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Beyond wax printing: The future of paper analytical device fabrication

Rachel M. Roller, Marya Lieberman *

University of Notre Dame, Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, IN 46556, United States

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ABSTRACT

Many microfluidic "lab on paper" devices have been demonstrated for point-of-use applications, but scalable fabrication methods are necessary for these devices to make it past the proof-of-concept stage and become viable commercial products. Commercially available wax printers such as the Xerox ColorQube presented a near-ideal compromise between cost, ease of prototyping, and throughput, but in 2016, these printers were discontinued, and now, alternative methods are needed. In this review, we survey current paper analytical device (PAD) fabrication methods through the lens of scalability, focusing on the tradeoffs between resolution, ease of prototyping, cost, and throughput. Categories discussed include handmade, semi-automated, batch, laboratory-based, printer-based, and roll-to-roll fabrication methods. Hand-, batch-, semi-automated, and laboratory-based fabrication methods suffer from low throughput and high hands-on labor requirements. Roll-to-roll methods are high throughput but costly, while printer-based methods offer a compromise between cost and throughput. By highlighting the comparative merits of existing methods, we hope to offer insight into what makes a method "scalable" or "manufacturable."

1. Introduction

Despite the burgeoning research interest in microfluidic paper analytical devices, or μPADs, for applications as diverse as point-of-care diagnostics [1,2], food and drug testing [3–8], environmental monitoring [9–12], and chemical education [13–21], very few μPADs successfully navigate the transition from "benchtop to bedside" to become a commercial or clinical product [22,23]. One reason for this is the lack of affordable, scalable fabrication methods. Numerous fabrication methods have been reported and reviewed in the literature [2,23–38], but the majority of these methods are limited by challenges related to cost, resolution, and the amount of infrastructure needed [39,40]. High-throughput methods, such as those used by producers of commercial lateral flow assays, require expensive equipment that can be a barrier to entry for new potential products, while small-scale methods used to develop μPADs in academic laboratories cannot produce the volume of devices needed to get beyond the proof-of-concept stage. What is needed is a middle ground between laboratory-scale and manufacturing-scale methods—something that could be termed "mid--scale manufacturing." Affordable, mid-scale methods can fill the gap between proof-of-concept studies and real-world applications, and, ideally, pave the way to transition successful μPADs to larger-scale production.

The goal of the present review is to examine the existing fabrication methods for paper-based analytical devices through the lens of mid-to-large-scale manufacturability, focusing on the tradeoffs between resolution, cost of equipment/materials, throughput, and ease of prototyping. Specifically, this review surveys the methods for creating the architecture (channels, test zones, and barriers) of the devices themselves; although reagent deposition is a vital facet of μPAD manufacturing, it is outside the scope of this review. Similarly, although low-cost devices can be made from a variety of materials, including glass, thread, plastic film, polydimethylsiloxane (PDMS), and others [41], this review focuses on devices made primarily of cellulosic paper.

Six main categories of fabrication methods will be discussed: hand-fabrication methods, semi-automated methods, batch methods, laboratory-based methods, printing methods, and roll-to-roll methods (see Fig. 1). By evaluating the strengths and weaknesses of each method, this review aims to 1) provide a guide to researchers deciding on an existing fabrication method for use in their own projects, and 2) highlight the continuing need for new fabrication methods that will enable affordable and scalable production of paper-based devices.

2. Tradeoffs in paper device fabrication

The usefulness of a given fabrication method depends on four

* Corresponding author.
E-mail address: mlieberm@nd.edu (M. Lieberman).

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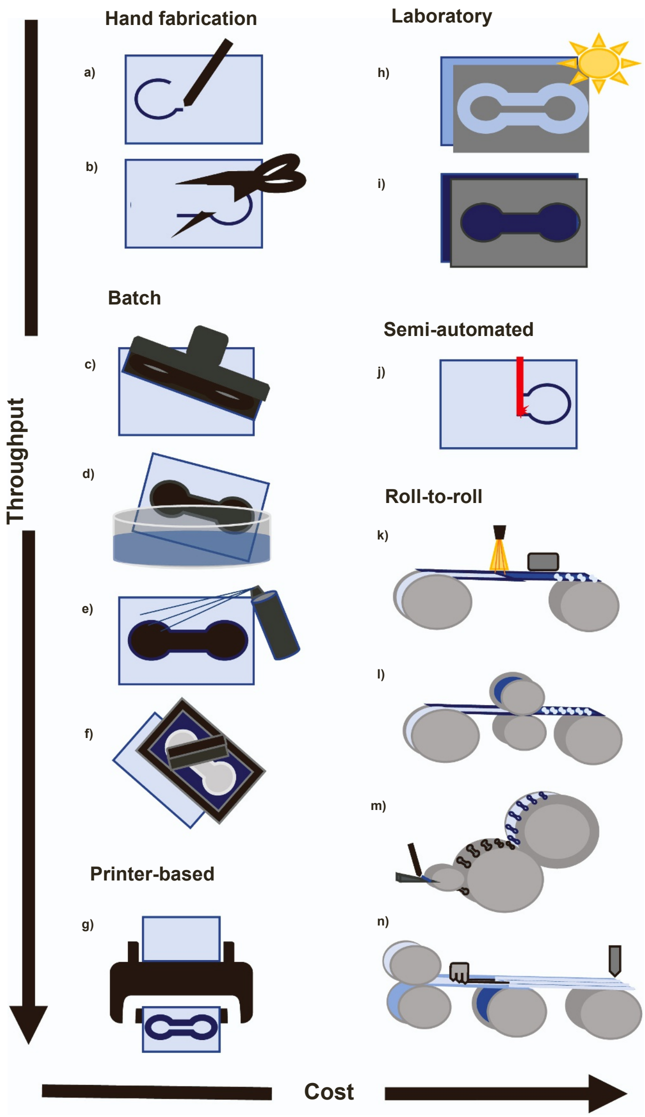

Fig. 1. Selected PAD fabrication methods. a) drawing, b) cutting, c) stamping, d) dipping, e) spraying, f) screen printing, g) printing (inkjet, wax, toner), h) photolithography, i) plasma treatment, j) laser cutting/treatment/plotting, k) liquid flame spray, l) roll-to-roll wax thermal transfer, m) flexography, n) air-gap.

primary factors: cost, throughput, ease of prototyping, and resolution.

2.1. Cost

For any fabrication method, the cost will depend largely on three factors: materials, equipment, and labor. Of these three factors, materials (paper, patterning materials, solid supports, and other consumables) are generally the least expensive. Obviously, specialty papers like the commonly-used Whatman 1 will be more costly than simple copy paper or paper toweling, but cellulosic papers in general are much less expensive than nitrocellulose membrane (see Section 3.1 for a discussion of paper types). The cost of patterning materials, meanwhile, varies widely. Materials such as paraffin wax and alkyl ketene dimer (AKD) are quite inexpensive, while photoresists can reach upwards of $3.00 per page. Approximate costs per page for common patterning materials are given in Table 1.

While consumables such as patterning materials and paper are an ongoing, per-device expense, equipment is typically a one-time, upfront investment. Equipment costs can range from a few dollars for small-scale hand-fabrication methods to tens of thousands of dollars for methods requiring specialized laboratory instruments or factory-scale equipment, as shown in Table 2. The cost of maintaining the equipment must also be considered.

Costs for materials and equipment are readily calculated; labor, however, is an often-overlooked expense. The vast majority of

Table 1
Patterning materials for paper device fabrication. Dollar-sign rankings are as follows: $ for < $0.10, $$ for $0.10–0.20, $$$ for $0.20–0.50, and $$$$ for > $3.00 per page. Cost per page estimated based on market prices for materials and any required solvents, assuming 40–100 % coverage of 8.5 × 11-inch paper. More details and calculations can be found in the supporting information.

Patterning Material	Compatible methods	Patterning principle	Solvent Compatibility	Cost per page
Wax (paraffin)	Drawing, 3D printing, dipping	Hydrophobic agent	Aqueous	$
AKD	Inkjet printing, plasma treatment	Chemical modification	Aqueous	$
Polystyrene	Inkjet printing, flexography	Hydrophobic agent	Aqueous	$
Laser toner	Laser printing	Hydrophobic agent	Aqueous, some organics	$$
TiO_2	Drawing, liquid flame spray	Pore blocking	Aqueous, some organics, low concentrations of alcohols	$$
PDMS	Plotting, inkjet printing, flexography	Pore blocking	Aqueous	$$
Silanes	Silanization	Chemical modification	aqueous, organics, surfactants	$$
Wax Ink	Wax printing	Hydrophobic agent	Aqueous	$$
Thermal Transfer Ribbon	Thermal transfer printing, roll-to-roll wax printing	Hydrophobic agent	Aqueous, surfactants	$$$
Parafilm	Hot embossing	Hydrophobic agent	Aqueous, organics, surfactants	$$$
Spray Lacquer	Spraying	Hydrophobic agent	Aqueous	$$$
Waterproof ink	Drawing, plotting, inkjet printing	Hydrophobic agent	Aqueous	$$$$
Photoresists (SU8)	Photolithography	Pore blocking	Aqueous	$$$$

Table 2
Equipment costs for paper device fabrication, ranked from low to high cost. Dollar-sign rankings are as follows: $ for < $50, $$ for $50-$500, $$$ for $500-$2000, $$$$ for $2,000-$10,000, and $$$$$ for > $10,000. Costs estimated from online prices and from quotes requested from equipment suppliers.

Equipment	Cost
Screen (for screen printing)	$
Stencil/mask	$
Stamp	$
3D printer (homebuilt)	$$
Craft plotter/cutter	$$
Inkjet printer (office grade)	$$
Laser printer	$$
Thermal transfer printer	$$
Wax printer	$$$
Laser cutter (benchtop)	$$$
UV lamp/chamber	$$$$
Photolithography equipment	$$$$$
Laser cutter (research grade)	$$$$$
Plasma cleaner	$$$$$
CVD chamber	$$$$$
Inkjet (research grade)	$$$$$

publications on fabrication methods contain no information on the time or labor required to produce their paper devices. In academic laboratories, which rely on cheap student labor, methods that produce only a handful of devices per hour may be acceptable, but for any practical application, the fabrication process must minimize hands-on labor and maximize throughput.

2.2. Throughput

Throughput may be the most important and least discussed aspect of PAD fabrication. Practically, throughput depends not only on how long it takes to produce a single device, but also on whether the method can be automated and what percentage of the produced devices can pass quality control. An automated process that produces devices more slowly than a human, but can run around the clock without hands-on intervention, may be a worthwhile investment, while a process that produces devices quickly, but with a high fail rate, is unlikely to be viable. Ideally, automated processes, despite the higher upfront costs of the equipment, will decrease cost in the long run by minimizing labor and maximizing reproducibility [30]. These parameters are largely ignored in the literature—very few papers contain any information whatsoever on the time and labor requirements or the reproducibility of a given method.

Different applications will require different levels of throughput. For early device development, prototyping, proof-of-concept studies, and educational laboratory exercises with small groups of students, only tens of devices may be needed. Most applications, however, require at least medium throughput. Early-phase clinical trials require hundreds of devices, while commercially viable products will need to be produced by the thousands or even millions. Malaria diagnostic tests, for example, are produced in lot sizes of at least 200,000 [42]. Scalability, then, is crucial for any paper device intended for real-world applications.

This point is illustrated by the fact that very few paper analytical devices are commercially available. Of the ten companies in the category of "Paper Diagnostics" listed in Grand View Research's most recent market analysis [43], the vast majority sold PAD-adjacent products like lateral flow assays. Only two offered PADs that were not simple lateral flow assays or dipsticks, and neither of these is still actively in business as of the time of this writing. Diagnostics for All (DFA), a company that started out of the Whitesides group at Harvard, offered tests for HIV, liver function, and nutrient deficiency, but now is in dormant mode, with no active employees [44]. DFA formerly used wax printing, but sought other methods when wax printing ceased to be a viable option for commercial-scale manufacturing. BiognostiX, the other PAD company in the Grand View report, started in 2011 but faced several challenges related to fabrication, trying both embossing and lamination before settling on polymer-molded coating [45]. After the three-year research and development phase, they were unable to launch successful commercialization, and their website is no longer active. This storyline—a promising proof-of-concept device failing to achieve commercialization despite years of funded innovation—is an all-too-common tale in the realm of paper microfluidics. While scalable fabrication is only one of the many factors that contribute to a company's success or failure, without medium- and high-throughput fabrication methods, paper devices simply cannot make the leap from proof-of-concept to point-of-care [22,33,46].

2.3. Ease of prototyping

Another key consideration when choosing a fabrication method is its conduciveness to prototyping—that is, how easy it is to alter the pattern of the device. For semi-automated methods (plotting, craft cutting, laser cutting, 3D printing) and printer-based methods (wax, laser, and inkjet printing), this is as simple as editing a computer-based file. Batch methods (stamping, dipping, spraying, screen printing) rely on simple tools like stamps and screens, which must be replaced for each new pattern. The cost of a new stamp, screen, or similar template is typically low, so altering the pattern is not unreasonable. Manufacturing-scale methods, such as roll-to-roll processing, may require entirely new equipment to produce a different pattern, which can be prohibitively expensive. Ideally, a method would have both high throughput and ease of prototyping, but in practice, there is often a tradeoff. Thus, it is important to choose a method that fits the stage (early development vs commercialization) of a given project.

2.4. Resolution

In paper device fabrication, resolution is typically reported in terms of the minimum functional channel and barrier widths—functional, in this case, meaning that channels wick fluid and barriers do not leak. Selected minimum channel and barrier widths are presented in Table 3.

Resolution matters because smaller features enable more efficient use of space. This allows multiplexing—multiple assays on a single device—and miniaturization. Smaller devices use less material, which can decrease the cost per device. However, there is often a tradeoff between a method's resolution and its cost [39]. A quick glance at Table 3 reveals that laser-based methods (laser cutting, laser ablation, laser-induced polymerization) give superior resolution; unfortunately, they also require expensive laser-cutting equipment. Similarly, photolithography achieves excellent resolution, but requires expensive reagents, specialized equipment, and trained personnel, all of which drive up cost. A method like wax dipping, on the other hand, uses cheap materials and minimal equipment, but cannot produce high-resolution devices. Not all applications, however, require high resolution—for most practical applications, channels and barriers in the millimeter range are perfectly acceptable [47]. Often, then, the cost and throughput of a method will be a higher priority than its resolution.

3. Paper device architecture

Before surveying the methods of making paper devices, it will be helpful to understand the basic structure and function of devices

Table 3
Smallest reported channel and barrier widths for selected paper device fabrication methods.

Method	Minimum Channel	Minimum Barrier	Reference
Laser cutting	139 ± 8 μm, 130 ± 11 μm, 103 ± 12 μm, 45 ± 6 μm, and 24 ± 3 μm depending on substrate	-	[48]
Laser ablation	80 μm	62 ± 1 μm	[49]
Photolithography	90 μm	250 μm	[50]
Laser-induced polymerization	120 μm	80 μm	[51]
FLASH photolithography	184 ± 12 μm	186 ± 13 μm	[52]
Wax printing	228 ± 30 μm	467 ± 33 μm	[53]
Silanization	233 ± 30 μm	137 ± 21 μm	[54]
Inkjet printing	272 ± 19 μm	425 ± 26 μm	[55]
Flexography	400 μm	400 μm	[56]
Laser printing	415 ± 35 μm	200 μm	[57]
Stamping	428 ± 21 μm	357 ± 28 μm	[58]
Thermal transfer printing	627 ± 2 μm	749 ± 31 μm	[47]
Wax dipping	639 ± 7 μm	-	[59,60]
Screen printing	670 ± 50 μm 256 ± 21 μm	380 ± 40 μm -	[61,62]
Plotting	2 mm	1 mm	[63]

themselves. This entails a discussion of the channels, typically formed from hydrophilic paper, and the barriers that confine the flow of fluid to the desired areas.

Paper has been used as a platform for chemical tests since at least the first century AD. The earliest report of a paper-based chemical test is found in Pliny the Elder's *Natural History,* which describes a test for ferrous sulfate adulteration in verdigris using "papyrus previously steeped in an infusion of plantgall" [64]. Through the subsequent centuries, paper was used for a variety of chemical applications, including indicator papers in the 1600 s [65], urinalysis dipsticks in the 1800 s [65], and paper chromatography in the 1900 s [66].

As the field of chemistry-on-paper progressed, however, it became apparent that patterning the paper with hydrophilic and hydrophobic regions to control fluid movement would enable more sophisticated analyses [67]. The first report of a patterned paper device was in 1937, when Yagoda used a heated copper stamp and paraffin wax to create hydrophobic barriers around circular test zones in paper [67]. Shortly thereafter, in 1949, Mueller and Clegg expanded on this concept to pattern hydrophobic barriers around a microfluidic channel for paper chromatography, creating the predecessor to today's microfluidic paper analytical devices, or μPADs [68]. (See Fig. 2 for a timeline of μPAD fabrication methods.) Details of paper and barrier types are discussed in the following two sections.

3.1. Paper

Paper is an attractive platform for microfluidics because it is inexpensive, lightweight, portable, renewable, and easily disposable. Unlike traditional materials for microfluidics, such as glass or PDMS, paper does not require an external pump to drive fluid flow [69]. Instead, paper microfluidics relies on spontaneous capillary action to move fluid through the device. As a first-level approximation, and assuming a constant channel width, the capillary flow in paper can be modeled with the Washburn Equation:

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

where L is the distance traveled by a liquid with surface tension γ and viscosity η through a medium with pore radius r and contact angle θ in time t [70,71]. The pertinent variables for the current discussion are the pore radius and the contact angle.

Contact angle is a measure of a material's affinity for a liquid. Hydrophilic materials have a contact angle of less than 90° with water, while hydrophobic materials have a contact angle greater than 90°, because the water wets the hydrophilic material but beads up on the surface of the hydrophobic material. Pore radius is a more complicated variable, as the Washburn equation was originally developed for capillary tubes, which have a uniform and easily measurable interior diameter, while paper is an inherently disordered system that contains pores with a variety of shapes and sizes [72,73]. However, nominal pore size and pore size distribution can be estimated from flow experiments and the bubble point method, or measured by methods such as mercury intrusion porosimetry [2,73,74]. The porosity, defined as the void volume of the paper, and the paper's thickness also affect how much liquid the paper can absorb [2].

All of these quantities—contact angle, pore size, porosity, thickness—will vary depending on the type of paper chosen, and can noticeably affect an assay's performance [75]. In the articles surveyed by the current review, Whatman 1 filter paper and Whatman 1 CHR were by far the most commonly used papers. This agrees well with the findings of Sharma et al. [25,41] and Singh et al. [37], who discussed the paper types most commonly used in paper devices.

3.2. Barriers

Li, Ballerini, and Shen's 2012 review provides a helpful breakdown of paper device fabrication methods into three major categories based on how the barriers are formed. Barriers can be formed by 1) physically blocking the pores of the paper, as in photolithography, 2) depositing a hydrophobic material, such as wax or polystyrene, or 3) chemically modifying the paper, as with alkyl ketene dimer (AKD) or silanes [27]. Table 1 in Section 2.1 categorizes patterning reagents based on this classification system. Broadly speaking, chemical modification produces barriers that are compatible with a wider range of liquids than depositing a hydrophobic agent [27]. Silanization, for instance, produces barriers that are robust against some organics and surfactants,[54] while wax-based barriers are typically only compatible with aqueous solutions [76].

Methods can be further broken down into additive and subtractive—additive methods selectively hydrophobize only the barriers, while subtractive methods hydrophobize the entirety of the paper before selectively restoring hydrophilicity to the channels [27,49]. For applications where residue in the hydrophilic zones would negatively affect assay performance, additive methods may outperform subtractive methods, because the hydrophilic zones are not exposed to the patterning reagents [77]. In general, additive methods also involve fewer steps and less waste of materials.

While this remainder of the present review categorizes fabrication methods based on their manufacturability rather than the physical principles of barrier formation, these classifications of additive/

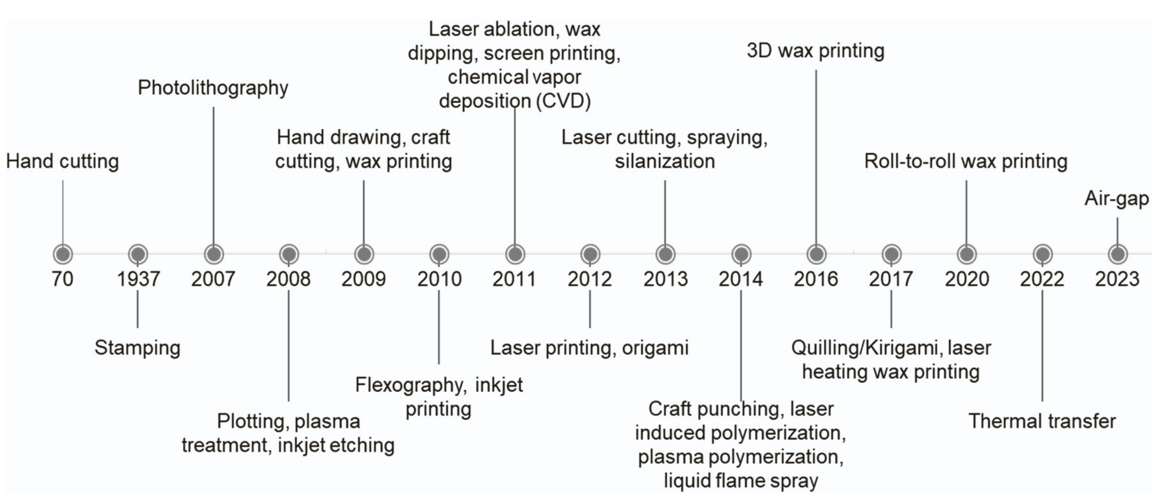

Fig. 2. History of paper device fabrication methods.

subtractive and pore blocking/hydrophobic material/chemical modification are valuable to keep in mind when evaluating the suitability of a method for a given application.

4. Fabrication methods

The rest of this review surveys paper device fabrication methods through the lens of manufacturability. Methods are placed in six broad categories—handmade, semi-automated, batch, laboratory-based, printer-based, and roll-to-roll fabrication—from low to high throughput. The practical pros and cons of the various methods are discussed based on their throughput, cost, ease of prototyping, resolution, and equipment and labor requirements.

4.1. Hand-fabrication methods

Fabricating paper devices by hand, whether by drawing, folding, or cutting, is simple, inexpensive, and virtually equipment free, but it is also slow, labor intensive, and lacking in resolution and reproducibility. Thus, hand-fabrication methods are useful for early prototyping, in educational contexts, and in low-resource settings, but not for any mid- or large-scale applications.

4.1.1. Hand cutting

One of the simplest low-tech fabrication strategies is to simply cut the desired pattern out of paper by hand. This is how the earliest devices were most likely fabricated (see Fig. 2).

In more recent years, Wang et al. used a hand-cutting technique to create a tree-shaped paper device with seven branches—six to create a calibration curve, and one for an unknown—for a semiquantitative protein assay [78]. In a 2020 study by Zhang et al., a hand-cut flower-shaped paper was pasted to a hydrophobic poly(vinyl) chloride (PVC) support [79].

As hand-cutting is a tedious and error-prone process, some researchers turn to craft punching. Mu et al. used a craft punch to cut flower-shaped devices out of nitrocellulose [80]. Since nitrocellulose is highly flammable, higher-throughput laser cutting is hazardous, so craft punching is a reasonable middle ground between hand-cutting and laser-cutting.

4.1.2. Origami

Another fabrication strategy is to use crafting methods such as origami, kirigami, and quilling to create paper devices. Several researchers have combined origami folding techniques with other methods such as wax printing to create multilayer, 3D devices [81–85]. Gao et al. used kirigami (paper cutting and folding) and quilling (paper rolling) to make "vertical" paper devices without the need for any chemicals or equipment [86]. Because these methods necessitate folding or otherwise manipulating the paper by hand, they are inherently labor-intensive, low-throughput techniques. Thus, they are useful in mainly in low-resource situations where reagent- and equipment-free fabrication is a higher priority than efficiency.

4.1.3. Hand drawing

In contrast to cutting-based methods, which use the edge of the paper as a physical boundary, drawing-based methods use a hydrophobic material, such as wax or various inks, to create hydrophobic barriers on paper. Lu et al. patterned paper by hand with a wax pen, either by free-form drawing or by tracing a pre-printed pattern to improve reproducibility [87]. Similarly, Nie et al. used an iron template as a stencil for more reproducible drawing with a permanent marker [88]. TiO_2-based correction pens were used by Mani et al. to hand draw hydrophobic barriers that were compatible with most aqueous and organic solvents [89].

Since hand-drawing and cutting are arguably the simplest fabrication methods, they are useful in classroom settings where students create their own paper microfluidic devices. For example, Ravgiala et al. used a craft-punching method in a high school class—students used simple color tests on craft-punched paper to analyze forensic evidence at a mock crime scene [90]. In an inquiry-based laboratory experiment reported by Armenta et al., students drew test zones on their own paper devices using permanent marker, wax pen, or even eyeliner [17], while one of the at-home experiments described by Roller et al. involved students drawing lateral flow devices with crayons [19]. Most applications, however, will require a higher-throughput fabrication method with higher resolution and reproducibility.

4.2. Semi-automated methods

Semi-automated methods, including plotting, craft cutting, laser cutting, and 3D printing, retain much of the easy prototyping of hand-fabrication methods, but they improve device reproducibility and decrease the amount of time and hands-on effort required to produce paper-based devices. Because these methods require access to equipment such as a plotter, laser cutter, or 3D printer, the upfront infrastructure costs are somewhat higher, but not as high as most laboratory or manufacturing methods (see Table 2).

4.2.1. Plotting

The simplest way to scale up hand-drawing is to use an x-y plotter to automate the drawing process. The first report of plotting as a μPAD fabrication method was in 2008, when Bruzewicz et al. used a plotter to deposit polydimethylsiloxane (PDMS) on a paper substrate [63]. The plotter used in that study was only compatible with one type of pen, so to obtain a pen that would dispense the PDMS solution, the researchers had to cast a replica of the plotter-compatible pen [63]. Later studies used plotters that were compatible with commercial pens; for example, Gallibu et al. used a commercially available Sharpie® permanent marker in a plotter [91], while Nuchtavorn et al. plotted with a technical drawing pen filled with in-house-formulated permanent inks to draw hydrophobic barriers [92]. The advantage of technical drawing pens is that the actual linewidth of the plotted barrier is nearly identical to the stated linewidth of the pen, allowing greater control over the final design than with felt-tipped pens [92]. Ghaderinezhad et al. took the automation one step further by fitting their plotter with a custom roller-based paper feeder to eliminate the need for a human operator to manually reload the paper in between sheets [93].

4.2.2. Craft cutting

Just as plotting increased the efficiency of drawing, craft cutting can make paper cutting more scalable. Fenton et al. used a cutting plotter to shape both nitrocellulose membrane and chromatography paper [94]. Because the chromatography paper was prone to tearing, the researchers had to use multiple overlapping "kiss cuts" to cut the substrate cleanly [94]. Giokas et al. found that small (2–15 mm) cuts in paper channels could be used to control the flow rate—cuts perpendicular to the channel reduced flow rate, while parallel cuts increased flow [95].

Craft cutters can be used to cut materials besides paper to speed up the fabrication process—the Remcho group used a craft cutter to pattern Parafilm® before melting it into paper to form hydrophobic barriers [96, 97]. Cassano et al. cut both paper and laminate film for a multi-layer device [98], and Yu et al. cut paper, tape, and PVC backing with a craft cutter [99]. The downside of these methods is that they require tedious alignment of the various layers by hand. To automate the alignment process when multiple fabrication steps are required, Rahbar et al. developed an optical scanning process that allows the plotter to align to registration marks printed on the substrate [100]. With this automatic alignment, the plotter was able to cut out and deposit reagents on wax-printed paper devices with high accuracy and precision. Because craft cutters are commercially available for $65 to $1500 (USD), they are an attractive low-cost tool for μPAD fabrication.[22] Given their relatively low speed, however, craft cutters and plotters are not viable for applications requiring medium-to-high throughput.

4.2.3. Laser cutting and laser treatment

Laser cutters are a versatile tool for paper device fabrication, as they can be used to cut, etch, polymerize, and more with excellent resolution. Depending on the laser power and speed, the laser can cut through the full thickness of the substrate, or merely etch the surface. Chitnis et al. took advantage of this flexibility by etching away the hydrophobic coating of wax paper, parchment paper, and palette paper to create hydrophilic zones [49]. The laser cutter could pattern features as small as 62 ± 1 μm, but the hydrophilic zones had to be coated with silica microparticles to enable fluid wicking [49]. Kim et al. [101] took the opposite approach with paper that had been partially fused with Parafilm®, etching away the paper but leaving the Parafilm® below. This ablative etching of Parafilm®-laminated paper achieved barriers as small as 137 ± 22 μm and functional channels 150 μm wide, and the resulting devices were compatible with both surfactants and organic solvents [101].

Nie et al. used a laser cutter to burn hollow microstructures through the thickness of the paper to act as hydrophobic barriers [102]. These devices were designed to remain attached to the surrounding paper, which provided enough mechanical stability that no solid support was needed, but also meant that no free-standing, fluidically isolated structures could be patterned. In contrast, most free-standing cut designs require some sort of solid support [103]. Cover and support layers are essential when laser cutting nitrocellulose membrane, as it is flammable in the presence of air, so Spicar-Mihalic et al. sandwiched nitrocellulose between two thin polymer layers during the cutting and etching process [104]. In Mahmud et al.'s 2018 study, backing paper with aluminum foil before laser cutting provided mechanical strength, but did not significantly affect the resolution or minimum channel width of the method [48]. After testing five paper types, they found that the minimum functional channel width depended on the size of the paper fibers—smaller fibers led to smaller functional channels [48]. Thus, Whatman filter paper grade 50 produced the largest channels (139 ± 8 μm), while nitrocellulose produced the smallest channels (24 ± 3 μm)—the narrowest reported channel for any literature method (see Table 3 above) [48].

Laser cutters are capable of more than just cutting—Sones et al. developed a laser-direct-write patterning technique, where paper is soaked in a photopolymer, treated with a laser to polymerize the desired hydrophobic zones, and washed to remove unpolymerized reagent from the remainder of the paper [51]. This method was adapted to create fluid delays [105] and 3D devices [106], as well as to pattern nitrocellulose membrane with channels down to 100 μm and barriers as narrow as 60 μm [107].

Another creative application of a laser cutter, "laser-heating-wax-printing" was reported by Le et al. [108] In their study, filter paper was rubbed by hand with a thin layer of solid paraffin wax, and then an inexpensive mini CO2 laser cutter was used to melt the wax where hydrophobic barriers were desired [108].

Laser-based methods have excellent resolution and reasonably good throughput, making them an attractive option for paper device fabrication. Given the high cost of freestanding laser cutters (>$20,000), laser cutting may be out of reach for some [22,104], but the advent of less-expensive benchtop models (~$2,000) and the increasing popularity of community maker-spaces are making laser cutters more accessible [104]. But, to be viable for mid-to-large-scale production, laser-based methods must be simplified and/or automated to minimize hands-on steps like backing, coating, and pre- and post-treatment.

4.2.4. 3D printing

3D printing with wax is a creative semi-automated method for PAD fabrication. Since commercial 3D printers are not designed to print wax, some modification is required. Using an open-source hardware approach, Pearce et al. converted a Prusa Mendel RepRap into a wax 3D printer by fitting it with a heated syringe pump to extrude Cerita wax with 20 μm accuracy [109]. The heated platform could then be used to further melt the wax into the paper [109]. The total cost for all of the components of the open-source hardware 3D wax printer was less than $90 [109]. Similarly, Chiang et al. created a custom copper print head for a Prusa i3 3D printer [110]. Because the wax is deposited in its molten form, no separate heating step is needed to reflow the wax; however, eight layers of wax were required to form a complete hydrophobic barrier on filter paper [110]. Building a custom wax 3D printer may be less convenient than using off-the-shelf equipment like a craft cutter, but the open-source hardware approach makes wax 3D printing accessible for many researchers. The need for multiple layers of wax, however, limits throughput for this innovative approach, as each additional pass increases the time required and introduces potential misalignment. Thus, while these methods are not currently suited for mid-to-large-scale production, further development to streamline the wax extrusion process and minimize the number of passes needed may increase their promise.

4.3. Batch methods

Batch methods, such as stamping, dipping, spraying, and screen printing, use minimal equipment (stamps, masks, screens) to streamline the fabrication process and improve reproducibility, but they still require substantial hands-on labor. Although throughput and resolution are limited, and prototyping is constrained by the need to fabricate the stamp, mask, or screen, these methods remain attractive for small-to-medium-scale production and use in low-resource settings because of their low cost and simplicity, and the fact that many of them do not require access to electricity.

4.3.1. Stamping

Stamping was the earliest method used to create hydrophobic barriers in paper. In 1937, Yagoda developed two different methods for stamping wax onto paper [67]. To pattern a simple circle, a metal tube was heated, brought into contact with a block of solid paraffin, and then pressed against the paper surface [67]. For more sophisticated patterns, a metal stamp was heated and used to transfer wax from a wax-impregnated tissue to the paper substrate [67]. The same wax stamping technique, with minor variations, was deployed by Mueller and Clegg in 1949 [68] and by the Coltro group in more recent years [111,112]. To more easily create the metal stamps required for wax stamping, Zhang et al. used printed circuit technology to etch the desired pattern into copper [113]. Even so, each design required a separate metal stamp. Zhang et al. partially solved this problem with "moveable type wax printing," which utilizes an iron stamp with moveable pattern elements to stamp multiple designs with a single tool [114].

Wax, of course, is not the only material for stamping hydrophobic barriers onto paper. Using a custom PDMS stamp, Curto et al. patterned paper with indelible fountain pen ink [115]. Akyazi et al. used a similar method, but stamped the paper on both sides three times [116]. Several researchers have stamped PDMS solution onto paper, since PDMS is flexible, readily available, and compatible with some organic solvents and surfactants. The main difference between these studies was how the stamp itself was fabricated. Because Sun et al. used art paper as their substrate, their method required a custom thermo-molded Teflon stamp to prevent the PDMS from sticking to the stamp rather than the smooth paper [117]. Dornelas et al. used a custom photopolymer rubber stamp [118], while He et al. developed flash foam stamp lithography, creating a custom photosensitive seal stamp by exposing a polyethylene-based flash foam to radiation [58]. Flash foam stamp lithography is extraordinarily inexpensive—the cost of one stamp was estimated to be $0.15, which makes rapid prototyping easy and affordable [58]. As these studies illustrate, the main difficulty with stamping-based methods is the design and fabrication of the stamp—beyond that, the process is simple, low-cost, and requires only minimally trained personnel. Throughput, however, is limited by the fact that devices are typically stamped one at a time. For any mid-to-large-scale application, efforts would need to be made to stamp multiple devices at one time with a large stamp and to automate the stamping process.

4.3.2. Dipping

Wax dipping involves sandwiching paper between a glass slide and an iron mask with a magnet before briefly dipping it in molten wax. The areas covered by the mask remain hydrophilic, while the remaining paper is saturated with hydrophobic wax [59,60]. A slightly more complex version of this process was reported by Nechaeva et al. [119]. The need to obtain a new iron mask for each pattern limits the ease of prototyping, but new masks cost only ~$0.35, so this is not a significant barrier [59]. However, only relatively simple patterns (no independent freestanding hydrophilic regions) can be fabricated with this method. As with other wax-based methods, resolution is limited, as the wax spreads laterally into the edges of the hydrophilic areas before cooling. Throughput is limited by the fact that the masking, dipping, and cooling steps must be performed for each individual device, but Songjaroen et al. were reportedly able to achieve a throughput of ~90 devices per hour [59]. This method, then, is best suited for smaller-scale applications where hands-on labor is not a concern.

4.3.3. Spraying

In spraying-based methods, spray paint or lacquer is used to hydrophobize paper. Nurak et al. developed a lacquer-spraying method by covering paper with a patterned iron mask held on by a magnet before spraying the exposed regions with acrylic gloss lacquer to create hydrophobic zones.[120] This method requires a custom iron mask, but a variation by Deng et al. took a different approach to create multilayer devices, using a hole punch and aerosol spray paint to pattern the hydrophobic layers [121]. Even distribution of the spray can be difficult, and the pattern quality is strongly influenced by the paper's porosity—Nurak et al. found that higher-porosity paper, such as Whatman 4, produced more well-defined patterns than lower-porosity paper, such as Whatman 1, because the lacquer penetrated the pores of the paper more quickly [120]. Like stamping, this method would be more viable for mid-scale manufacturing if it were automated, for instance, with a roll-to-roll method.

4.3.4. Screen printing

Screen printing is a method commonly used to pattern textiles, but it has also been adapted to rapidly produce paper analytical devices. In traditional screen printing, ink is pressed through a patterned screen onto the substrate using a squeegee. Dungchai et al., however, screen printed µPADs by rubbing solid candle wax through a patterned screen onto paper and then heating the paper on a hotplate to melt the wax and create hydrophobic barriers [122]. Namwong et al. adapted this method in a classroom setting by having students melt the wax with a hair dryer [123]. Several patterning reagents are compatible with screen printing—Sameenoi et al. used a solution of polystyrene in toluene [61]. Mohammadi et al.[124] and Shangguan et al. [125] used PDMS, and Kajornklin et al. used waste rubber from recycled latex gloves dissolved in gasoline [62]. PDMS requires a heating step to cure the polymer after screen printing, and according to Shangguan et al., rapid heating increases resolution by limiting lateral spread of the PDMS solution [125]. Screen printing can also be used to deposit functional polymers and electrodes [126].

The advantage of screen printing is that it is a simple and relatively high-throughput method that can potentially be automated for scaling up production. Resolution varies by patterning material—polystyrene gave 670 ± 50 µm channels and 380 ± 40 µm barriers [61], while rubber latex created channels as small as 256 ± 21 µm [62]. Although screens must be custom-made and are prone to wearing out after several uses, they are quite inexpensive (~$5) [122]. Because of the nature of screen printing, however, large volumes of patterning reagent are required, and some waste is inevitable. Screens must also be cleaned in between uses, increasing the time and labor involved. The screen-printing methods currently reported in the literature are not intended for large-scale production, but given that screen-printing is used commercially by companies such as T-shirt manufacturers, this method holds great promise for producing large numbers of µPADs.

4.4. Laboratory methods

Laboratory methods require specialized equipment and/or reagents typically found primarily in research laboratories. Such methods include photolithography, silanization, plasma treatment, chemical vapor deposition, and modification of omniphobic or superhydrophobic paper. While several of these methods have been adapted to be more manufacturable, most remain useful only for laboratory-scale proof-of-concept studies.

4.4.1. Photolithography

Photolithography uses UV light and a patterned mask to selectively polymerize a photoresist. In the Whitesides group's notable 2007 study, paper was soaked in a solution of SU8 photoresist, prebaked to remove the solvent, exposed to UV light through a photomask with the desired pattern, post-baked to cross-link the UV-exposed regions, treated with propylene glycol monomethyl ether acetate (PGMEA) and propanol to remove unpolymerized photoresist, and exposed to oxygen plasma to restore hydrophilicity to the unexposed regions [127]. This method is capable of very high resolution, but it requires specialized equipment, expensive SU8 photoresist, and many tedious steps. Thus, a year later, Martinez et al. reported a simplified method called Fast Lithographic Activation of Sheets, or FLASH, which used a homemade photoresist, a low-cost photomask made of transparency film and construction paper, and a simple UV lamp or even sunlight, with no need for plasma treatment or a cleanroom [52].

Other groups likewise reported modifications to the photolithographic method. OuYang et al. created a simplified photolithographic method in which paper was hand-coated with a thinned SU8 solution, covered with a photomask, exposed to UV light, and then washed with acetone to remove unpolymerized photoresist [128]. In 2010, Klasner et al. developed a novel siloxane- and acrylate-based polymer blend that enabled fabrication of narrower features than traditional SU8 in three minutes per device [50]. No plasma treatment step was needed—devices were simply rinsed with acetone after exposure.[50] Unlike most devices created with photolithography, which can crack when bent, Klasner et al.'s devices were flexible and could be flexed without damage.

Several researchers have found creative ways to use photolithography to make 3D µPADs. Martinez et al. stacked layers of photoresist-patterned paper with double sided tape to create 3D devices [129]. The original design required gaps between layers to be filled with cellulose powder to enable fluid flow [129], but a later modification turned that liability into an asset by using the open channels as programmable fluid gates [130]. Another approach by Yu et al. combines photolithography with hot embossing to fabricate 2D and 3D Parafilm®-based paper devices [131]. Mora et al. found that by exposing the photoresist-impregnated paper to UV through a photomask from both sides, it was possible to create 3D channels within a single sheet of paper [132].

He et al. developed dynamic mask photocuring, which uses a desktop stereolithography 3D printer to achieve similar results to photolithography, but without the need for a predesigned mask [133]. Paper is soaked in a UV-sensitive resin, and then the 3D resin printer uses a liquid crystal display as a dynamic mask to expose the paper to UV light in the desired pattern [133].

Photolithography has excellent resolution, but requires specialized equipment, expensive reagents, and multiple steps. In addition, since the paper is exposed to patterning reagents and solvents, residue may negatively impact assay performance [77]. While much work has been done to simplify the process and reduce the cost, photolithography remains more suited to the laboratory than to mass production.

4.4.2. Plasma treatment

Plasma treatment typically involves hydrophobizing the entire paper surface, and then exposing select regions to plasma to restore hydrophilicity. In a 2008 study by Li et al., paper was soaked in a solution of alkyl ketene dimer (AKD), a commercial paper-sizing agent, and baked to cure the AKD [134]. The hydrophobic sheets were then sandwiched between metal masks and treated with plasma to create hydrophilic zones [134]. A related method published by Obeso et al. used poly (hydroxybutyrate), a biodegradable polymer, to create superhydrophobic paper, which was patterned with hydrophilic regions using plasma treatment [135].

Unlike plasma treatment, which uses plasma to selectively remove a patterning material to restore hydrophilicity, plasma polymerization uses plasma to deposit a hydrophobic polymer onto paper. Kao and Hsu developed a rapid, one-step fabrication process in which paper was sandwiched between a positive and a negative metal mask before plasma polymerization with C4F8 [136]. The advantage of this approach is that the hydrophilic regions of the paper are not exposed to the patterning material.

One downside of these methods is that they require toxic solvents such as heptane [134] and chloroform [135], or environmentally hazardous fluorocarbons [136] for patterning. They also rely on expensive plasma reactors, which require skilled technicians and carefully chosen operating conditions. For these reasons, these methods remain suited nearly exclusively for smaller-scale laboratory studies.

4.4.3. Silanization

In silanization, silanes covalently bond to the hydroxyl groups of cellulose to render the paper surface hydrophobic. To obtain hydrophobic/hydrophilic contrast, He et al. silanized paper with a solution of octadecyltrichlorosilane (OTS) in heptane, then exposed it to UV light for 90 min through a quartz mask [54]. The areas covered by the quartz remained silanized, while the UV-exposed regions became hydrophilic once more [54]. Asano and Shiraishi took a similar approach, but used a 3D printed photomask in place of the more expensive quartz mask [137]. Yan et al. used plasma rather than UV irradiation to restore hydrophilicity to the pattern.[138].

Cai et al. developed two different silanization techniques that rely on a paper mask rather than on UV or plasma treatment. In one, the entire paper surface is silanized, and then a separate paper mask soaked in a NaOH etching solution is placed in contact with the hydrophobic silanized sheet [139]. The areas touching the paper mask become hydrophilic as the NaOH solution etches away the silane [139]. In the other approach, the paper mask is soaked with the trimethoxyoctadecylsilane patterning reagent [140]. When paper is sandwiched between the soaked mask and glass slides and heated, the areas touching the mask are silanized, while the rest of the paper remains hydrophilic [140]. These two paper-mask-based methods substantially reduce the cost and equipment requirements for silanization, making it more attractive for resource-limited settings than most laboratory-scale methods, but they remain fairly low-throughput methods.

4.4.4. Chemical vapor deposition

Chemical vapor deposition (CVD) is a process in which vapor-phase polymer precursors are used to deposit thin solid films on a substrate surface. In the context of μPAD fabrication, CVD is used to deposit a hydrophobic layer, usually in combination with a mask or inhibitor to pattern the hydrophilic regions. Gupta and coworkers developed several applications of CVD, including directed deposition, which used metal salts as inhibitors [141]. CVD of functional polymers, which deposited ionizable polymers for cation/anion separation and a UV-sensitive polymer as a fluid-control switch [142], and initiated chemical vapor deposition (iCVD), which created fluoropolymer barriers that were compatible with organic solvents [143]. They found that the metal salts used to inhibit deposition could be patterned by hand-painting (low resolution, but simple), spraying with a stencil (rapid, but wasteful), or photolithography (high resolution, but complex).[143].

Rather than using metal salt inhibitors, Demirel and Babur sandwiched paper between metal masks and magnets before depositing poly (chloro-*p*-xylene) using iCVD [144]. Lam et al. took an even more low-tech approach in their CVD of trichlorosilane—in their method, hydrophilic zones were simply covered with vinyl tape, which could later be removed by heating [145]. The advantage of these physical masking approaches is that they are simpler than metal salt inhibition and require fewer steps, although they may increase the amount of hands-on labor. However, CVD inevitably requires specialized equipment, and its throughput is limited by the number of devices that can fit in the deposition chamber at one time. CVD, then, is best used only for small-scale, laboratory-based applications.

4.4.5. Omniphobic/superhydrophobic paper

The last category of laboratory-scale techniques covered here involves modification of hydrophobic paper. Unlike the other paper-based devices discussed in this review, these devices do not rely on spontaneous capillary flow. Instead, they either use gravity to move droplets across a superhydrophobic surface when the device is tilted [146], or they require an external pump to move fluid through enclosed hydrophobic channels, similar to conventional microfluidic devices [147, 148].

The first class of these devices use slightly less hydrophobic regions to direct droplets across a superhydrophobic surface. Balu et al. fabricated their own paper, used plasma etching to modify the roughness and a fluorocarbon film to render the surface superhydrophobic, and then wax printed patterns [149]. Because the printed wax was more hydrophilic than the paper surface, water droplets that beaded up on the paper surface could be guided by the patterned wax when the device was held at an angle [149]. Barona and Amirfazli made a similar device with regular copy paper and inkjet printing—the paper was rendered superhydrophobic by spraying with a nanocomposite film, then patterned with commercial inkjet ink to create less-hydrophobic zones [150]. Sousa and Mano used poly(hydroxybutyrate) in chloroform to create superhydrophobic paper, then drew hydrophilic zones by hand with a coal pencil or water-based marker, or printed them with commercial inkjet ink [151]. Elsharkawy et al. also used inkjet ink to pattern the hydrophilic areas, but they used sandpaper dropcast with a fluoroacrylic copolymer as the superhydrophobic surface [146].

The second class of these devices rely on pumps to drive fluid flow through channels. Glavan et al. created omniphobic paper by vapor-phase deposition of fluorinated organosilanes [152], carved channels into the omniphobic paper with a craft cutter, and enclosed the channels with tape [147]. Thuo et al. used embossing and cut-and-stack methods to form channels in silanized paper [148]. Because these devices rely on an external pump, their applicability is limited.

The advantage of these superhydrophobic approaches is that droplets are not absorbed by the paper and thus are available for later analysis [146,152]. However, as they require environmentally hazardous halogenated compounds, many steps, and specialized equipment, they remain laboratory-scale methods.

4.5. Printer-based methods

Printer-based methods have the potential to offer an ideal compromise between ease of prototyping and scalability, as they offer reasonably high throughput and the ability to print almost any designed pattern. While a few of these methods require expensive equipment such as UV chambers or research-grade inkjets, many use commercially available printers, which are affordable and accessible to nearly any researcher.

4.5.1. Wax printing

Wax printing uses solid-ink printers, such as Xerox's Phaser and ColorQube product lines, to deposit a wax-based ink, which can then be melted into the paper to create hydrophobic zones. This method was first reported for paper in 2009 by Carrilho et al. [76] and Lu et al. [87], and applied to nitrocellulose membranes by Lu et al. in 2010 [153]. Because of its simplicity, relatively low cost, ease of prototyping, and scalability, wax printing was rapidly adopted as the method of choice for many researchers [1,76]. Innovations included backing the wax-printed devices with a printed wax layer [71] instead of packing tape [154], as well as forming enclosed channel s[155,156] or 3D structures in a single sheet [157] by carefully designed double-sided printing. After Noh et al. used droplets of paraffin dissolved in hexane as "meters" to slow fluid flow [158]. Jang et al. discovered that the paper's permeability and lateral flow rate could be controlled by varying the intensity of printed wax [159].

Wax printing typically has lower resolution than many other techniques because the wax spreads laterally when heated [76], but Yeh et al. and Tenda et al. showed that resolution could be improved by heating the wax-printed paper with a laminator rather than a hotplate or oven [53,160]. Strong et al. addressed the resolution problem by shrinking wax-printed paper with periodate to obtain miniaturized devices [161].

Wax-printed sheets can be layered to construct 3D devices. Schilling et al. wax printed devices and folded them, using printed laser toner as a thermal adhesive between the layers [162]. Rather than assembling devices individually, which is tedious and time-consuming, Lewis et al. stacked entire sheets with spray adhesive and then cut the stacks into many individual devices [163]. Xiao et al. adapted the principles of bookbinding by sewing or stapling together stacks of wax-printed sheets, a method which, in principle, is mass-producible [164].

Shortfalls of wax printing include the wax's sensitivity to heat and inability to contain alcohols and organic solvents. The biggest problem with wax printing, however, is that Xerox discontinued its line of solid-ink printers in 2016, and no other company has adopted the technology. Thus, while wax printing was once hailed as one of the most mass-producible fabrication methods because of its low cost, ease of prototyping, user-friendliness, commercially available equipment, and medium-to-high throughput [23], wax printers are becoming increasingly scarce, and successor technologies are needed.

4.5.2. Laser printing

Laser printing is a potentially promising printer-based technology for μPAD fabrication. Printer toner is composed of resin, which, when heated after printing, can melt and reflow to serve as an adhesive between paper layers [162] an encapsulator around paper channels [165], or even a hydrophobic barrier similar to wax.[166] The heating step can be performed with a thermal laminator [162], a hotplate [167], or an oven [57]. Functional barriers as small as 200 μm and channels as narrow as 415 μm were reported by Ghosh et al.[57].

Laser printing is convenient, as the only equipment required is a commercially available laser printer and a heat source. The method, however, has several shortcomings. Often multiple coats of toner are required [165], which increases fabrication time and introduces alignment issues. Toner formulations vary greatly—Ruiz et al. reported that out of the five toners they tested, only two produced functional devices, and there was no clear pattern that would enable them to predict whether or not a given toner would work [47]. Perhaps most concerningly, the long baking times (Shi et al. call for a five-hour heat treatment at 150 °C [166]) and/or high temperatures (200 °C for one hour in Ng and Hashimoto's 2020 method [168]) required to melt the toner have been reported to pyrolyze the paper [168], which severely limits the utility of the technique.

4.5.3. Thermal transfer printing

Thermal transfer printing, most often used to print receipts and shipping labels, uses heat to transfer a wax-based ink from a ribbon to a paper substrate. A custom-built roll-to-roll version of this technique will be discussed in the next section, but recently, Ruiz et al. tested a cheap ($250), commercially available thermal transfer printer designed to print on 8.5 × 11 in. sheets of paper [47]. The authors found that one-sided printing on Whatman 1 chromatography paper followed by baking for 15 min at 90 °C produced functional devices with channels as narrow as 627 ± 2 μm and barriers as small as 749 ± 31 μm [47]. This technique retains many of the advantages of wax printing—rapid prototyping, low cost, low hands-on labor—with the added benefit of portability—the printer is only 1.5 pounds and runs on a rechargeable battery, and thus would work for printing in a field setting [47]. While not suitable for mass production, thermal transfer printing offers a viable alternative to wax printing for prototyping and small-batch production.

4.5.4. Inkjet printing

Inkjet printing is a powerful tool for μPAD fabrication, as it can be used both to pattern hydrophobic barriers and to deposit reagents [32]. As reagent deposition is beyond the scope of this review, however, only hydrophilic/hydrophobic patterning will be discussed here. The earliest inkjet-based approach was inkjet etching, reported by Abe et al. in 2008 [169]. Paper was soaked in a solution of poly(styrene) in toluene to render it hydrophobic [169]. The hydrophilic pattern was etched by printing toluene with a modified piezo-driven inkjet printer [169]. After ten print cycles, the printed toluene dissolved the poly(styrene) sufficiently to render the pattern hydrophilic [169]. Wang et al. used inkjet etching to pattern paper with a hydrophobic methylsilsesquioxane (MSQ) sol gel technique [170]. The paper was soaked in methyltrimethoxysilane (MTMS) and air dried, then etched with an NaOH/glycerol solution using an inkjet printer to achieve hydrophilic/hydrophobic contrast [170]. Since the inkjet etching method requires separate soaking and printing steps as well as multiple print cycles, most inkjet-based methods focused on printing hydrophobic material directly.

In 2010, Li et al. used the paper-sizing agent AKD dissolved in heptane as the ink in a reconstructed commercial inkjet printer [171,172]. Upon heating for 8 min at 100 °C, AKD forms covalent bonds with the cellulose hydroxyl groups, rendering the AKD-printed patterns hydrophobic [171,172]. When relatively thin Whatman 4 chromatography paper was used, one printing cycle of one side was sufficient to form functional hydrophobic barriers [171]. Since AKD is one of the cheapest hydrophobic patterning materials available [172], this method is particularly attractive for fabricating μPADs for low-resource settings, and has been used by several other research groups [10,173].

Inkjet printers have also been used to print PDMS [174], hydrophobic sol-gel derived methylsilsesquioxane (MSQ) [170], silicone resin [175], and permanent marker ink [176]. Maejima et al. even used an environmentally benign UV-curable ink in an off-the-shelf tank-style inkjet printer [55]. Each of these materials comes with unique challenges and advantages. MSQ and silicone resin are resistant to surfactants, and therefore can be used for cell-lysing experiments, but MSQ requires research-grade printers and a long cure time [170]. Silicone resin, on the other hand, can be printed with inexpensive commercial inkjets and has a rapid cure time, but the catalyst and siloxane solutions must be printed with two separate printers to avoid the ink polymerizing prematurely and clogging the printer [175].

Inkjet printing offers excellent resolution and easy prototyping, as well as rapid fabrication times. The main challenges with inkjet methods center around the choice of ink, as inks must a) lie in a narrow range of viscosity values (1–40 cps) to be printable [174,177] and b) be chemically compatible with the printer components to avoid damaging the equipment [171]. While some inkjet methods require research-grade printers, many are compatible with off-the-shelf or slightly modified commercially available inkjets, making inkjet printing an accessible method for most researchers. Since most inkjets are capable of printing several pages per minute, inkjet printing can have good mid-scale throughput. Given the low cost and ubiquitous availability of commercial inkjets, the ease of prototyping varied patterns, and the medium-to-high throughput, inkjet printing may be the most directly comparable successor technology to fill the mid-scale manufacturing gap left by wax printing.

4.6. Roll-to-roll methods

Roll-to-roll methods are the most conducive to large-scale manufacturing—many print houses and commercial test strip manufacturers use roll-to-roll patterning methods. Prototyping, however, is difficult, given the need to produce a new template for each pattern, and equipment costs can be prohibitive.

4.6.1. Flexography

Several approaches to roll-to-roll μPAD fabrication have been developed. In flexography, the anilox roll transfers ink from a reservoir to a patterned plate on the plate roll. The printing plate then presses against the paper on the impression roll to print the pattern. Olkkonen et al. produced paper-based devices with flexographic roll-to-roll printing of polystyrene [56]. While at least two printing cycles were required to produce hydrophobic barriers, ink spreading was negligible, leading to high resolution [56]. Määttänen et al. used flexography to pattern paper with PDMS [174]. Six layers of PDMS were needed to make functional hydrophobic barriers, and a heating step was required to cure the polymer [174]. The Sikes group used roll-to-roll printing of a UV-curable resin to create devices that could be folded into four-layer flow-through assays [178]. The advantage of flexography is its compatibility with a wide variety of patterns. The need for multiple print cycles somewhat hinders the utility of the technique, but flexography remains highly promising for large-scale paper device production.

4.6.2. Liquid flame spray

Liquid flame spray is another roll-to-roll-compatible method for μPAD fabrication—paper is hydrophobized by continuous liquid flame spray of titanium dioxide nanoparticles in a roll-to-roll process, then selectively made hydrophilic again by UV exposure [179], heat, or plasma [180]. While the nanoparticle flame spray step is roll-to-roll compatible, additional treatment steps require more hands-on time. For this method to be viable for large-scale production, the post-treatment steps would need to be integrated into the roll-to-roll process.

4.6.3. Roll-to-roll wax printing

Another roll-to-roll approach, reported by Liu et al., uses a wax ribbon to thermally transfer wax patterns to a roll of newsprint paper [181]. Unlike the thermal transfer printer used by Ruiz et al., which was compatible with chromatography paper [47], this roll-to-roll wax printer requires thin newsprint, because the commercial wax ribbons do not deposit enough wax to create complete hydrophobic barriers in thicker paper. The authors posit, however, that if the method were scaled up to manufacturing scale, thicker wax ribbon could be produced, which would allow printing on thicker paper types [181]. Recently, Monju et al. took a step in this direction by performing thermal transfer with a homemade wax ribbon consisting of paraffin, synthetic wax, and ethylene-vinyl acetate copolymer resin [182]. The thicker ribbon enabled barrier formation on a variety of paper substrates, including thicker papers [182]. Roll-to-roll wax printing allows for easy prototyping, as the thermal printhead will print any computer-generated pattern. The method is inherently wasteful, however, as much of the wax on the ribbon is discarded. Although an additional heating step is required after printing, this method is very rapid (the roll-to-roll printer can produce ~10 devices per second) and low cost ($200 initial investment in the printing equipment, $4 per 80-meter roll of wax ribbon) [181], making it highly attractive for producing large numbers of devices.

4.6.4. Roll-to-roll air-gap fabrication

The air-gap design [183] takes inspiration from the roll-to-roll lamination techniques used by manufacturers of commercial products such as pH strips and aquarium test strips. The air-gap PAD consists of hydrophilic paper test zones fixed to a hydrophobic backing with double-sided adhesive, separated by an "air gap" rather than a traditional hydrophobic barrier [183]. Currently, the air-gap method can produce only straight lanes and square dot features, but these architectures have wide applicability. The authors partnered with a commercial test strip manufacturer to produce the devices, which were compatible with aqueous solutions, including surfactants, but not with organic solvents [183]. The advantage of this method is its high-volume, relatively low-cost production—the authors report that a single manufacturing run produced 2700 linear feet of product (roughly 162, 000 devices) for as little as $0.03-$0.05 per device [183]. The disadvantage is that it uses industrial equipment unavailable to many researchers. The authors point out, however, that this barrier can be overcome by collaborating with commercial manufacturers to use their roll-to-roll equipment [183].

5. Discussion and conclusions

The lack of scalable paper device fabrication methods is one of the biggest hurdles facing the field of paper microfluidics today. Hand-fabrication methods, such as drawing, cutting, or folding, are cheap, equipment-free, and offer unlimited flexibility, but since they lack reproducibility and are too time consuming to be scalable, they are best suited for prototyping and classroom applications. Semi-automated methods, such as plotting and craft cutting, and batch methods, including stamping, spraying, dipping, and screen printing, use inexpensive equipment to improve reproducibility and throughput, but are still only suited for small-to-medium-scale applications. Laboratory-based methods, such as photolithography, chemical vapor deposition, silanization, plasma treatment, and modification of superhydrophobic or omniphobic paper, may produce excellent resolution, but they are often time consuming and require hazardous chemicals, specialized equipment, and trained personnel. Printer-based methods, such as wax, laser, and inkjet printing, offer convenient prototyping and good throughput, and, if they use a commercially available printer, provide an accessible option for μPAD fabrication. Roll-to-roll methods rapidly produce large numbers of devices, but they rely on expensive, specialized equipment.

If the field of paper microfluidics is to move beyond endless proof-of-concept studies and begin to produce devices for real-world applications, researchers must shift the focus away from small-scale prototyping and toward mid-to-large-scale fabrication methods. For too long, researchers have focused on factors such as resolution and novelty, while downplaying the crucial importance of throughput and the cost of labor and equipment. For simple prototyping, low-tech methods like hand fabrication may be sufficient, but commercialization will require high-throughput manufacturing-scale methods like roll-to-roll processing. For the numerous applications that fall somewhere between these two extremes, in the range we term mid-scale manufacturing, methods will require both medium-to-high throughput and affordable, easy-to-use equipment. In the past, wax printing filled this need for mid-scale production, but with the growing scarcity of wax printers, successor technologies are needed, and inkjet-based methods are a promising alternative. Future research should focus on making more mid-scale and high-throughput production methods accessible to researchers and entrepreneurs alike.

CRediT authorship contribution statement

Rachel M. Roller: Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Marya Lieberman:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marya Lieberman reports financial support was provided by National Science Foundation. Rachel M. Roller reports financial support was provided by Howard Hughes Medical Institute. Rachel M. Roller reports financial support was provided by USAID. Marya Lieberman and Rachel M. Roller are co-inventors on patent #63/373,059 pending to University of Notre Dame. Marya Lieberman is the Founder and CEO of Paper Analytics, LLC, a not-for-profit that sells paper analytical devices for education and pharmaceutical screening at cost.

Data availability

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2023.134059.

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Rachel M. Roller is a chemistry Ph.D. candidate at the University of Notre Dame. Her collaborative work using paper microfluidics to promote inquiry-based chemical education with the MICRO Project has impacted over 2000 students at 30+ colleges and universities. Her recent work on the air-gap PAD focuses on developing scalable manufacturing strategies for paper analytical device fabrication.

Marya Lieberman's group at the University of Notre Dame designs and implements technologies for performing chemical analysis in low-resource settings. A leader in the field of paper microfluidics, she has published 90 peer-reviewed papers, including paper-based tests for substandard and falsified pharmaceuticals, illicit drugs, nutrients, and environmental toxins.