

Review

Applications of 3D Printing in Paper-Based Devices for Biochemical and Environmental Analyses

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Abstract: Paper-based analytical devices (PADs) have received considerable attention due to their affordability, portability, and ease of use, making them suitable for on-site and point-of-care testing. The conventional fabrication of PADs has been explored for years to enhance their performance in sensing applications. Recently, to facilitate the automated production of PADs and support their practical use, 3D printing technology has been applied to fabricate PADs. Integrating 3D printing with PADs allows for precise fabrication without human intervention, improves fluidic control, and enables the development of complete devices. This technology allows for the printing of 3D parts that can be integrated with smartphones, making portable sensing applications of PADs more feasible. This mini-review highlights recent advancements in the application of 3D printing techniques to PADs. It focuses on their use in detecting biochemical analytes and monitoring environmental pollutants. Additionally, this review discusses the challenges and future possibilities of integrating 3D printing with PADs.

Keywords: paper-based analytical devices; 3D printing; biochemical analysis; environmental analysis



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1. Introduction

Microfluidic paper-based analytical devices (PADs) provide excellent opportunities for biochemical analysis and environmental monitoring. These devices are reported to meet the “ASSURED” criteria set by the World Health Organization (WHO) [1], which stands for affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users. As a result, PAD platforms have emerged as an ideal solution for cost-effective, flexible, and portable analysis. In conventional microfluidic PADs, microchannel networks with both hydrophobic and hydrophilic features can be fabricated on paper substrates using various methods, including photolithography [2–5], wax printing [6–9], screen printing [9–14], and inkjet printing [15–20]. The creation of hydrophilic channels and hydrophobic barriers on paper substrates enables the flow of samples through capillary action, allowing them to reach the detection zone without the need for external driving power, such as a pump. This capillary force ensures that the sample is distributed evenly within the detection zone, which enhances accuracy [21]. Following this, chemical reactions that occur result in a change in signal within the detection area. Due to their versatility in handling a variety of samples, including biological fluids (such as sweat, saliva, blood, tears, and urine), DNA, bacteria, pesticides, and heavy metals, PADs have demonstrated significant potential for analysis in biochemistry and environmental monitoring [22,23].

3D printing, basically additive manufacturing technology, is a one-step production method that enables the creation of complex designs using a variety of materials. This technology is increasingly influencing research in chemistry laboratories. 3D printing facilitates the production of useful devices, holders, and laboratory appliances with unique functions, properties, and designs. Particularly, in analytical chemistry, 3D printing technology has been extensively utilized in applications such as the separation and sensing capabilities of microfluidic devices [24–26], immunosensors [27], and biosensors [28–30]. It offers advantages over traditional manufacturing methods by enhancing personal efficiency in making custom parts. This technology bridges the gap between designers and manufacturers and makes it easier to create replacement parts for specific laboratory instruments. Furthermore, 3D printing technology paves the way for a more economical, facile, and time-effective fabrication process while also reducing chemical waste. The 3D printing techniques most relevant to the fabrication of microfluidic devices include stereolithography, multijet modeling, fused deposition modeling, and selective laser sintering, all of which have been discussed in notable review papers [31–34].

The integration of 3D printing technology with the fabrication of PADs is unique and offers several advantages, yet it has not been extensively reviewed. A very limited number of reviews have addressed this unique integration. Typically, 3D printing is used to create 3D parts that facilitate the development of complete PADs. For instance, it has been utilized in portable smartphone-based sensing platforms that detect analytes in biological fluids and pesticides through bio-chemiluminescence and fluorescence signals, respectively [35,36]. One of the earliest studies showcasing the application of 3D printing in PAD fabrication was conducted by He and coworkers in 2015, who used a desktop stereolithography (SL) 3D printer to create microfluidic PADs through dynamic mask photo curing [37]. This mini-review paper aims to summarize the recent research on the integration of 3D printing technology in PAD fabrication (Figure 1). We begin by discussing conventional methods for fabricating PADs to provide a foundational understanding of standard fabrication processes. Subsequently, we examine two primary categories for incorporating 3D printing into the fabrication of PADs: (1) creating hydrophilic channels using 3D-printed parts and (2) constructing fully developed portable PADs by printing 3D parts that facilitate biochemical analysis and environmental monitoring. We also compared the role of 3D printing technology in the fabrication of PADs and microfluidic devices to demonstrate the improvement of PADs following the integration of 3D printing technology. Finally, we address the challenges and future prospects of using 3D printing in the fabrication of PADs.

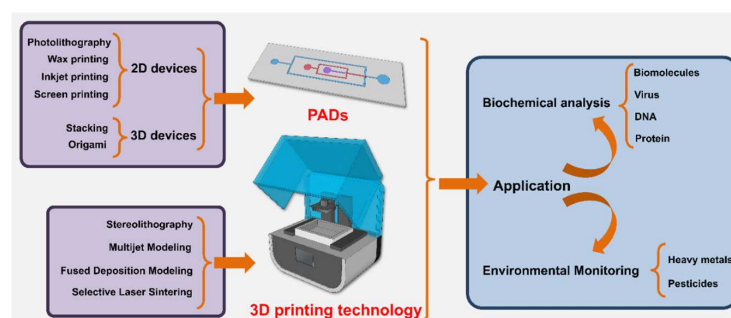


Figure 1. Schematic diagram illustrating the integration of PAD fabrication with 3D printing technology in biochemical and environmental analyses.

2. Fabrication of Paper-Based Analytical Devices (PADs)

The primary principle behind the fabrication of PADs is the formation of hydrophilic pathways on paper substrates by applying hydrophobic patterns. This can be achieved through the use of hydrophobic reagents or physical barriers [38]. PADs can be classified into two categories: 2D and 3D designs, based on their structural layouts. Com-

mon 2D PADs are fabricated using techniques such as photolithography [2–5], screen printing [9–14], wax printing [6–9], and inkjet printing [15–20]. In contrast, 3D PADs are constructed by stacking layers of patterned papers. These layers can be assembled together using adhesive tapes or sprays [39–41] or by employing a simple folding technique known as origami [42–47]. This origami technique not only allows for effective assembly but also minimizes the risk of contamination by simply folding the paper.

2.1. Fabrication of 2D PADs

The first research proposing the use of patterning paper to detect glucose and protein in urine was reported in 2007, utilizing a photolithography method (Figure 2A). In the conventional photolithography process, chromatography paper was immersed in SU-8 photoresist and subsequently baked to completely remove cyclopentanone. The paper was then exposed to UV light through a photomask and baked to cross-link the photoresist. Unpolymerized photoresist was removed by washing with propylene glycol monomethyl ether acetate and propan-2-ol, followed by an oxygen plasma treatment to enhance the hydrophilicity [3]. However, this fabrication process is time-consuming and labor-intensive and requires sophisticated instruments. To achieve a faster, more convenient, and simpler fabrication process, a method called fast lithographic activation of sheets (FLASH) was also developed. The FLASH approach could replace conventional photolithography without the need for a clean room or specialized facilities [4].

To simplify the complexity of the photolithography process, wax has been used to create hydrophobic barriers on porous paper. Wax printing is a straightforward and cost-effective method that can be performed using a wax printer [6–9,48]. In this process, the wax pattern is directly printed onto the paper surface, and when heated on a hot plate, the solid wax melts and penetrates the porous paper, forming hydrophobic barriers that define the hydrophilic areas, as illustrated in Figure 2B [6]. Wax patterning on paper can be accomplished through three methods: (1) using a wax pen for painting, (2) printing with an inkjet printer, and (3) using a wax printer [8]. These wax printing techniques are simple and do not require a clean room, chemicals, or specialized equipment.

Similar to wax printing, inkjet printing is considered as a time-saving and low-cost alternative to photolithography, requiring no special instruments. This technique not only allows us to create hydrophilic patterns but also to generate conductive patterns for fabricating electrodes by substituting the ink in the cartridge with conductive printing ink. Inkjet printing can create patterned channels and dispense the reagents needed for sensing applications [17,18,20,49]. For example, Abe K. et al. used a commercial inkjet printer to dispense sensing inks for the simultaneous determination of pH levels, total protein, and glucose concentration in urine, as shown in Figure 2C [49]. However, it is important to note that the cartridges and printers can be damaged by organic solvents, which have strong dissolving properties. Organic solvents such as hexane, heptane, or toluene that are used in printing solutions can cause significant damage to the inkjet printers [50].

Screen printing is a technique used to transfer ink onto a substrate via an ink-blocking stencil. This stencil creates areas of mesh that allow ink or other printable materials to be pushed through the mesh to form an image on the substrate [10]. Due to the versatility of the screening printing technique in the choice of stencil designs and patterning agents, this technique enables the creation of various functional microfluidic PADs. For instance, Dungchai et al. utilized wax and carbon as inks in their screen printing process to fabricate microfluidic PADs for the colorimetric and electrochemical detection of glucose and total iron in human serum samples [9]. On the other hand, Sammeenoi et al. employed polystyrene to create a 3D hydrophobic barrier that defined a hydrophilic analysis zone. Specifically, they prepared a polystyrene solution by dissolving it in toluene, which

was then passed through the screen and allowed to penetrate to the bottom of the paper, resulting in the formation of a hydrophobic area, as shown in Figure 2D [11].

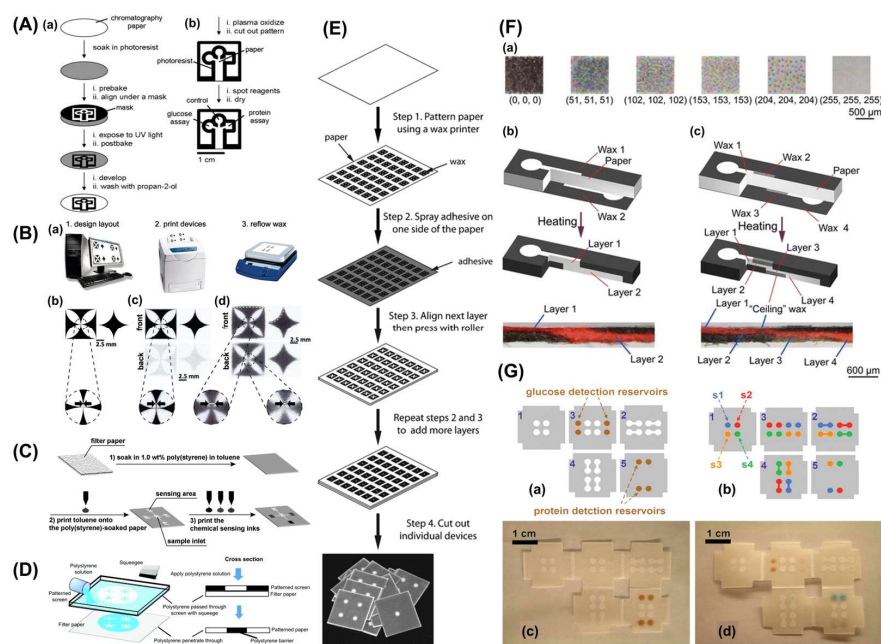


Figure 2. Demonstration of 2D PAD fabrication through (A–D) techniques. (A) Photolithography process (a) to pattern SU-8 photoresist into paper and (b) bioassays modified from patterned paper (reprinted with permission from ref [3]. Copyright 2007, WILEY). (B) Pattern hydrophobic barriers in paper by wax printing with (a) representation of three basic steps and (b–d) details for each step (reprinted with permission from ref [6]. Copyright 2009, American Chemical Society). (C) Inkjet printing (reprinted with permission from ref [49]. Copyright 2008, American Chemical Society). (D) Screen printing (reprinted with permission from ref [11]. Copyright 2014, Royal Society of Chemistry). Demonstration of 3D PAD fabrication through (E–G) techniques (reprinted with permission from ref [40,51]. Copyright 2014, Springer and Copyright 2014, Royal Society of Chemistry). (F) 3D PADs modified with (a) wax microdots printed at different RGB (or grayscale) levels contain (b) two-layer and (c) three-layer channels. (G) Origami technique illustrating (a) design of network used for assaying glucose and BSA, (b) location of four injected samples with (c) unfolded device containing assay reagents dried in detection reservoirs and (d) unfolded device after completion of the assay (reprinted with permission from ref [52]. Copyright 2011, American Chemical Society).

2.2. Fabrication of 3D PADs

While 2D PADs exhibit great performance in sensing applications on their flat surfaces, they face limitations in conducting multiple assays on a small device, distributing samples rapidly, and using minimal sample volumes [40]. As a result, 3D PADs are emerging as promising alternatives to perform complicated operations with compact configurations and improve detection performance compared to 2D PADs.

Traditionally, 3D PADs are created by stacking 2D PADs formed using the techniques discussed earlier, along with a double-sided tape or adhesive layer, as demonstrated in Figure 2E [39–41]. However, this approach involves lengthy processes of alignment, bonding, and punching holes to assemble the patterned 2D layers. To address this limitation, Li and coworkers developed a double-sided printing pattern process that constructed three layers of channels on a single paper substrate. This method allowed for the simultaneous detection of glucose, lactate, and uric acid while reducing the number of paper layers needed (Figure 2F) [51].

Origami is an artistic form that involves folding paper. The term originated from Japanese folding paper art, and it has become widely utilized for the fabrication of 3D PADs. The fundamental principle of origami is to create a 3D object from a single flat sheet of paper

through a series of folding steps [42–47,52,53]. Unlike stacking methods, the origami approach offers a straightforward and rapid assembly process for constructing 3D PADs. Liu and coworkers developed this technique to fabricate 3D microfluidic PADs from a single flat sheet of paper using a single step of photolithography. This origami technique was demonstrated for the colorimetric and fluorescence sensing of glucose and protein, as shown in Figure 2G [52].

3. Application of 3D Printing Technology in PAD Preparation

As a substrate material, paper and other porous hydrophilic materials exhibit many advantages over traditional materials, such as power-free fluid transport through capillary force, reasonably high surface area to volume ratio to enhance detection limit, and the ability to store reagents in their active form within the fiber network [54]. The first PAD was prepared by Martinez and coworkers in 2007 using a photomask to pattern photoresist on paper to construct and define a hydrophilic area [3]. However, challenges persist in PAD production, which can significantly hinder the commercialization of these devices, making it challenging to transition from laboratory development to delivering products to customers. For instance, these challenges encompass the performance factors of PADs such as sample loss, inadequate flow control, sensitivity, and selectivity. Another challenge is that human involvement in the fabrication process of PADs can lead to considerable variations between each PAD. To address these issues, 3D printing technology offers a promising solution for the large-scale production of PADs using an automated 3D printing system, thereby minimizing discrepancies caused by human error. 3D printing technology has already been utilized in the large-scale production of plastic products due to its affordability, ease of operation, and ability to print complex shapes with intricate details. Furthermore, due to the aforementioned advantages over conventional fabrication processes, 3D printing has been exploited in PAD fabrication. The automatic production of patterned PADs to create hydrophobic barriers can be improved to reduce human factors [37,55–62]. Additionally, design flexibility and rapid prototyping offer 3D printing technology considerable advantages over traditional fabrication processes. Finally, the weak mechanical nature of paper substrates, which limits their practical applications, can be supplemented by parts printed from a 3D printer, providing extra support for the paper substrate and facilitating the signal measurement processes [36,63–66]. The following sections will provide an insightful perspective on how 3D printing technology can be utilized to enhance the practical applications of PADs.

3.1. Preparation of Fluidic Channels

One primary application of 3D printing is the creation of hydrophobic regions to construct fluidic channels. The first method for patterning hydrophilic channels using 3D printing was developed by He and coworkers in 2015 [37]. They introduced the fabrication of PADs using dynamic mask photo curing (DMPC), which was accomplished with a desktop stereolithography (SL) 3D printer. Specifically, a filter paper substrate was immersed in UV resin, followed by a curing process in the SL 3D printer. By taking advantage of the laser beam in the SL 3D printer system, the resin was cured in the UV-exposed regions that were covered by a dynamic mask with a negative channel pattern, thereby creating hydrophobic barriers (Figure 3A). Meanwhile, the uncured resin was washed away with anhydrous alcohol to yield complete PADs [37]. Fu and coworkers also employed this method to develop 3D PADs in 2019 [56]. They made a slight modification to the original principle. During the fabrication process, a printing pause was implemented between two layers to allow the paper to be inserted into the resin tank. As the resin on the second layer adhered to the paper, it would spontaneously bond with the previously cured paper layer during the curing process. This technique eliminates human involvement and potential errors associated with stacking or folding methods, enabling automatic bonding and alignment between different layers [56]. A conventional 3D printer can also be useful

in the fabrication process of PADs by printing photomasks, which are then exposed to UV light to create patterns (Figure 3B) [55], or by printing substrates with open channels [57]. Recent research explored reusable microfluidic PADs that were filled with cellulose powder to create hydrophilic channels. After each experiment, the powder could be washed away, allowing the channel to be reused. These studies highlighted a flexible approach for conveniently producing photomasks or channels of various sizes and shapes through 3D printing technology. Another affordable approach for creating hydrophobic barriers is by utilizing the hydrophobic properties of plastic printing. Sreenivasan and coworkers used polycaprolactone (PCL) ink [58], while the Espinosa research group employed wax filament to generate functional hydrophobic barriers on paper substrates [62]. Additionally, a creative method involved using a 3D pen to fabricate PADs [60]. In their research, hydrophobic barriers were created with a 3D pen that had high chemical resistance, except to ethanol. After the drawing step, a flashlight was used to cure the polymer on the paper surface, resulting in the formation of hydrophobic barriers (Figure 3C).

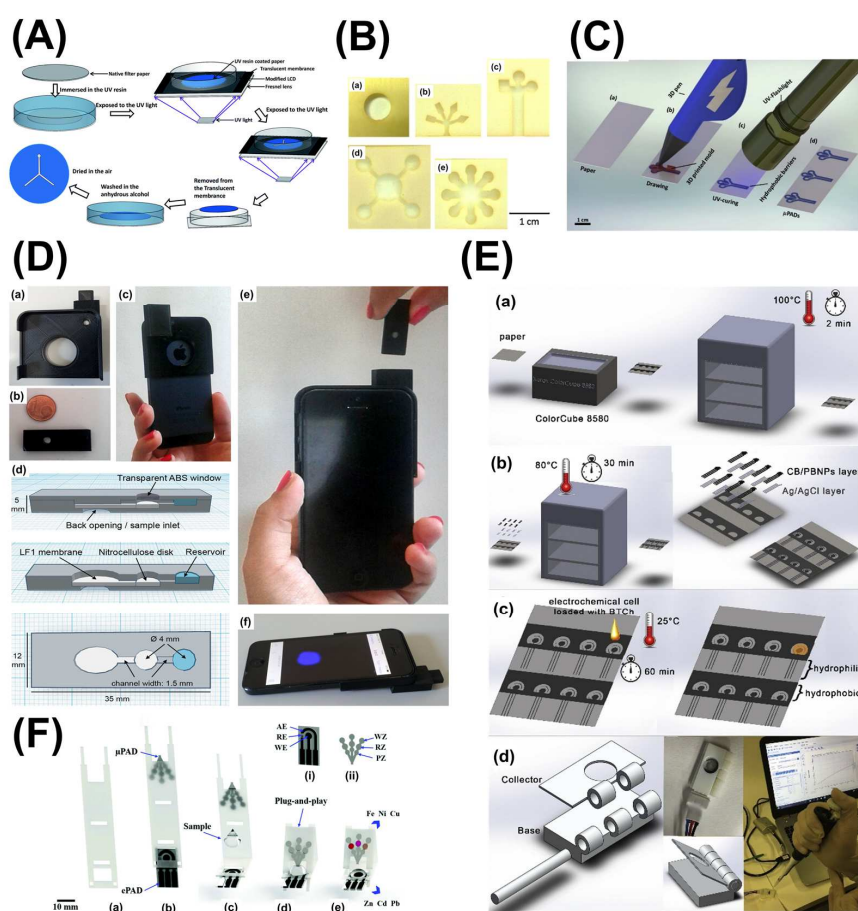


Figure 3. (A) The fabrication of PADs using an SL 3D printer (reprinted with permission from ref [37]. Copyright 2015, Royal Society of Chemistry). (B) Photomasks (a–e) printed from a 3D printer with various sizes and shapes (reprinted with permission from ref [55]. Copyright 2015, Elsevier). (C) PAD fabrication using a commercial 3D pen (reprinted with permission from ref [60]. Copyright 2020, Elsevier). (D) Demonstration of (a–c) 3D-printed PAD supports and (b) the respective cutaway drawings to (e–f) record bio-chemiluminescence signal from smartphone-integrated device (reprinted with permission from ref [35]. Copyright 2014, American Chemical Society). (E) The screen-printing fabrication (a–c) and assembling process (d) of PADs into 3D printed holder and connect to portable potentiostat to run electrochemical detection (reprinted with permission from ref [66]. Copyright 2018, Elsevier). (F) Demonstration of (a) 3D-printed PAD support and holder, (b–e) integration of colorimetric and electrochemical detection modes for heavy metals (reprinted with permission from ref [63]. Copyright 2021, Royal Society of Chemistry).

3.2. Development of Integrated PADs

3D printing technology is increasingly being used to construct partially or fully integrated devices for portable and practical sensing applications. Roda and coworkers were the first to integrate 3D printing technology with smartphones to detect cholesterol, bile acid, and oral fluids by imaging bio-chemiluminescence signals on PADs [35]. This 3D printing process was fundamental for developing a portable system to assemble directly onto a smartphone. Specifically, the accessories for this system included a mini cartridge and a mini darkbox, both of which were extruded using a 3D printer with black thermoplastic acrylonitrile butadiene styrene (ABS) polymer (Figure 3D) [35]. A similar strategy was applied for visualizing thiram on paper strips by recording fluorescence signals through a portable smartphone-based sensing platform [36]. In the case of electrochemical PADs, a complete set of components was produced using a 3D printer to enable the electrochemical detection of cholinesterase in serum [66], as illustrated in Figure 3E. In addition, a dual detection mode that combined colorimetric and electrochemical methods was implemented using a 3D-printed support. Each detection mode was performed separately at opposite ends of the support, allowing for the simultaneous detection of multiple heavy metals in river water. Specifically, the analyte was drop-cast onto the PAD's surface, and the device was folded over onto the electrochemical PAD. This configuration facilitated the vertical flow of the sample to the surface of the electrochemical PAD through the arm of the support (Figure 3F) [63].

4. Comparison of 3D-Printed PADs and 3D-Printed Microfluidic Devices in Sensing Applications

Before diving into the differences between the analytical performance of 3D-printed PADs and 3D-printed microfluidic devices, we briefly discuss the advantages of PADs over conventional analytical methods. Though traditional analytical techniques such as ELISA, HPLC, GC-MS, LC-MS, etc., have been introduced and proven to achieve high precision and sensitivity for diagnostic applications, they are not widely accessible to people living in developing countries or remote regions due to their high cost, bulky instrument, periodic maintenance, and need for trained personnel. To overcome these challenges, a need for simple, economical, and instrument-free devices such as PADs is being developed to address the growing need for biochemical and environmental monitoring. For instance, Ma and coworkers introduced a rapid detection of clenbuterol in milk using ELISA-based PADs. This device is simple, cheap, and equipment-free and can be performed by untrained users. The device produced a colorimetric signal, which is detectable by the naked eye, and it only required a small volume of sample (1.5–6% amounts used in conventional ELISA) and a shorter analysis time (less than 1 h), with a low limit detection of 0.2 ppb in water and food samples [14].

Table 1 presents the criteria essential for constructing effective sensing platforms. To emphasize the unique advantages of combining 3D printing technology with PADs, this section will compare 3D-printed PADs and 3D-printed microfluidic devices based on the criteria relevant to their sensing applications.

The sensitivity and reproducibility of 3D-printed PADs are relatively moderate compared to those of 3D-printed microfluidic devices. Additionally, 3D-printed PADs are restricted by inherent capillary forces and the porous structure of the paper substrate, resulting in lower sensitivity and reproducibility compared to their counterparts. In contrast, precise flow control and uniform channel dimensions enable 3D-printed microfluidic devices to enhance reaction kinetics and minimize the variation in flow and reaction conditions. Moreover, 3D-printed microfluidic devices also offer good control in mixing through a pump compared to natural mixing resulting from the capillary force in PADs,

which mainly depends on porosity, cellulosic fiber density, and the thickness of the paper substrate [67].

Table 1. Comparison of analytical performance aspects between 3D-printed PADs and 3D-printed microfluidic devices.

Criteria	3D-Printed PADs	3D-Printed Microfluidic Devices
Sensitivity	Moderate—limited by capillary force	High—allow precise flow control
Reproducibility	Moderate—influenced by paper properties	High—consistent and less variable
Response time	Fast—few minutes, due to capillary force	Variable—optimized by external pump
Multiplexing capability	Moderate—feasible with patterned zones but limited by cross-contamination between different zones	High—complex design allows parallel assays with minimal cross-contamination.
Stability	Moderate—can be improved through the incorporation of 3D printing technology	High—depend on starting materials
Portability	High	Moderate

The stability of the devices is also a crucial factor that can affect their analytical performance. Due to the hydrophilicity nature of cellulose in paper substrates, PADs are sensitive to humidity and susceptible to degradation from moisture and chemicals. However, their mechanical properties can be improved significantly due to 3D-printed supports, as mentioned in the above sections. Meanwhile, the chemical and environmental resistance of 3D-printed microfluidic devices depends on their starting materials, but generally, they are better than those of PADs.

Additionally, due to the lightweight and compact nature of cellulose, PADs exhibit better portability as opposed to 3D-printed microfluidic devices, which are made of rigid materials such as ABS, acrylonitrile, or PLA, making them bulkier and heavier. Another factor that makes PADs more portable is the minimal equipment required, while their counterparts need external equipment such as pumps, detectors, or power supplies. In summary, 3D-printed PADs are ideal for low-cost, disposable, and field-deployable applications, particularly in resource-limited settings. Their simplicity and rapid response make them suitable for on-site biochemical and environmental monitoring, though sensitivity and reproducibility are comparatively lower. On the other hand, 3D-printed microfluidic devices offer superior sensitivity, high precision, reproducibility, and integration with sophisticated detection methods. These devices are preferred for clinical diagnostics and applications requiring multiplexing automation.

These two types of devices take advantage of 3D printing technology, but the way they apply 3D printers is significantly different. While a 3D printer is used to construct 3D-printed microfluidic devices entirely, it provides a role in functionalizing PADs and integrating 3D-printed parts for 3D-printed PADs.

One challenge with 3D-printed microfluidic devices is the resolution limitation of current 3D printers. This can result in rough surface profiles in the microchannels. For instance, after removing the supporting parts, we can observe prominent rough ridges around the inner wall of 3D-printed microchannels, causing dead volumes and inconsistent surface modifications, as shown in Figure 4A [68]. Another concern arising from 3D print-

ing is the absorption on the surface of 3D-printed devices. Most of the 3D-printed materials are made up of acrylate and ABS, which can absorb biomolecules such as proteins and lipids, leading to a significant loss in analytes of interest and thus falsifying our results [69]. Therefore, functionalization on 3D-printed devices is often required for practical analysis applications. Regarding 3D-printed microfluidic devices, two strategies can be applied to perform functionalization, such as post-printing and pre-printing functionalization. While post-printing relates to the immobilization of reactive substances by adsorption or covalent interactions with/without pre-adsorbed cross-linkers, pre-printing functionalization directly incorporates reactive substances into the raw starting materials. Although they can improve biological activity, electrical conductivity, and chemical reactivity, the functionalization process substantially encounters various limitations, such as complicated and time-consuming procedures, leading to compromises between the thermostability and miscibility of the incorporated substances with the starting materials, as well as the leakage of the incorporated substance [70].

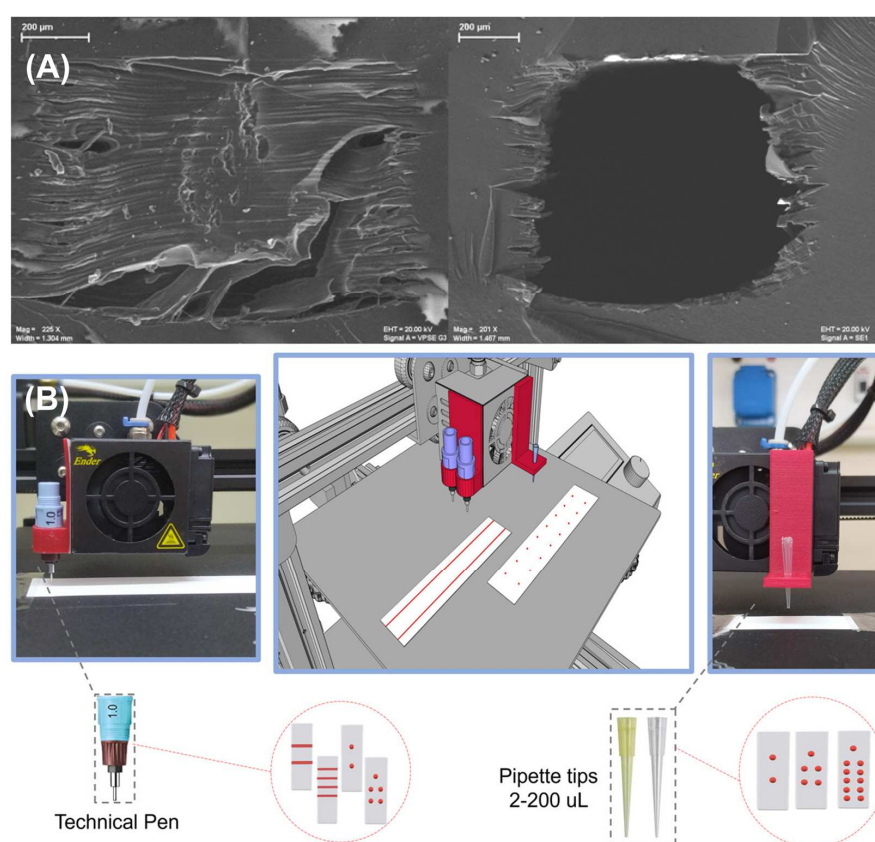


Figure 4. (A) Cross-section SEM images of the channel after printing with the presence of supporting materials (**left**) and after removing supporting materials (**right**) (reprinted with permission from ref [68]. Copyright 2015, American Chemical Society). (B) The photograph and schematic illustration of the dispensing reagent configuration through a technical pen connected to a commercial 3D printer (reprinted with permission from ref [71]. Copyright 2024, American Chemical Society).

Meanwhile, the modification of 3D-printed PADs is more facile than that of 3D-printed microfluidic devices due to their inherent hydrophilic nature with abundant hydroxyl functional groups from the paper substrate. For instance, as mentioned in Section 3, we can apply 3D printing technology to create hydrophobic barriers on paper by either direct formation on paper or through photomasks. Another significant improvement in using a 3D printer to modify the surface of the paper substrate is dispensing reactive substances on the paper surface. For instance, Kalligosfyri and coworkers integrated an affordable 3D printer with technical

pens for the precise and reproducible deposition of reagents such as proteins, antibodies, and oligonucleotides to detect single- and double-stranded DNA on cellulose paper (Figure 4B) [71].

5. Biochemical and Environmental Analyses

As discussed so far, we overviewed how PADs are fabricated and how 3D printing technology can be applied to enhance the feasibility of PADs in practice. While PADs can be chemically modified for application in various fields due to their flexible manufacturing processes and environmentally friendly attributes, their primary application lies in biochemical analysis and environmental monitoring. PADs enable the detection of a wide range of biochemical targets, including cancer biomarkers such as carcinoembryonic antigen (CEA) [47,72–74] and prostate-specific antigen (PSA) [75], as well as DNA [76–78]. They can also detect antioxidants [79], bacteria [80], and common analytes like glucose, lactate, and uric acid in biological samples [81–86]. In addition, PADs have been employed for the environmental monitoring of heavy metals [63,85,87–89] and pesticides, such as organophosphate [90–92], dithiocarbamate [36], and phenthoate [93]. Furthermore, many studies have utilized 3D printing technology integrated with PADs to construct complete devices for practical applications in biosensors and environmental monitoring. Thus, this section provides a detailed insight into the advantages of 3D printing technology in biochemical and environmental analyses. Table 2 summarizes research that has employed 3D printing technology in the fabrication of PADs for biochemical and environmental analyses.

Table 2. Summary of research utilizing 3D printing technology in biochemical analysis and environmental monitoring using PADs.

Application of 3D Printing Technology		Target	Limit of Detection	Linear Range	References
Creation of microfluidic channels	Biochemical analysis	Glucose	0.3 mM	1–10 mM	[64]
			0.8 mM	n.a.	[56]
			0.05 mM	0–1 mM	[60]
		Cholesterol	0.3 mM	0–15 mM	[94]
			0.2 mM	0.2–1 mM	[64]
			0.3 mM	0.3–1 mM	[64]
		Triglyceride	0.3 mM	0.3–1 mM	[64]
		Albumin	3.5 µM	n.a.	[56]
	Environmental analysis	Dopamine	n.a.	0–50 µM	[59]
		Virus derivatives	5.23–38.17 nM	n.a.	[61]
Construction of integrated devices	Biochemical analysis	Nitrite	4.8 µM	5–100 µM	[60]
		Heavy metals	Cu: 0.07 mM	0.3–1.8 mM	[60]
			Fe: 0.21 mM	0.3–1.8 mM	
	Environmental analysis	Cholesterol	20 mg/dL	140–386 mg/dL	[35]
		Cholinesterase	0.1 IU/mL	1–12 IU/mL	[66]
		5-hydroxytryptophan	50 nM	0.165–150 µM	[65]
		Pesticide (Thiram)	59 nM	0–1 µM	[36]
		Heavy metals	Fe: 0.1 mg/L	1–20 mg/L	[63]
			Ni: 0.3 mg/L	1–50 mg/L	
			Cu: 0.2 mg/L	1–25 mg/L	
			Zn: 10.5 µg/L	0.1–1.4 mg/L	
			Cd: 1.3 µg/L	0.01–1.4 mg/L	
			Pb: 0.9 µg/L	0.01–1.4 mg/L	

5.1. Biochemical Analysis

The use of 3D printing technology holds great promise for the fabrication of biosensors. For example, 3D-printed components have been demonstrated to facilitate the assembly of smartphone cameras for bio-based PADs through bio-chemiluminescence signals [35]. Ad-

ditionally, they could serve as holders to simplify the detection processes for glucose [64], dopamine [59,62], and cholinesterase in serum [66]. In addition to traditional 2D PAD fabrication, digital light processing–stereolithography (DLP–SLA) 3D printing has been adopted to fabricate 3D PADs for sensing glucose and albumin [59]. The Park research group successfully developed 3D PADs using DLP printing to simultaneously detect glucose, cholesterol, and triglycerides in whole blood [94]. To address color interference in colorimetric assays, a plasma separation membrane (PSM) was implemented to filter out red blood cells (RBCs) [94]. Specifically, the PSM was coated with parylene C to prevent its dissolution in organic solvents during the 3D printing process. The coated PSM was then layered on top of the paper substrate. Detection zones and a reservoir were printed onto both the paper and PSM through liquid photopolymerization within a DLP printer. Figure 5A illustrates the schematic design of the PADs, while Figure 5B shows the simultaneous colorimetric detection of biomarkers in blood. The feasibility of PADs was tested with whole blood samples from patients suffering from diabetes mellitus. This technique showed similar analytical performance in glucose detection to that of a commercial glucometer, with a correlation coefficient of less than 10%, demonstrating the clinical application of PADs to whole blood samples of patients. The detection limit of PADs mentioned in this research was also comparable with other 3D-assisted PADs, as shown in Table 2. Similar analytical performance towards glucose detection in whole blood was also achieved compared to that of a noble metal-modified sensing platform (0.1 mM) [95] or wax-printed PADs (0.3 mM [41], 0.7 mM [39]). Park’s research group also applied a similar DLP printing technique to fabricate 3D PADs for the simultaneous detection of glucose, cholesterol, and triglyceride with LODs of 0.3, 0.2, and 0.3 mM, respectively. The LOD of cholesterol is lower than that of inkjet-printed PADs (0.57 mM) [96] and higher than that of carbon dot-modified PADs (0.014 mM) [97]. Additionally, an intriguing application of 3D printing technology in bio-based PADs involved the use of a commercial 3D pen [98]. In this study, Sousa and coworkers demonstrated how a 3D pen could be used to dispense conductive ink. This ink was prepared by mixing graphite powder, polymer resin, and acetone, which was then applied to a paper surface using a plastic stencil. Furthermore, a 3D-printed mold was prepared to design a microfluidic guide, as illustrated in Figure 5C. This device was employed to measure the levels of lactate, nitrite, and salivary amylase in saliva samples [98].

5.2. Environmental Analysis

3D printing-based PADs offer a cost-effective and eco-friendly solution for environmental monitoring. There is an increasing number of studies exploring the integration of 3D printing technology with PAD fabrication. These studies demonstrated the detection of various substances, including nitrite [37,57,60], heavy metals [50,63], and pesticides [36]. For instance, He and coworkers demonstrated the use of SL 3D printing for the fabrication of PADs by utilizing UV light to cure resin applied to a paper substrate. This process created a hydrophobic barrier [37]. The device was applied for the colorimetric detection of nitrite ions, with the detection zones showing color changes in response to the nitrite concentration, as illustrated in Figure 5D. The color changes were converted into grayscale images that corresponded to the nitrite levels. This technique overcomes the environmental sensitivity of PADs fabricated via wax printing. For instance, if the temperature reaches over 50 °C, the wax-printed PADs cannot be used since wax barriers will melt. Additionally, wax-printed PADs also reduce the flexibility of paper due to the brittleness of wax. The presented device fabricated using the SL 3D printer mentioned above shows a detection limit of 4.8 µM, which is comparable to a previous report from the Cardoso research group with a detection limit of 5.6 µM for nitrite detection [99]. A dual detection method combining colorimetric and electrochemical analyses for heavy metals was achieved using a support structure printed via a 3D printer. At both ends of this support, PADs for colorimetric and

electrochemical detection were attached. When an analyte was injected into the colorimetric PADs, it flowed vertically through the support arm and reached the electrochemical PADs. Figure 5E illustrates the simultaneous colorimetric detection of heavy metals, including Fe, Ni, and Cu. The limit of detections (LODs) for Fe, Ni, and Cu was found to be 0.1, 0.3, and 0.2 mg/L, respectively [63]. The analytical performance of the 3D-integrated PADs was demonstrated through the detection of heavy metals in river water samples. The reliability of this method was also compared to a reference technique, atomic absorption spectroscopy (AAS). The metal concentration found using the multiplexed PADs revealed good agreement with those obtained from the AAS technique. This device also exhibited similar analytical performance for Ni (0.24 mg/L [100]) and Zn (0.03 mg/L [101]) detection with previous research. In summary, incorporating 3D printing technology in PAD fabrication not only preserves or improves analytical performance in biochemical and environmental analyses but also enhances the feasibility of transferring PADs into commercial products.

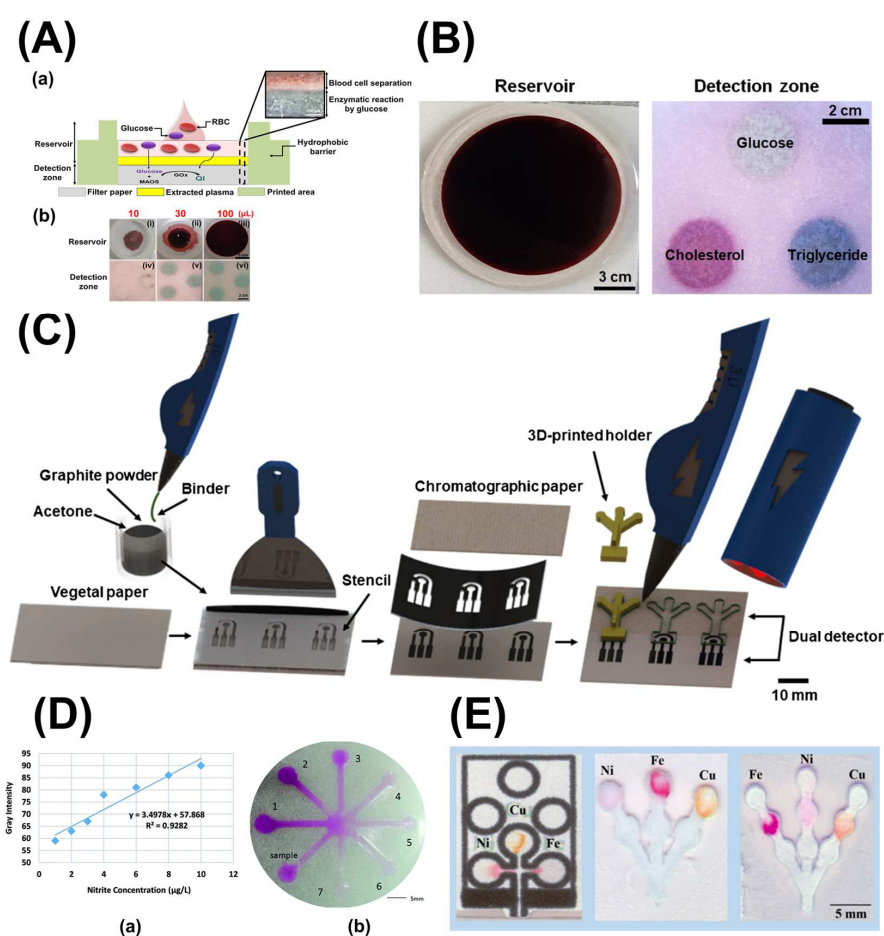


Figure 5. (A) (a) Schematic illustration filtering out blood cells to detect glucose in separated plasma and (b) color generation on the detection zone with different amounts of whole blood samples. (B) Simultaneous colorimetric detection of multiple biomarkers, including glucose, cholesterol, and triglyceride in whole blood samples using 3D PADs (reprinted with permission from ref [94]. Copyright 2019, American Chemical Society). (C) Fabrication of PADs from 3D pen polymeric resin for dual colorimetric and electrochemical PADs for detection of lactate, nitrite, and salivary amylase in saliva samples (reprinted with permission from ref [98]. Copyright 2023, Springer). (D) Colorimetric assay of nitrite via color-reaction (a) curve for nitrite ion and (b) image of the testing PAD. Calibration curve for nitrite ions and optical image of PADs fabricated from SL 3D printing after being exposed to different concentrations of nitrite (reprinted with permission from ref [37]. Copyright 2015, Royal Society of Chemistry). (E) Simultaneous colorimetric detection of Fe, Ni, and Cu in river water samples (reprinted with permission from ref [63]. Copyright 2021, Royal Society of Chemistry).

6. Challenges in 3D Printing with PADs

The incorporation of 3D printing in the fabrication of PADs has been explored using various techniques and strategies aimed at efficiently producing high-quality PADs. The performance of PADs can also be significantly improved by incorporating advanced materials that are compatible with 3D printing, enhancing the functionality of traditional PADs. The incorporation of multi-material 3D printing on paper substrates can enhance the robustness and reproducibility of PADs while significantly reducing the fabrication time [102]. However, the higher costs associated with multi-material printers compared to single-material printers pose a challenge for their application in large-scale production. This limitation could hinder the widespread adoption of the advanced 3D printing techniques in the commercial development of PADs despite their potential advantages. Additionally, 3D printing allows for greater creativity in design, geometry, and fabrication techniques, enabling the creation of diverse types of PADs that meet customer demands. It is essential, however, to carefully consider the types of 3D printers we use, the materials for printing, and how we can access high-quality 3D printers [103]. The resolution of 3D printers also plays a crucial role in determining the performance of PAD products. For example, low-resolution 3D printers tend to produce masks or parts that are of low quality, often resulting in significant size deviations. This can adversely affect microfluidic channels in PADs and prevent the designs from fitting properly into smartphones for portable PADs. Furthermore, the regular maintenance of 3D printing equipment is essential to ensure the proper operation of PADs. One common issue is a clogged nozzle, which can occur due to filament jams or the accumulation of debris and dust. This can lead to extrusion problems or even damage to the 3D printer. To prevent clogs, it is important to clean the nozzle regularly using a needle or wire brush to remove any residue after printing. Bed leveling is also a crucial issue and should be calibrated periodically before using 3D printers. If the distance between the nozzle and the print bed is inappropriate, it can result in warping, curling, or poor adhesion, negatively impacting the quality of the printed objects. Many components of 3D printers, including the nozzle, belts, and print beds, can be broken or worn out over time. Therefore, it is important to inspect these parts regularly and replace them when necessary. Using high-quality replacement parts can also prolong the life of 3D printers. Furthermore, proper lubrication is vital, as many parts can be broken down due to inadequate lubrication. Rails and bearings must be lubricated to operate smoothly and minimize friction.

7. Conclusions

The rise of 3D printing offers greater flexibility and reusability in fabricating PADs. With a single fabrication process, it is possible to create hydrophilic channels on PADs through the 3D printing of masks or patterns. In this mini-review, we highlighted the strengths of 3D printing technology in the fabrication of PADs and also briefly discussed the conventional fabrication methods of PADs such as photolithography, wax printing, screen printing, and inkjet printing techniques. Specifically, hydrophilic channels can be created using 3D-printed masks or can be formed directly through a dynamic mask photo-curing process, which is carried out with a desktop SL 3D printer. By fully leveraging 3D printing technology, we can also design customized equipment for specific experiments, including cells, holders, and supports. This enables us to develop complete devices that integrate fabricated PADs with signal readers, such as smartphones, thereby making them suitable for portable sensing applications in biochemical and environmental analyses. Future developments can focus on incorporating advanced materials, such as biodegradable materials, into the 3D printing process, enhancing the automation of PAD production, and commercializing PADs at more affordable prices. Another area for further improvement can be

centralized on printing multiple materials in a single fabrication process to simultaneously create hydrophilic channels and modify the working area with functional materials. This approach could reduce fabrication time while enhancing the analytical performance of PADs. The increasing number of publications utilizing 3D printing for PAD fabrication reflects the growing popularity of this integration. In the future, further advancements in 3D printing technology combined with PADs could lead to a novel generation of PADs with improved performance by integrating components through 3D printing.

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