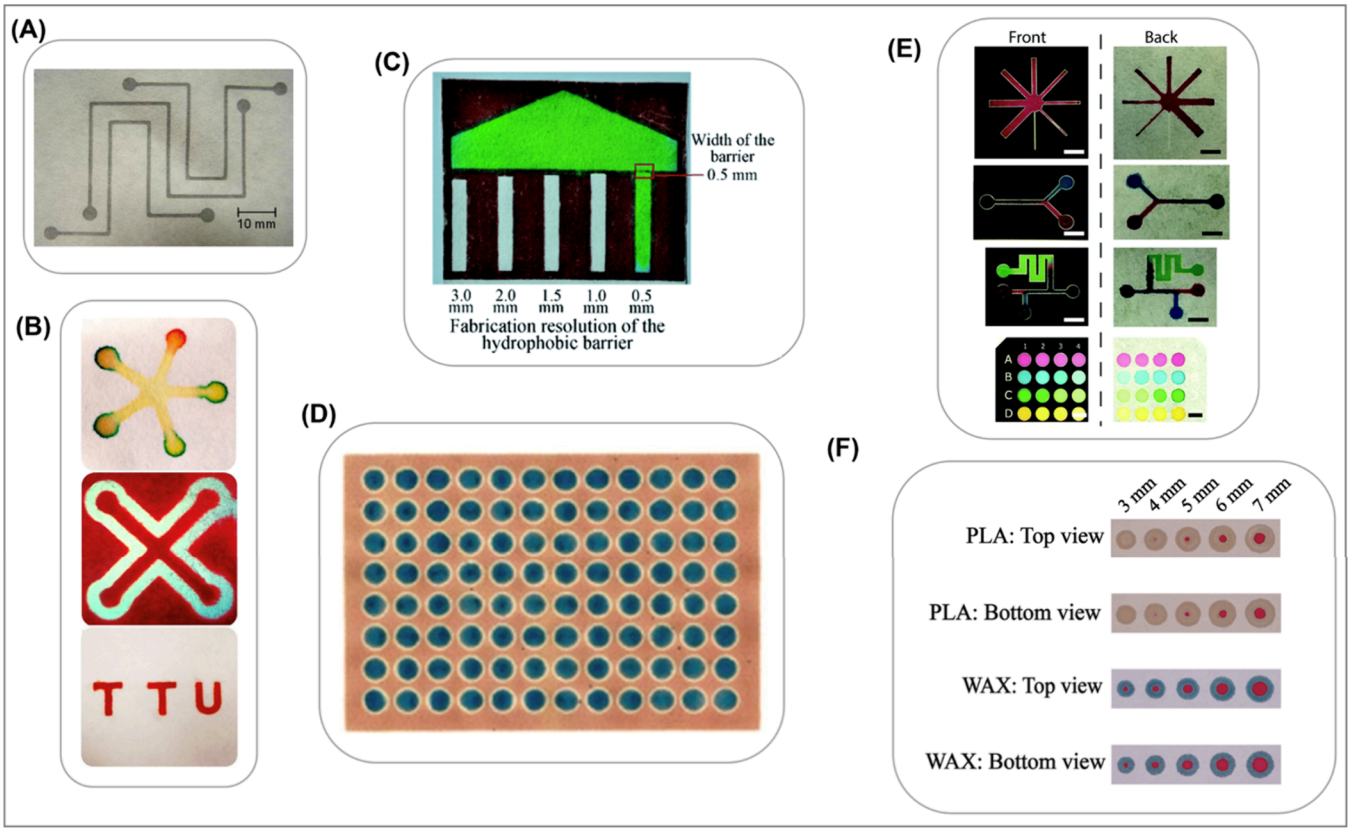


Figure 3. Different configurations of patterns on paper-based substrates for microfluidics applications. A) Inkjet printer printed patterns. Reprinted with permission from ref. [36]. Copyright 2010, Elsevier B.V. B) Chemical vapor deposition of hydrophobic barriers. Reprinted with permission from ref. [37]. Copyright 2017, Springer Nature. Licensed under CCBY. C) Laser-induced selective wax patterning. Reprinted with permission from ref. [38]. Copyright 2019, Royal Society of Chemistry Publishing. Licensed under CCBY-NC. D) Wax printing of a 96-well paper-based multiplatform. Reprinted with permission from ref. [39]. Copyright 2019, Elsevier B.V. E) Fabrication of microfluidic channels via a toner laser printer. Reprinted with permission from ref. [40]. Copyright 2020, Royal Society of Chemistry Publishing. Licensed under CCBY-NC. F) Creation of 3D printed hydrophobic barriers using polylactic acid (PLA) and wax filaments. Reprinted with permission from ref. [41]. Copyright 2022, Elsevier B.V.

The design of immunosensors with more complex configurations has been achieved by folding different layers of paper to realize one origami structure for the simultaneous and selective detection of various diseases.

multilayered PAD was introduced by Wang et al. for gastric cancer detection.[48] The research group fabricated a PAD consisting of a splitting layer, a detection layer, and a blotting layer by combining different grades of chromatographic paper. The rotatable detection layer served as a switch that enables the sample to flow or to be incubated in the sensing area. Both 2D and 3D sensors enable sophisticated and intelligent multichannel formations. In conclusion, the above aspects provide the capability to regulate reagent deposition, manage reagent storage, and control sample volume, resulting in a more efficient analyte detection.[49]

The origin of paper-based materials, their manufacturing process, and their surface functionalization affect not only the properties of the paper-based substrates but also the overall environmental footprint of a POC device. During the COVID-19 pandemic, apart from dealing with biological waste, managing single-use plastic waste was a real challenge. Tons of plastic residue generated from test kit packaging and components were mismanaged contributing to future pollution and posing a burden on the environment.[50,51] Approximately 40% of the single-use plastic wastes ended up in a landfill, with only 16% being recycled, and 19% of them leaked in the ecosystem.[52] As a result, new alternatives such as bioplastics, paper-based materials, and other sustainable technologies are crucial to be applied for POC devices manufactured for diagnosis and health monitoring.[53]

As a result, new alternatives such as bioplastics, paper-based materials, and other sustainable technologies are crucial to be applied for POC devices manufactured for diagnosis and health monitoring.

Among the characteristics of paper-based materials used in the construction of sensors is their environmental friendliness. In comparison to existing materials commonly used in POC devices, such as plastic, cotton, glass-fiber, epoxy substrates, toxic metallic foils, or other nonbiodegradable sources, paper-based materials consist of a more sustainable choice for sensor

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

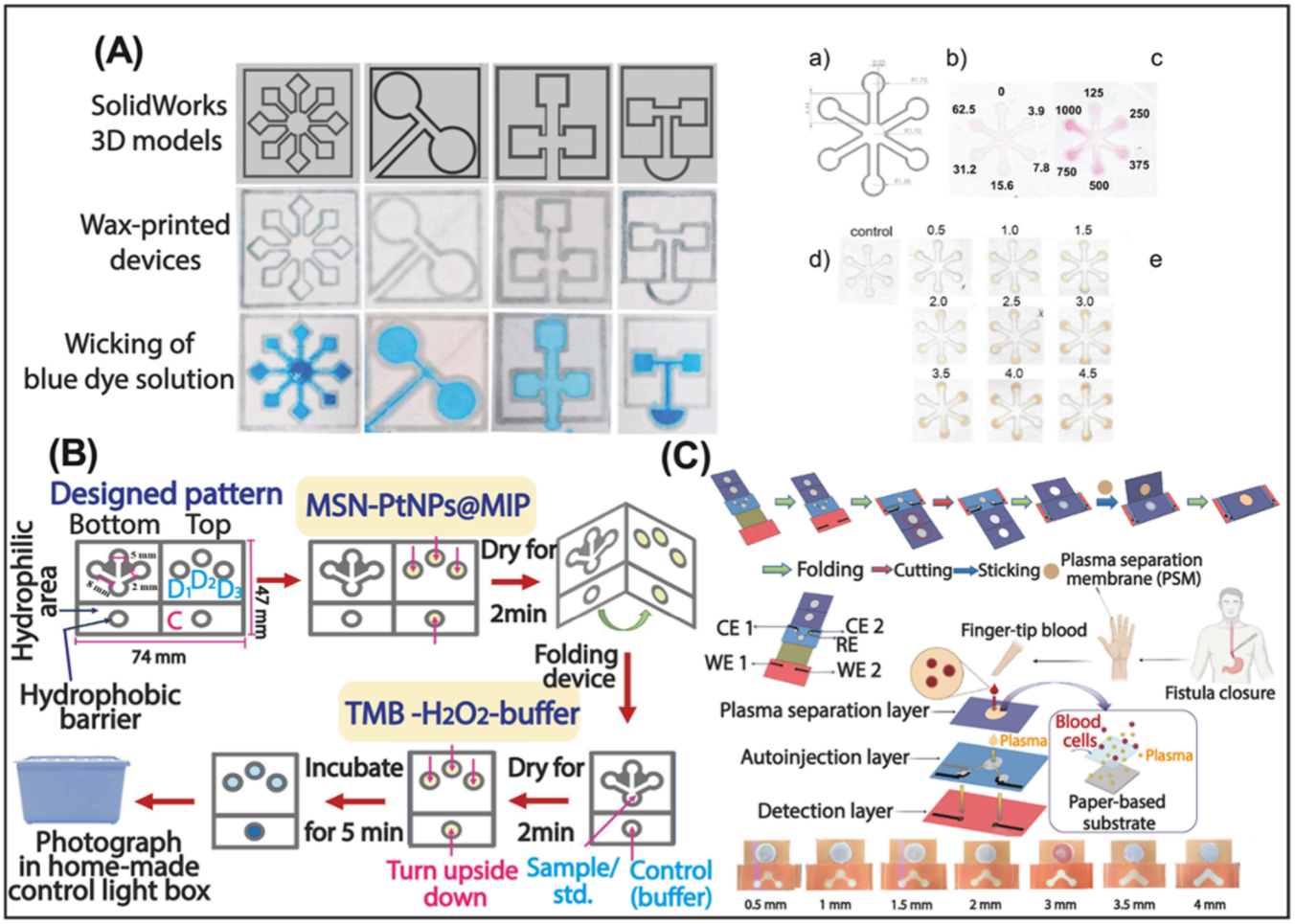


Figure 4. Examples of various PADs' configurations. A) Different configurations of 3D wax printing of microfluidic PADs for glucose and nitrite assays. Reprinted with permission from ref. [42]. Copyright 2019, Elsevier B.V. B) Origami 3D-microfluidic paper-based analytical device. Reprinted with permission from ref. [43]. Copyright 2023, Elsevier B.V. C) Multifunctional self-driven origami paper-based integrated microfluidic chip. Reprinted with permission from ref. [44]. Copyright 2022, Elsevier B.V.

applications. More specifically, the versatility of paper-based materials extends beyond their role as substrates for the sensing areas in POC devices, as they can also be exploited for the fabrication of functional electronic materials serving as green electronics alternatives to conventional sensor electronics and for packaging of the POC devices.[54]

Moreover, paper-based substrates can be combined with biobased surface functionalization reagents such as chitosan,[55] with biodegradable cases, with paper-based printed electronics, and with nontoxic inks and solvents, noble metals, and carbon-based materials for the various electronic components if needed.[56,57] By integrating all these applications of paper-based materials in one POC device, it is easier to increase the sustainability of POC diagnostics.

Based on the advantageous properties of the paper-based materials, mentioned in the previous sections, paper-based sensors can be handled more easily for proper disposal. Considering the above, in terms of environmental impact, the footprint of paper-based sensors is significantly low. Consequently, paper-based sensors can contribute to the reduction of the overall environmental waste.

Other than being used as a substrate or for the construction of the sensing area of the POC devices, paper-based materials can be exploited also for multiple applications, integrated in a single diagnostic test, such as collection or preparation of the sample, isolation of analytes, and reagent storage. With the use of filter paper, multiple analytes, such as uric acid and ascorbic acid, might be separated from the sample upon contact with the eluent, moving through the fibers of the paper by capillary action. As reported, the separation efficacy is affected by the thickness and the length of the paper used.[58] Sample preconcentration is exploited to improve the assay sensitivity. This can be performed by drop casting[59] on filter paper or by letting the sample flow through a filter paper disc with the help of peristaltic pumps.[60]

When nucleic acid is the target molecule, paper substrates can also be used to extract the nucleic acid from the total sample. The high binding affinity of cellulose paper with DNA makes it an ideal substrate for extraction. Since 2000, cellulose paper material has been commercially available for nucleic acid isolation. Filter paper has also been applied as an alternative to glass fiber in DNA extraction kits providing a lower cost of the assay.[61] Various biomolecules, such as nucleic acids, proteins, antibodies, nanoparticles conjugates, etc., can be stored in paper-based substrates by simply drying on them. This application facilitates on-site testing without needing any storage equipment. The reagent storage/immobilization step is very crucial for the POC devices, as it determines their proper function, and it can be physically or chemically performed.[62]

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

Table 1. Reported Paper-Based POC Devices Exploiting Various Sensing Strategies

Sensing strategy	Application	Analytical performance	Usage of paper-based material	Ref
Colorimetric Detection				
Paper-based ELISA	exosomes detection and isolation	R^2 = 0.95 (exosome standard) R^2 = 0.92 (human serum)	reagent/sample reservoirs	65
Enzymatic mediated chromogenic reaction	uric acid and glucose detection	LOD = 0.03 mg/dL (uric acid) LOD = 0.6 mg/dL (glucose)	-flow control -reagent stability	66
pH change-based colorimetric PADs	chronic kidney disease biomarker detection	LOD = 0.032 mM (ammonium) LOD = 0.049 mM (urea)	reagent stability	67
Enzymatic mediated chromogenic reaction	simultaneous glucose and cholesterol detection	LOD = 0.16 mM (glucose) LOD = 0.57 mM (urea)	-reagent storage -controllable deposition	68
Electrochemical Detection				
Paper-based multiplex electrochemical platform	simultaneous detection of analytes in drugs	LOD = 0.04 mM (paracetamol) LOD = 0.22 mM (caffeine) LOD = 0.40 mM (ascorbic acid)	-flow control -flexible electronics	71
Origami, graphene-modified aptasensor	EGFR biomarker detection	LOD = 5 pg/mL	flow and volume control	72
Origami PAD, enzymatic mediated reaction	wound infection monitoring	LOD = 5.04 nM (H_2O_2)	-flow control -sample treatment	73
Paper-based electrochemical platform	detection of copper ions	LOD = 4 ppb	-reagent storage -sample collection -analyte preconcentration	74
Fluorescence Detection				
Fluorescent PAD	histidine detection	LOD = 1.8 μM	-reagent storage -flow control	75
Carbon dots-based florescent platform	folic acid detection	LOD = 0.28 μM	-reagent deposition/storage -reusable platform	76
PNA-based fluorescent platform	hepatitis C virus DNA detection	LOD = 5 pmol	-reagent deposition -flow control -washing steps	77
LFA fluorescent assay	rheumatoid arthritis antibodies detection	LOD = 0.4U/mL	-reagent deposition -flow control	78
Chemiluminescent Detection				
Paper-based chemiluminometric device	genotyping of single nucleotide polymorphisms	25 fmol amplified target DNA	-reagent deposition -flow control	79
Paper-based cell-free toehold switch biosensor	SARS-CoV-2 RNA detection	10 nM RNA	sample/reagent storage	80
Gold nanoparticle-based, paper-based chemiluminescent immunodevice	acute myocardial infarction biomarkers detection	LOD = 0.06 pg/mL (heart-type fatty acid-binding protein) LOD = 0.3 pg/mL (cardiac troponin I) LOD = 0.4 pg/mL (copeptin)	-reagent deposition -flow control -analyte separation	81
Multiplex paper-based electrochemiluminescence platform	microRNA detection	LOD = 5.7 fM (miRNA-155) LOD = 4.2 fM (miRNA-126)	-flow control	82

All the different and smart device configurations are combined with various detection methods (Table 1) such as colorimetry, electrochemistry, fluorescence, chemiluminescence, etc. providing the required selectivity and sensitivity needed in the field of diagnostics.

The reported paper-based devices for POC applications can be exploited for a wide range of biomarker screening and detection, responsible for cancer, cardiovascular diseases, infectious diseases, etc. The biomarkers of interest include DNA target sequences, enzymes, proteins as tumor biomarkers, exosomes, microRNAs, viral RNA, etc. POC devices are able to provide the detection of the above biomolecules that are clinically significant.[63]

Colorimetric assays on paper-based materials including both LFAs and PADs allow eye detection and also semiquantification of the analytes as the signal readout can be achieved via portable and cost-effective equipment such as smartphones and conventional scanners.[64]

For instance, paper-based ELISA has been reported for the isolation and colorimetric semiquantitative detection of exosomes in serum samples. A biotinylated specific antibody was immobilized for exosome capture on the device sensing area. The exosomes have been detected through a sandwich ELISA assay containing a horseradish peroxidase (HRP)−antibody conjugate (Figure 5A).

In the presence of the analyte, a blue color appears due to the chromogenic reaction of the TMB substrate with HRP. In this example, the hydrophilic paper wells, which have been patterned through wax printing, serve as reservoirs for reagent/sample retaining. The reagent/sample volume loss has been limited, and as a result, the reaction in the presence of the analyte has been more stable. This platform characteristic

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

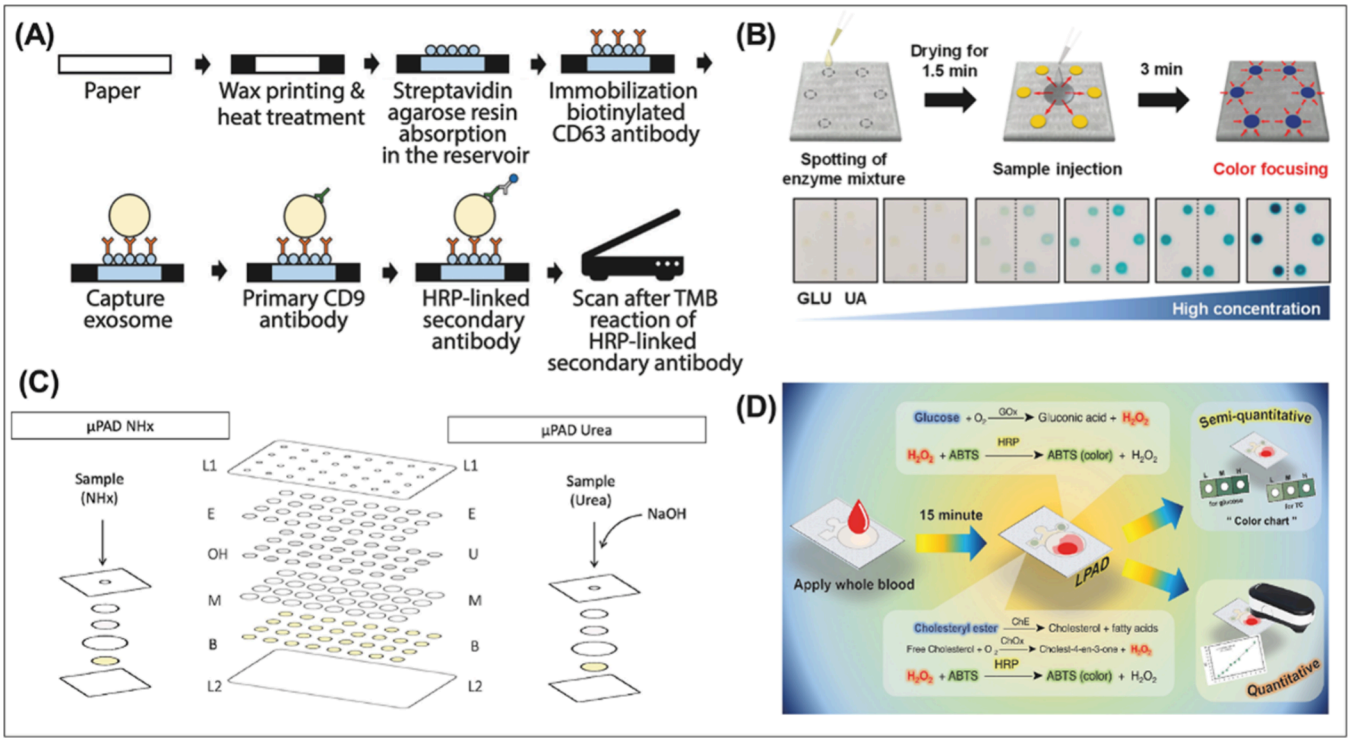


Figure 5. Examples of various colorimetric paper-based devices. A) Paper-based ELIZA assay for isolation and detection of exosomes. Reprinted with permission from ref. [65]. Copyright 2020, Royal Society of Chemistry Publishing. B) Paper-based color focusing platform for uric acid and glucose detection. Reprinted with permission from ref. [66]. Copyright 2022, American Chemical Society. C) Laminated microfluidic PAD platform for chronic kidney disease biomarker colorimetric detection. Reprinted with permission from ref. [67]. Copyright 2023, Elsevier B.V. Licensed under CCBY. D) Laminated PAD for simultaneous total cholesterol and glucose determination. Reprinted with permission from ref. [68]. Copyright 2023, Elsevier B.V.

provides the possibility for application by resource-limited countries.[65]

In another example of a colorimetric sensor, the authors have developed a simple paper-based platform for quantification of uric acid and glucose in urine samples with a limit of detection 0.03 and 0.6 mg/dL, respectively, covering the physiological target ranges. The paper-based substrate was treated with chitosan oligosaccharide lactate providing an advantageous enzyme stability and high color intensity by decreasing the flow rate (Figure 5B). The analytes were detected by specific enzymatic reaction in the presence of chromogenic substrates for the colorimetric visualization. The chitosan treatment led to pore blocking, allowing liquid flow control. Consequently, without any specific patterns, signal losses were eliminated by employing asymmetric flow. Finally, this paper-based platform exhibited a color focusing effect where localized enzymatic reactions and eventually analyte detection can occur.[66]

Recently, novel colorimetric microfluidic assays have been fabricated. In this category belong the pH change-based colorimetric PADs that have been developed for chronic kidney disease biomarker detection down to the millimolar level.[67] Ammonium and urea have been detected via a colorimetric assay by the use of the bromothymol blue indicator using saliva samples as the case of study. The PAD platform consists of 32 detection areas and 4 layers of stacked filter paper supported by a laminated pouch, as reported in Figure 5C. The different paper layers have been utilized for the following distinct purposes: sample separation, reagent storage, and overall reaction stability. This configuration enables direct sample analysis without the necessity of pretreatment steps. However, the manual assembly of the sensing platform may lead to poor reproducibility, which can only be ensured with the use of replicates. A different PAD configuration, having a Mickey Mouse shape, for simultaneous glucose and total cholesterol in blood samples has been also developed, as shown in Figure 5D. The PAD configuration has been achieved with the use of a homemade knife plotter preparing more than 140 PADs in less than 10 min. The analytes were simultaneously detected within 15 min with HRP enzymatic activity by the color change of the ABTS substrate in the presence of H_2O_2. In this case, the filter paper enabled the precise and controlled reagent deposition, offering the advantage of simultaneous detection of two analytes. The stability of the reagents and the overall enzymatic reaction were enhanced by laminating the PAD. Also a paper color chart has been attached of the laminated PAD for semi-quantitative analysis with an LOD of 0.16 mM for glucose and 0.57 mM for total cholesterol.[68]

Additional possibilities toward the development of paper-based sensing architectures are based on the adoption of electrochemical transducers. ECSs have been widely applied to different fields like clinical, environmental, food, etc.[69,70] A main advantage related to the electrochemical measurements is due to the fact that colored/turbid matrices do not interfere with the detection. This feature is of high relevance in the field of diagnostics: in fact, the analysis of complex matrices like blood, serum, urine, and saliva often represents a limitation for colorimetric methods: the combination with paper-based substrates is also capable of creating a synergic boost, i.e.

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

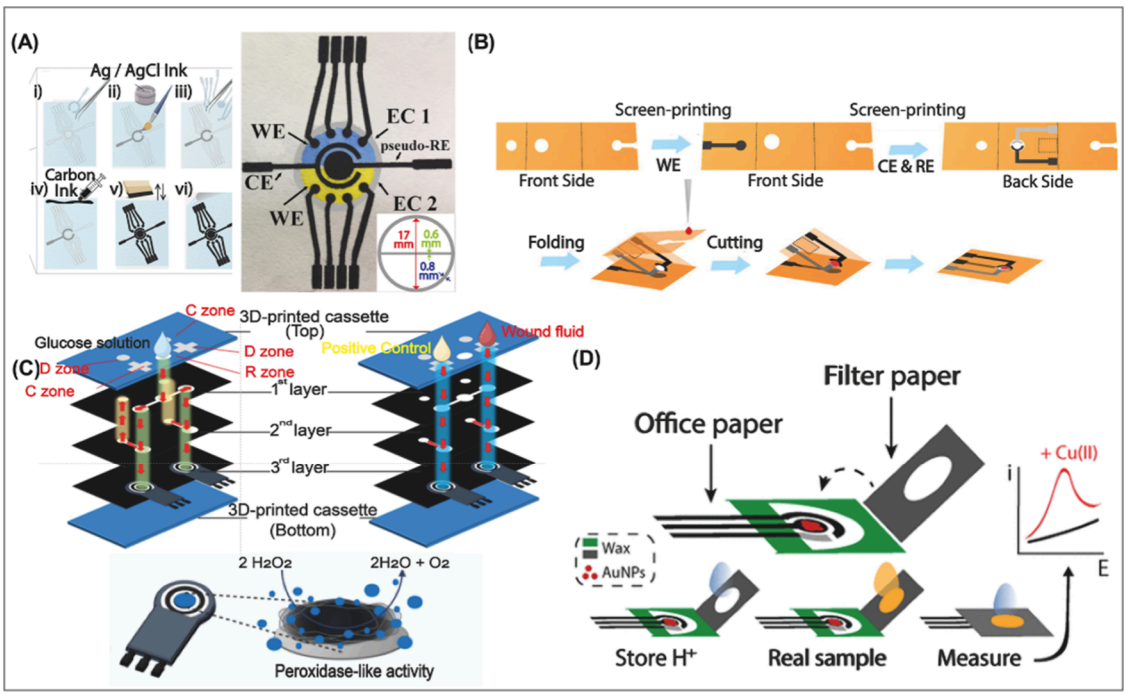


Figure 6. Example of EC sensing strategies with application to paper-based diagnostics. A) Paper-based ECSs for simultaneous multiplex detection of drugs components. Reprinted with permission from ref. [71]. Copyright 2019, Elsevier B.V. B) Electrochemical paper-based aptasensor, with 3D formation, for label-free and ultrasensitive detection of EGFR. Reprinted with permission from ref. [72]. Copyright 2020, Pubmed Central. Licensed under CCBY. C) Smartphone-based 3D origami paper-based electrochemical assay for myeloperoxidase activity wound infection assessment. Reprinted with permission from ref. [73]. Copyright 2024, Elsevier B.V. D) Novel paper-based electrochemical sensing platform for the detection of copper ions in real matrices. Reprinted with permission from ref. [74]. Copyright 2022, American Chemical Society.

removal of gross impurities. For instance, a paper-based multiplex electrochemical platform has been reported for the detection of paracetamol, caffeine, and ascorbic acid in drugs with a limit of detection (LOD) of 0.04 0.22, and 0.40 mM, respectively. This smart and flexible paper-based configuration (Figure 6A) enabled the simultaneous detection of three analytes on the same device, consisting of an array of working electrodes, and did not require electrode modification steps. The electrodes that have been printed on the filter paper make the device versatile and sustainable. Finally, the one-step wax barrier design allowed easy modification and handling of the diagnostic device.[71]

Also, 3D origami architectures are very common for developing ECSs because they can be used for reagent storage, preconcentration, and also for multiple reactions/interactions on the same sensor. For instance, an EC paper-based aptasensor for the detection of epidermal growth factor receptor (EGFR) in serum samples has been reported.[72] The electrode surface has been modified with an anti-EGFR aptamer, and the detection occurs through an antibody–antigen interaction with this label-free approach, yielding an LOD of 5 pg/mL. The 3D origami served as a pump enabling the sample flow through the sensor for analyte detection. Additionally, the integration of hydrophobic patterns into the origami configuration significantly decreased the required sample volume (Figure 6B). In the same context of device configuration, another 3D origami based on a smartphone-readout has been recently reported in the literature. The authors reported a portable, 3D origami paper-based EC sensor for wound infection monitoring (Figure 6C). Myeloperoxidase (MPO) activity was captured through immobilized MPO antibodies. Glucose oxidase has been used for H_2O_2 production, while a Prussian blue/MXene composite has been used for catalyzing H_2O_2 detection. In this example, the 3-layer origami which combines NC membrane and filter paper allowed reagent storage in the first and second layers and analyte detection via the paper-based electrodes on the last layer. The hydrophilic channels ensured the proper and controlled flow of all of the reagents through the origami layers. Finally, the last layer was responsible for the arrival of the analyte on the working electrode surface for the detection step. Considering all the above, this advantageous configuration can be exploited in low-resources areas.[73] Furthermore, another novel paper-based electrochemical platform has been reported exploiting the combination of office paper with screen-print electrodes and a filter paper disk for reagent storage, sample collection, and analyte preconcentration (Figure 6D). It should be noted that the mechanical stability of office paper has been powered by the porosity-based properties of filter paper, to obtain a superior sensing platform. As a result, an all-in-one 2D platform has been introduced, and it has been applied toward copper ion detection in serum samples, reaching an LOD of 4 ppb.[74]

The use of fluorescent molecules, conjugates, materials, or nanoparticles can improve the sensitivity of the POC devices in comparison to colorimetric assays providing sensitive analyte quantification. Portable readout equipment has also been integrated with fluorescent PADs to facilitate the performance of the measurements. An example of a selective fluorometric PAD has been reported for direct detection of histidine in urine samples with an LOD of 1.8 μM. Briefly, histidine reacted with o-phthalaldehyde (OPA), and this produces a

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

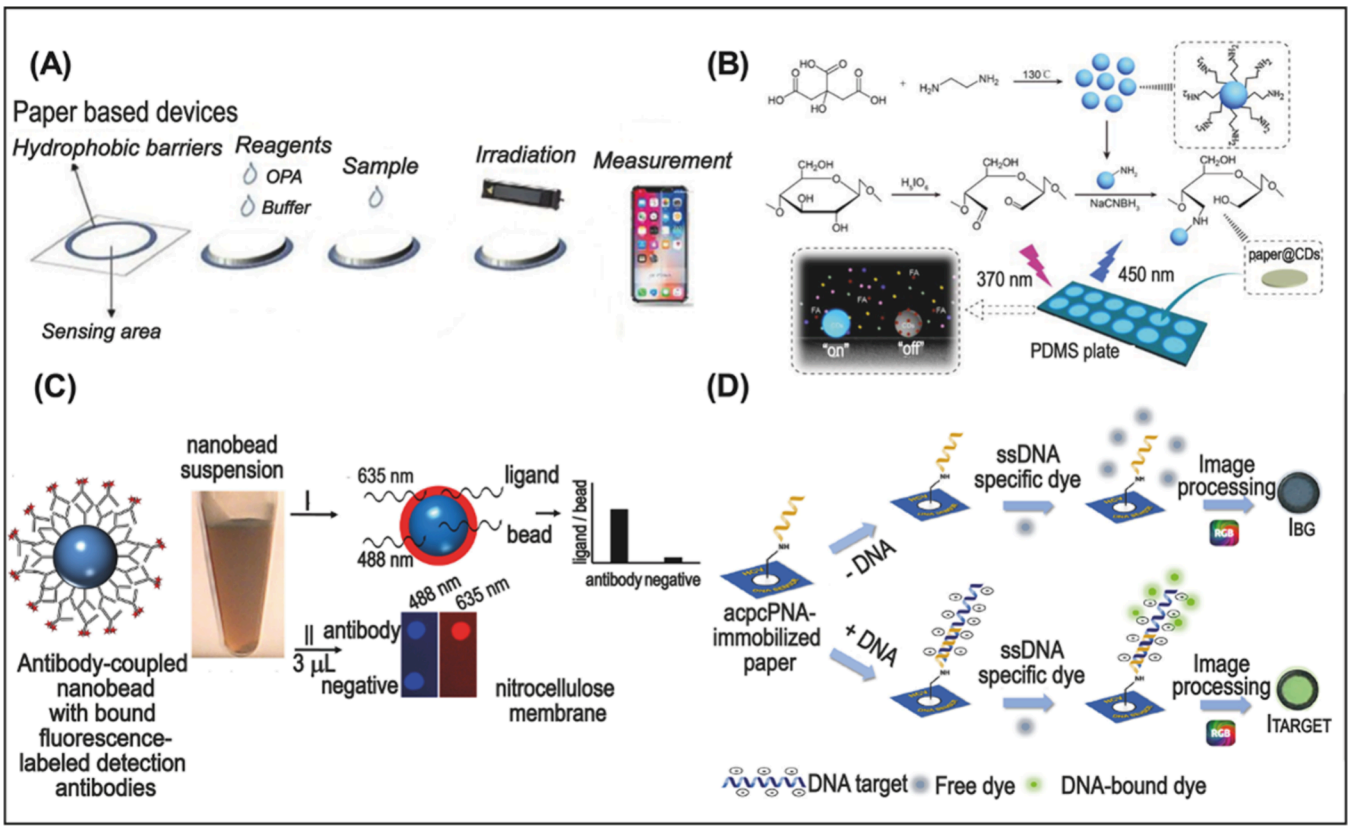


Figure 7. Example of fluorometric paper-based devices. A) Equipment-free, paper-based fluorometric platform for the detection of histidine. Reprinted with permission from ref. [75]. Copyright 2019, Elsevier B.V. B) Fluorometric paper-based device exploiting carbon dot nanoparticles for folic acid detection. Reprinted with permission from ref. [76]. Copyright 2020, Springer-Verlag GmbH Germany. C) Poly(methyl methacrylate) NP-based fluorescence LFA for rheumatoid arthritis antibody detection in serum. Reprinted with permission from ref. [77]. Copyright 2021, Elsevier B.V. D) Paper-based fluorescence device exploiting specific pyrrolidinyl peptide nucleic acid probes for hepatitis C virus cDNA detection. Reprinted with permission from ref. [78]. Copyright 2021, Elsevier B.V.

fluorescence emission following UV lamp radiation, as reported in Figure 7A. The filter paper used as a substrate was characterized by higher thickness and mass per area in comparison to other paper-based materials. These two specific advantageous properties of the filter paper used enabled homogeneous reagent deposition and assay stability.

In this research work, the paper substrate was used for reagent storage and also volume limitation with the exploitation of hydrophobic barriers, and the signal has been acquired through the use of a conventional smartphone.[75] Another approach of a simple pattern fluorometric paper-based device exploits carbon dots (CDs) nanoparticles (NPs) properties with the platform's surface functionalization for folic acid detection in urine down to 0.28 μM as the detection limit (Figure 7B). The sustainable paper-based substrate that was used for the CDs fluorophore anchoring was attached on the PDMS plate providing an ultimate cost-effective hybrid sensing platform. When folic acid has been present, it quenched the fluorescence of the CDs providing a "signal off" sensing device. Cellulose paper ensured the assay stability and reproducibility. Interestingly, the limitation of single-use POC devices is addressed with the use of this particular material, making the platform potentially reusable up to 5 times.[76]

Another approach of a fluorescence paper-based device based on the LFA principle was recently reported. This fluorescent and NP-based LFA has been introduced for rheumatoid arthritis (RA) antibodies detection in human serum with a calculated LOD of 0.4 U/mL. Poly(methyl methacrylate) NPs, that are biocompatible, were doped with fluorescence dye and labeled with a specific antibody for the RA biomarker (Figure 7C). The research group exploited the inexpensive LFA paper-based components by integrating them with a fluorescent assay. As a result, the limited sensitivity of the traditional LFA appeared to be improved. However, the main drawback of the method is represented by the need of readout equipment, more specifically a fluorescence scanner or stimulated emission depletion microscope.[77]

An additional example of a paper-based fluorescent sensor with a 2D architecture has been reported by Teengam et al.[78] The NC substrate was functionalized, through covalent bonds, with a specific pyrrolidinyl peptide-based nucleic acid probe. The device was developed for the detection of hepatitis C virus cDNA (single stranded) down to the pmol level, as reported in Figure 7D. The signal response has been obtained with a fluorescence camera connected to a smartphone. In this POC device example, an NC substrate was employed for reagent immobilization, and an absorbent pad was utilized to facilitate the washing step in the assay, leading to the subsequent detection of the target.

Chemiluminescent detection is also exploited in various assays because of the low background of chemiluminescence phenomena, which often represents an advantage for obtaining more sensitive architectures with lower limits of detection. The

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

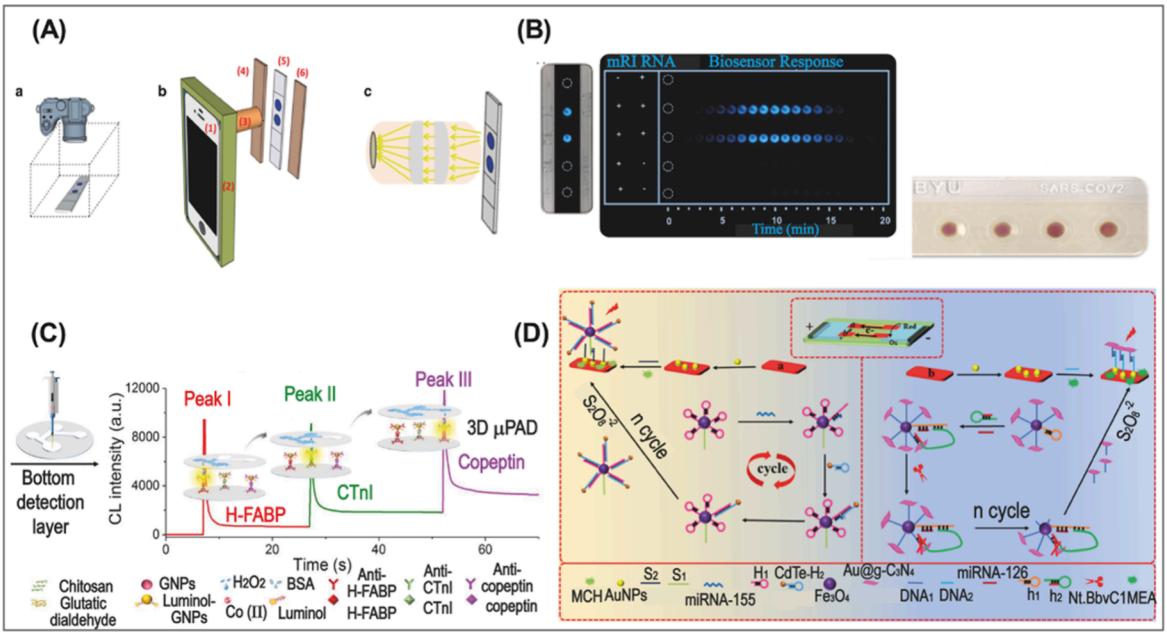


Figure 8. Example of chemiluminescent detection strategies in paper-based devices. A) Paper-based chemiluminometric assay for single nucleotide polymorphism genotyping in clinical samples. Reprinted with permission from ref. [79]. Copyright 2016, Springer-Verlag GmbH Germany. B) Paper-based toehold switch platform for bioluminescent detection of SARS-CoV-2 RNA in human saliva. Reprinted with permission from ref. [80]. Copyright 2021, Elsevier B.V. C) Multiplex microfluidic immunodevice for the simultaneous detection of acute myocardial infarction biomarkers. Reprinted with permission from ref. [81]. Copyright 2020, Elsevier B.V. D) Paper-based electrochemiluminescence platform for multiplex microRNA detection. Reprinted with permission from ref. [82]. Copyright 2020, American Chemical Society.

main limitations are represented by the use of ad hoc probes and the necessity of various washing steps to avoid nonspecific signals. However, the use of paper-based substrates could represent the right substrate for going beyond the state of the art. For instance, a paper-based chemiluminescent assay for single nucleotide polymorphisms in DNA isolated from clinical samples has been developed. The genotyping occurs within the primer extension reaction with the use of a specific polymerase that extends the primer only if it is fully complementary. Biotin molecules have been incorporated during the reaction so that the products have been detected from the streptavidin-HRP conjugate. When the chemiluminogenic substrate of the HRP has been added, the emitted light was captured by a smartphone (Figure 8A). In this case, all the properties of paper-based LFAs have been integrated with chemiluminescence to overcome the limitation of low sensitivity in the traditional LFA where detection occurs (mainly) with the naked eye. Paper-based materials served for reagent immobilization, washing steps, and control of the liquids flow during the whole assay. This was achieved through capillary action that ensured a continuous flow of the reagents.[79]

A plethora of diagnostic devices was developed during and after the COVID-19 pandemic. The following reported work is an example of this type of diagnostic platform (Figure 8B) for SARS-CoV-2 RNA detection in human saliva. Toehold switch riboregulators have been engineered to express the protein Nanoluc as a reporter. The signal has been generated just by the addition of the sample, and the light emitted has been captured by a digital camera. Herein, the paper material was used as an inexpensive substrate for the diagnostic device, and also it enabled long-term reagent storage.[80] PADs have also been combined with chemiluminescent strategies. Here has been presented an example of a multiplex microfluidic immunodevice for the simultaneous detection of acute myocardial infarction biomarkers down to the pg/mL concentration level. The developed device had a 3D-PAD formation that allowed dual amplification strategy with AuNPs and primary antibody conjugates and a Co(II) catalyst secondary antibody (Figure 8C). With the addition of H_2O_2, blue light was emitted and captured by a luminescence analyzer. This 3D-PAD consisted of two paper layers. The first patterned microfluidic layer served for sample addition and separation. The second layer, the detection layer, consisted of three detection areas. The above features enabled the simultaneous detection of the target molecules.[81]

Another important detection method is the electrochemiluminescence (ECL) strategy. ECL is called the phenomenon of the generated species on electrodes that triggers light emission. Recently, a representative example of an ECL platform for multiplex detection of microRNAs has been developed by Wang et al.[82] Two target microRNAs were simultaneously detected on this microfluidic platform. Quantum dots and AuNPs were used as dual ECL probes with a bipolar electrode strategy, as reported in Figure 8D. The specific hairpin probes, after the target has been recognized, hybridize with each other enabling target strand replacement and thus recycling of the target. In this work, the paper substrate was exploited for the fabrication of multiple hydrophilic channels; therefore, the fabricated multichannel paper-chip was suitable for multiple microRNA detection down to femtomolar levels. The multichannel paper-chip offered an additional benefit by ensuring the platform's proper function and eliminating the potential for false positive results.

Paper is a cost-effective, easy-to-use, versatile, and environmentally friendly material that can be used as a substrate for the preparation of sustainable POC devices. These analytical

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

devices have already proven, during the pandemic crisis, that they can facilitate the burden of health care units and hospitals. Sophisticated and smart device configuration combined with cost-effective paper-based materials is a valuable contribution in the field of diagnostics. Cutting edge technologies provide an unlimited option for paper-based POC fabrication regarding the usage of novel materials and assay design. As a result, these tools preserve the economy as well as the health system and, in some cases, can also expand patient life expectancy. These devices can also be accessible to resource-limited countries as they provide the possibility of multiple biomarker screening for affordable disease and therapy monitoring. While POC devices provide numerous benefits and facilitate access to personalized medical care and treatment, the downside is that the associated waste products pose an environmental burden, and they need appropriate management. It is important to note that the usage of paper-based sensors can offer environmental advantages because of their ecofriendliness and sustainability, but the overall environmental impact can vary based on specific materials, manufacturing processes, and disposal methods.

The advantages of paper allow the development of a paper-based POC device that complies with the ASSURED criteria and eventually will be used near the patient. As discussed above, the emerging tools that exist lead to many different shapes of patterning on paper-based substrates. The hydrophobic barriers, which form hydrophilic areas, can monitor the flow rate and the volume of reagents/samples. They also provide the possibility of adding prestored reagents, on paper substrates, even during the analysis. Additionally, the analysis of biomarkers in complex biological fluids is facilitated by paper stacking and folding thanks to the feasibility of sample separation and isolation within the 3D paper networks.

The limitations of paper-based substrates in various sensing strategies such as colorimetric detection might be low LODs, narrow linear range, usually semiquantitative results, etc. These challenges can be addressed by incorporating paper substrates into more sensitive sensing strategies such as electrochemistry and chemiluminescence.

Paper-based materials can be also combined with advanced, new nanomaterials and portable imagers/detectors to further improve the analysis of emerging important biomarkers. The above enhancements are crucial for the implementation of POC devices in real clinical practice and routine.

Paper-based materials can be also combined with advanced, new nanomaterials and portable imagers/detectors to further improve the analysis of emerging important biomarkers. The above enhancements are crucial for the implementation of POC devices in real clinical practice and routine.

Paper-based diagnostic tools are portable and simple, and usually no power consumption is needed. The previous characteristics make POC tools attractive for numerous applications. However, there are some future challenges and limitations that need to be addressed for developing new emerging POC devices. Some of the limitations that exist are the mechanical strength of the paper-based POC devices, the reagent evaporation, the assay sensitivity and detection accuracy, the fabrication variabilities between the same POC devices that can affect the reproducibility, the analysis of complex biological fluids, etc. Among the various configurations and surface functionalization of paper-substrates that are exploited to overcome the above limitations, artificial intelligence and computational simulations[83,84] can also contribute to POC devices' improvement. These approaches can optimize the POC device design. Moreover algorithm training for results interpretation and multiplex pattern recognition can facilitate the diagnostic results and improve the detection capability. Considering the above, the integration of advanced paper substrates and artificial intelligence technologies to diagnostics will introduce devices that will be used in real clinical applications, providing more robust and precise results.

AUTHOR INFORMATION

Corresponding Author

Stefano Cinti − *Department of Pharmacy, University of Naples "Federico II", Naples 80131, Italy; BAT Center - Interuniversity Center for Studies on Bioinspired Agro-Environmental Technology, University of Naples "Federico II", Naples 80055, Italy;* orcid.org/0000-0002-8274-7452; Email: stefano.cinti@unina.it

Author

Panagiota M. Kalligosfyri − *Department of Pharmacy, University of Naples "Federico II", Naples 80131, Italy;* orcid.org/0000-0002-8287-059X

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmaterialslett.3c01474

Author Contributions

CRediT: **Panagiota M Kalligosfyri** conceptualization, data curation, resources, writing-original draft; **Stefano Cinti** conceptualization, funding acquisition, project administration, supervision, writing-original draft.

Notes

The authors declare no competing financial interest.

Biographies

Panagiota Kalligosfyri received her BSc degree (2011-2015) in Chemistry and her MSc degree (2015-2017) in Analytical Chemistry and Nanotechnology from the Department of Chemistry of Patras University. She obtained her Ph.D. from the same University in 2021. She has 8 years of experience as a teaching assistant in the preparation and performance of weekly undergraduate laboratory exercises. She was a Postdoctoral Researcher for 2 years in the Laboratory of Instrumental Chemical Analysis (Department of Chemistry, University of Patras). Her current position is a Postdoctoral researcher in the uninanobiosensors group in the Department of Pharmacy of Naples Federico II University. Her research focuses on the design, construction, and validation of novel biomedical sensing devices for diagnosis, prognosis, and monitoring of diseases.

Stefano Cinti is an Associate Professor at the Department of Pharmacy, University of Naples "Federico II". He obtained a Ph.D. in Chemical Sciences in 2016 in the group headed by Prof. Giuseppe Palleschi at the University of Rome "Tor Vergata". He leads the uninanobiosensors Lab (uninanobiosensors.com) at the University of Naples "Federico II", and his research interests include the development of electrochemical sensors, portable diagnostics, paper-based devices, and nanomaterials. During his research activity, he had

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

the opportunity to spend a period abroad in Finland, U.K., the U.S., Germany, and Spain. He has published more than 80 papers in peer-reviewed journals, with an H-index of 35 and > 4000 citations. Among all the prizes and certificates, in 2022, he was awarded the early career award from the International Society of Electrochemistry in Analytical Electrochemistry; in 2022, he was awarded the Biosensors 2022 Young Investigator Award; in 2023, he was awarded a Sensor Division Early Career Award by ECS; and in 2022, he was recognized as the World's Top 2% Scientists. He is the coordinator of the Chemical Cultural Diffusion group of the Italian Chemical Society. He is the Chair of AMYC-BIOMED, a multidisciplinary conference for young chemists in the biomedical sciences. He is very active in communicating science to nonspecialized audiences through TV shows, radio, and magazine.

■ ACKNOWLEDGMENTS

The research leading to these results received funding from AIRC under MFAG 2022−ID. 27586 project—P.I. (S.C.). P.M.K. acknowledges AIRC for funding her postdoctoral Fellowship.

■ ABBREVIATIONS

POC = Point-of-care
LFA = Lateral flow assay
PADs = Microfluidic paper-based analytical devices
3D = Three-dimensional
ELISA = Enzyme-linked immunosorbent assay
PCR = Polymerase chain reaction
ECS = Electrochemical sensor
NC = Nitrocellulose membrane
SEM = Scanning electron microscopy
2D = Two-dimensional
HRP = Horse radish peroxidase
PDMS = Polydimethylsiloxane
TZ = Test zone
CZ = Control zone
LOD = Limit of detection
EGFR = Epidermal growth factor receptor
MPO = Myeloperoxidase
AuNPs = Gold nanoparticles
CDs = Carbon dots
NPs = Nanoparticles
RA = Rheumatoid arthritis
ECL = Electrochemiluminescence

■ REFERENCES

(1) Naseri, M.; Ziora, Z. M.; Simon, G. P.; Batchelor, W. ASSURED-compliant Point-of-care Diagnostics for the Detection of Human Viral Infections. *Rev. Med. Virol* **2022**, *32* (2), e2263.
(2) Scott, S.; Ali, Z. Fabrication Methods for Microfluidic Devices: An Overview. *Micromachines (Basel)* **2021**, *12* (3), 319.
(3) Müller, R. H.; Clegg, D. L. Automatic Paper Chromatography. *Anal. Chem.* **1949**, *21* (9), 1123−1125.
(4) Leuvering, J. H. W.; Thal, P. J. H. M.; Waart, M. van der; Schuurs, A. H. W. M. Sol Particle Immunoassay (SPIA). *J. Immunoassay* **1980**, *1* (1), 77−91.
(5) OLSZYNKO-GRYN, J. Thin Blue Lines: Product Placement and the Drama of Pregnancy Testing in British Cinema and Television. *British Journal for the History of Science* **2017**, *50* (3), 495−520.
(6) Martinez, A. W.; Phillips, S. T.; Butte, M. J.; Whitesides, G. M. Patterned Paper as a Platform for Inexpensive, Low-Volume, Portable Bioassays. *Angew. Chem., Int. Ed.* **2007**, *46* (8), 1318−1320.
(7) Martinez, A. W.; Phillips, S. T.; Whitesides, G. M. Three-Dimensional Microfluidic Devices Fabricated in Layered Paper and Tape. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (50), 19606−19611.
(8) Das, S.; Gagandeep; Bhatia, R. Paper-Based Microfluidic Devices: Fabrication, Detection, and Significant Applications in Various Fields. *Rev. Anal Chem.* **2022**, *41* (1), 112−136.
(9) Liu, H.; Crooks, R. M. Three-Dimensional Paper Microfluidic Devices Assembled Using the Principles of Origami. *J. Am. Chem. Soc.* **2011**, *133* (44), 17564−17566.
(10) Zhang, T.; Ding, F.; Yang, Y.; Zhao, G.; Zhang, C.; Wang, R.; Huang, X. Research Progress and Future Trends of Microfluidic Paper-Based Analytical Devices in In-Vitro Diagnosis. *Biosensors (Basel)* **2022**, *12* (7), 485.
(11) Jafry, A. T.; Lim, H.; Lee, J. Basic Paper-Based Microfluidics/Electronics Theory; Lee, J. H., Eds.; Springer: Singapore, 2021; Chapter 2, pp 7−39.
(12) Wang, L.; Xu, T. Editorial: Integrated Point-of-Care Testing (POCT) Systems: Recent Progress and Applications. *Front Bioeng Biotechnol* **2022**, *10*, 851675.
(13) Carstens, F.; Gamelas, J. A. F.; Schabel, S. Engineering Microfluidic Papers: Determination of Fibre Source and Paper Sheet Properties and Their Influence on Capillary-Driven Fluid Flow. *Cellulose* **2017**, *24* (1), 295−309.
(14) Then, W. L.; Garnier, G. Paper Diagnostics in Biomedicine. *Rev. Anal Chem.* **2013**, *32* (4), 269.
(15) Cytiva. https://www.cytivalifesciences.com/en/us/solutions/lab-filtration/knowledge-center/a-guide-to-whatman-filter-paper-grades (accessed 2023-02-21).
(16) Park, J.; Shin, J.; Park, J.-K. Experimental Analysis of Porosity and Permeability in Pressed Paper. *Micromachines (Basel)* **2016**, *7* (3), 48.
(17) Kar, S.; Das, S. S.; Laha, S.; Chakraborty, S. Microfluidics on Porous Substrates Mediated by Capillarity-Driven Transport. *Ind. Eng. Chem. Res.* **2020**, *59* (9), 3644−3654.
(18) Hassan, S.; Tariq, A.; Noreen, Z.; Donia, A.; Zaidi, S. Z. J.; Bokhari, H.; Zhang, X. Capillary-Driven Flow Microfluidics Combined with Smartphone Detection: An Emerging Tool for Point-of-Care Diagnostics. *Diagnostics* **2020**, *10* (8), 509.
(19) Fridley, G. E.; Holstein, C. A.; Oza, S. B.; Yager, P. The Evolution of Nitrocellulose as a Material for Bioassays. *MRS Bull.* **2013**, *38* (4), 326−330.
(20) Cinti, S.; Moscone, D.; Arduini, F. Preparation of Paper-Based Devices for Reagentless Electrochemical (Bio)Sensor Strips. *Nat. Protoc* **2019**, *14* (8), 2437−2451.
(21) Chagas, C. L. S.; Costa Duarte, L.; Lobo-Júnior, E. O.; Piccin, E.; Dossi, N.; Coltro, W. K. T. Hand Drawing of Pencil Electrodes on Paper Platforms for Contactless Conductivity Detection of Inorganic Cations in Human Tear Samples Using Electrophoresis Chips. *Electrophoresis* **2015**, *36* (16), 1837−1844.
(22) Cinti, S.; De Lellis, B.; Moscone, D.; Arduini, F. Sustainable Monitoring of Zn(II) in Biological Fluids Using Office Paper. *Sens Actuators B Chem.* **2017**, *253*, 1199−1206.
(23) Moccia, M.; Caratelli, V.; Cinti, S.; Pede, B.; Avitabile, C.; Saviano, M.; Imbriani, A. L.; Moscone, D.; Arduini, F. Paper-Based Electrochemical Peptide Nucleic Acid (PNA) Biosensor for Detection of MiRNA-492: A Pancreatic Ductal Adenocarcinoma Biomarker. *Biosens Bioelectron* **2020**, *165*, 112371.
(24) Caratelli, V.; Ciampaglia, A.; Guiducci, J.; Sancesario, G.; Moscone, D.; Arduini, F. Precision Medicine in Alzheimer's Disease: An Origami Paper-Based Electrochemical Device for Cholinesterase Inhibitors. *Biosens Bioelectron* **2020**, *165*, 112411.
(25) Evans, E.; Gabriel, E. F. M.; Coltro, W. K. T.; Garcia, C. D. Rational Selection of Substrates to Improve Color Intensity and Uniformity on Microfluidic Paper-Based Analytical Devices. *Analyst* **2014**, *139* (9), 2127−2132.
(26) Zhou, S.; Tu, D.; Liu, Y.; You, W.; Zhang, Y.; Zheng, W.; Chen, X. Ultrasensitive Point-of-Care Test for Tumor Marker in Human Saliva Based on Luminescence-Amplification Strategy of Lanthanide Nanoprobes. *Advanced Science* **2021**, *8* (5), 2002657.

(27) Yuan, C.; Zhao, Y.; Xi, X.; Chen, Y. Non-Destructive Screening of Sodium Metabisulfite Residue on Shrimp by SERS with Copy Paper Loaded with AgNP. *Biosensors (Basel)* **2023**, *13* (6), 575.
(28) Pelton, R. Bioactive Paper Provides a Low-Cost Platform for Diagnostics. *TrAC Trends in Analytical Chemistry* **2009**, *28* (8), 925−942.
(29) Kong, F.; Hu, Y. F. Biomolecule Immobilization Techniques for Bioactive Paper Fabrication. *Anal Bioanal Chem.* **2012**, *403* (1), 7−13.
(30) Cimmino, W.; Migliorelli, D.; Singh, S.; Miglione, A.; Generelli, S.; Cinti, S. Design of a Printed Electrochemical Strip towards MiRNA-21 Detection in Urine Samples: Optimization of the Experimental Procedures for Real Sample Application. *Anal Bioanal Chem.* **2023**, *415* (18), 4511−4520.
(31) Patari, S.; Mahapatra, P. S. Liquid Wicking in a Paper Strip: An Experimental and Numerical Study. *ACS Omega* **2020**, *5* (36), 22931−22939.
(32) Parolo, C.; Sena-Torralba, A.; Bergua, J. F.; Calucho, E.; Fuentes-Chust, C.; Hu, L.; Rivas, L.; Álvarez-Diduk, R.; Nguyen, E. P.; Cinti, S.; Quesada-González, D.; Merkoçi, A. Tutorial: Design and Fabrication of Nanoparticle-Based Lateral-Flow Immunoassays. *Nat. Protoc* **2020**, *15* (12), 3788−3816.
(33) Kalogianni, D. P. Lateral Flow Assays for Food Authentication; Pudake, R. N., Jain, U., Kole, C., Eds.; Springer: Cham, 2021; pp 343−363.
(34) Sher, M.; Zhuang, R.; Demirci, U.; Asghar, W. Paper-Based Analytical Devices for Clinical Diagnosis: Recent Advances in the Fabrication Techniques and Sensing Mechanisms. *Expert Rev. Mol. Diagn* **2017**, *17* (4), 351−366.
(35) Yamada, K.; Henares, T. G.; Suzuki, K.; Citterio, D. Paper-Based Inkjet-Printed Microfluidic Analytical Devices. *Angew. Chem., Int. Ed.* **2015**, *54* (18), 5294−5310.
(36) Li, X.; Tian, J.; Garnier, G.; Shen, W. Fabrication of Paper-Based Microfluidic Sensors by Printing. *Colloids Surf. B Biointerfaces* **2010**, *76* (2), 564−570.
(37) Lam, T.; Devadhasan, J. P.; Howse, R.; Kim, J. A Chemically Patterned Microfluidic Paper-Based Analytical Device (C-MPAD) for Point-of-Care Diagnostics. *Sci. Rep* **2017**, *7* (1), 1188.
(38) Zhang, Y.; Liu, J.; Wang, H.; Fan, Y. Laser-Induced Selective Wax Reflow for Paper-Based Microfluidics. *RSC Adv.* **2019**, *9* (20), 11460−11464.
(39) Bagheri, N.; Cinti, S.; Caratelli, V.; Massoud, R.; Saraji, M.; Moscone, D.; Arduini, F. A 96-Well Wax Printed Prussian Blue Paper for the Visual Determination of Cholinesterase Activity in Human Serum. *Biosens Bioelectron* **2019**, *134*, 97−102.
(40) Ng, J. S.; Hashimoto, M. Fabrication of Paper Microfluidic Devices Using a Toner Laser Printer. *RSC Adv.* **2020**, *10* (50), 29797−29807.
(41) Suvanasuthi, R.; Chimnaronk, S.; Promptmas, C. 3D Printed Hydrophobic Barriers in a Paper-Based Biosensor for Point-of-Care Detection of Dengue Virus Serotypes. *Talanta* **2022**, *237*, 122962.
(42) Chiang, C.-K.; Kurniawan, A.; Kao, C.-Y.; Wang, M.-J. Single Step and Mask-Free 3D Wax Printing of Microfluidic Paper-Based Analytical Devices for Glucose and Nitrite Assays. *Talanta* **2019**, *194*, 837−845.
(43) Amatatongchai, M.; Thimoonnee, S.; Somnet, K.; Chairam, S.; Jarujamrus, P.; Nacapricha, D.; Lieberzeit, P. A. Origami 3D-Microfluidic Paper-Based Analytical Device for Detecting Carbaryl Using Mesoporous Silica-Platinum Nanoparticles with a Molecularly Imprinted Polymer Shell. *Talanta* **2023**, *254*, 124202.
(44) Sun, S.; Luo, J.; Zhu, Y.; Kong, F.; Mao, G.; Ming, T.; Xing, Y.; Liu, J.; Dai, Y.; Yan, S.; Yang, Y.; Cai, X. Multifunctional Self-Driven Origami Paper-Based Integrated Microfluidic Chip to Detect CRP and PAB in Whole Blood. *Biosens Bioelectron* **2022**, *208*, 114225.
(45) Gong, M. M.; Sinton, D. Turning the Page: Advancing Paper-Based Microfluidics for Broad Diagnostic Application. *Chem. Rev.* **2017**, *117* (12), 8447−8480.
(46) Sun, K.; Fan, Y.; Hebda, M.; Zhang, Y. Origami Microfluidics: A Review of Research Progress and Biomedical Applications. *Biomedical Materials & Devices* **2023**, *1*, 388.
(47) Colozza, N.; Caratelli, V.; Moscone, D.; Arduini, F. Origami Paper-Based Electrochemical (Bio)Sensors: State of the Art and Perspective. *Biosensors (Basel)* **2021**, *11* (9), 328.
(48) Wang, K.; Yang, J.; Xu, H.; Cao, B.; Qin, Q.; Liao, X.; Wo, Y.; Jin, Q.; Cui, D. Smartphone-Imaged Multilayered Paper-Based Analytical Device for Colorimetric Analysis of Carcinoembryonic Antigen. *Anal Bioanal Chem.* **2020**, *412* (11), 2517−2528.
(49) Yao, Z.; Coatsworth, P.; Shi, X.; Zhi, J.; Hu, L.; Yan, R.; Güder, F.; Yu, H.-D. Paper-Based Sensors for Diagnostics, Human Activity Monitoring, Food Safety and Environmental Detection. *Sensors & Diagnostics* **2022**, *1* (3), 312−342.
(50) Aragaw, T. A.; Mekonnen, B. A. Understanding Disposable Plastics Effects Generated from the PCR Testing Labs during the COVID-19 Pandemic. *Journal of Hazardous Materials Advances* **2022**, *7*, 100126.
(51) Mittal, M.; Mittal, D.; Aggarwal, N. K. Plastic Accumulation during COVID-19: Call for Another Pandemic; Bioplastic a Step towards This Challenge? *Environmental Science and Pollution Research* **2022**, *29* (8), 11039−11053.
(52) Khoo, K. S.; Ho, L. Y.; Lim, H. R.; Leong, H. Y.; Chew, K. W. Plastic Waste Associated with the COVID-19 Pandemic: Crisis or Opportunity? *J. Hazard Mater.* **2021**, *417*, 126108.
(53) Hosseini, E. S.; Dervin, S.; Ganguly, P.; Dahiya, R. Biodegradable Materials for Sustainable Health Monitoring Devices. *ACS Appl. Bio Mater.* **2021**, *4* (1), 163−194.
(54) Piro, B.; Tran, H. V.; Thu, V. T. Sensors Made of Natural Renewable Materials: Efficiency, Recyclability or Biodegradability—The Green Electronics. *Sensors* **2020**, *20* (20), 5898.
(55) Kishnani, V.; Park, S.; Nakate, U. T.; Mondal, K.; Gupta, A. Nano-Functionalized Paper-Based IoT Enabled Devices for Point-of-Care Testing: A Review. *Biomed Microdevices* **2022**, *24* (1), 2.
(56) Sudheshwar, A.; Malinverno, N.; Hischier, R.; Nowack, B.; Som, C. The Need for Design-for-Recycling of Paper-Based Printed Electronics - a Prospective Comparison with Printed Circuit Boards. *Resour Conserv Recycl* **2023**, *189*, 106757.
(57) Ahamed, A.; Ge, L.; Zhao, K.; Veksha, A.; Bobacka, J.; Lisak, G. Environmental Footprint of Voltammetric Sensors Based on Screen-Printed Electrodes: An Assessment towards "Green" Sensor Manufacturing. *Chemosphere* **2021**, *278*, 130462.
(58) Carvalhal, R. F.; Simão Kfouri, M.; de Oliveira Piazetta, M. H.; Gobbi, A. L.; Kubota, L. T. Electrochemical Detection in a Paper-Based Separation Device. *Anal. Chem.* **2010**, *82* (3), 1162−1165.
(59) Bagheri, N.; Cinti, S.; Nobile, E.; Moscone, D.; Arduini, F. Multi-Array Wax Paper-Based Platform for the Pre-Concentration and Determination of Silver Ions in Drinking Water. *Talanta* **2021**, *232*, 122474.
(60) Satarpai, T.; Shiowatana, J.; Siripinyanond, A. Paper-Based Analytical Device for Sampling, on-Site Preconcentration and Detection of Ppb Lead in Water. *Talanta* **2016**, *154*, 504−510.
(61) J Shetty, P. The Evolution of DNA Extraction Methods. *Am. J. Biomed Sci. Res.* **2020**, *8* (1), 39−45.
(62) Shetty, P. J. The Evolution of DNA Extraction Methods. *Am. J. Biomed Sci. Res.* **2020**, *8* (1), 39−45.
(63) Puiu, M.; Mirceski, V.; Bala, C. Paper-Based Diagnostic Platforms and Devices. *Curr. Opin Electrochem* **2021**, *27*, 100726.
(64) Land, K. J.; Smith, S.; Peeling, R. W. Unmet Diagnostics Needs for the Developing World. In *Paper-based Diagnostics*; Land, K., Eds.; Springer International Publishing: Cham, 2019; pp 1−21.
(65) Lee, J.; Kim, H.; Heo, Y.; Yoo, Y. K.; Han, S. Il; Kim, C.; Hur, D.; Kim, H.; Kang, J. Y.; Lee, J. H. Enhanced Paper-Based ELISA for Simultaneous EVs/Exosome Isolation and Detection Using Streptavidin Agarose-Based Immobilization. *Analyst* **2020**, *145* (1), 157−164.
(66) Jang, H.; Park, J.-H.; Oh, J.; Kim, K.; Kim, M.-G. Advanced Colorimetric Paper Sensors Using Color Focusing Effect Based on Asymmetric Flow of Fluid. *ACS Sens* **2019**, *4* (4), 1103−1108.
(67) Ferreira, F. T. S. M.; Mesquita, R. B. R.; Rangel, A. O. S. S. On-Hand Tool for Ammonium and Urea Determination in Saliva to

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198

Monitor Chronic Kidney Disease - Design of a Couple of Microfluidic Paper-Based Devices. *Microchemical Journal* **2023**, *193*, 109102.
(68) Prakobkij, A.; Sukapanon, S.; Chunta, S.; Jarujamrus, P. Mickey Mouse-Shaped Laminated Paper-Based Analytical Device in Simultaneous Total Cholesterol and Glucose Determination in Whole Blood. *Anal. Chim. Acta* **2023**, *1263*, 341303.
(69) Miglione, A.; Lorenzo, R. Di; Grumetto, L.; Spinelli, M.; Amoresano, A.; Laneri, S.; Cinti, S. An Integrated Electrochemical Platform Empowered by Paper for Fast Nickel Detection in Cosmetics. *Electrochim. Acta* **2022**, *434*, 141332.
(70) Cioffi, A.; Mancini, M.; Gioia, V.; Cinti, S. Office Paper-Based Electrochemical Strips for Organophosphorus Pesticide Monitoring in Agricultural Soil. *Environ. Sci. Technol.* **2021**, *55* (13), 8859−8865.
(71) de Oliveira, T. R.; Fonseca, W. T.; de Oliveira Setti, G.; Faria, R. C. Fast and Flexible Strategy to Produce Electrochemical Paper-Based Analytical Devices Using a Craft Cutter Printer to Create Wax Barrier and Screen-Printed Electrodes. *Talanta* **2019**, *195*, 480−489.
(72) Wang, Y.; Sun, S.; Luo, J.; Xiong, Y.; Ming, T.; Liu, J.; Ma, Y.; Yan, S.; Yang, Y.; Yang, Z.; Reboud, J.; Yin, H.; Cooper, J. M.; Cai, X. Low Sample Volume Origami-Paper-Based Graphene-Modified Aptasensors for Label-Free Electrochemical Detection of Cancer Biomarker-EGFR. *Microsyst Nanoeng* **2020**, *6* (1), 32.
(73) Permpoka, K.; Purinai, P.; Cheerasiri, C.; Rojpalakorn, W.; Nilaratanakul, V.; Laiwattanapaisal, W. Smartphone-Enabled 3D Origami-Fluidic Paper-Based Electrochemical Detection of Myeloperoxidase Activity for Assessing Wound Infection. *Sens Actuators B Chem.* **2024**, *398*, 134712.
(74) Miglione, A.; Spinelli, M.; Amoresano, A.; Cinti, S. Sustainable Copper Electrochemical Stripping onto a Paper-Based Substrate for Clinical Application. *ACS Measurement Science Au* **2022**, *2* (2), 177−184.
(75) Tarara, M.; Tsiasioti, A.; Tzanavaras, P. D.; Tsogas, G. Z. Development of an Equipment Free Paper Based Fluorimetric Method for the Selective Determination of Histidine in Human Urine Samples. *Talanta* **2022**, *249*, 123685.
(76) Li, W.; Zhang, X.; Miao, C.; Li, R.; Ji, Y. Fluorescent Paper-Based Sensor Based on Carbon Dots for Detection of Folic Acid. *Anal Bioanal Chem.* **2020**, *412* (12), 2805−2813.
(77) Schmidt, C.; Borcherding, H.; Thiele, T.; Schedler, U.; Werner, F.; Rödiger, S.; Roggenbuck, D.; Schierack, P. Fluorescence-Encoded Poly(Methyl Metharcylate) Nanoparticles for a Lateral Flow Assay Detecting IgM Autoantibodies in Rheumatoid Arthritis. *Anal. Biochem.* **2021**, *633*, 114389.
(78) Teengam, P.; Nisab, N.; Chuaypen, N.; Tangkijvanich, P.; Vilaivan, T.; Chailapakul, O. Fluorescent Paper-Based DNA Sensor Using Pyrrolidinyl Peptide Nucleic Acids for Hepatitis C Virus Detection. *Biosens Bioelectron* **2021**, *189*, 113381.
(79) Spyrou, E. M.; Kalogianni, D. P.; Tragoulias, S. S.; Ioannou, P. C.; Christopoulos, T. K. Digital Camera and Smartphone as Detectors in Paper-Based Chemiluminometric Genotyping of Single Nucleotide Polymorphisms. *Anal Bioanal Chem.* **2016**, *408* (26), 7393−7402.
(80) Hunt, J. P.; Zhao, E. L.; Free, T. J.; Soltani, M.; Warr, C. A.; Benedict, A. B.; Takahashi, M. K.; Griffitts, J. S.; Pitt, W. G.; Bundy, B. C. Towards Detection of SARS-CoV-2 RNA in Human Saliva: A Paper-Based Cell-Free Toehold Switch Biosensor with a Visual Bioluminescent Output. *N Biotechnol* **2022**, *66*, 53−60.
(81) Li, F.; Guo, L.; Hu, Y.; Li, Z.; Liu, J.; He, J.; Cui, H. Multiplexed Chemiluminescence Determination of Three Acute Myocardial Infarction Biomarkers Based on Microfluidic Paper-Based Immunodevice Dual Amplified by Multifunctionalized Gold Nanoparticles. *Talanta* **2020**, *207*, 120346.
(82) Wang, F.; Liu, Y.; Fu, C.; Li, N.; Du, M.; Zhang, L.; Ge, S.; Yu, J. Paper-Based Bipolar Electrode Electrochemiluminescence Platform for Detection of Multiple MiRNAs. *Anal. Chem.* **2021**, *93* (3), 1702−1708.
(83) Rath, D.; Toley, B. J. Modeling-Guided Design of Paper Microfluidic Networks: A Case Study of Sequential Fluid Delivery. *ACS Sens* **2021**, *6* (1), 91−99.
(84) Gasperino, D.; Baughman, T.; Hsieh, H. V.; Bell, D.; Weigl, B. H. Improving Lateral Flow Assay Performance Using Computational Modeling. *Annual Review of Analytical Chemistry* **2018**, *11* (1), 219−244.

https://doi.org/10.1021/acsmaterialslett.3c01474
ACS Materials Lett. 2024, 6, 1184−1198