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Review

Recent advances in low-cost microfluidic platforms for diagnostic applications

The use of inexpensive materials and cost-effective manufacturing processes for mass production of microfluidic devices is very attractive and has spurred a variety of approaches. Such devices are particularly suited for diagnostic applications in limited resource settings. This review describes the recent and remarkable advances in the use of low-cost substrates for the development of microfluidic devices for diagnostics and clinical assays. Thus, a plethora of new and improved fabrication methods, designs, capabilities, detections, and applications of microfluidic devices fabricated with paper, plastic, and threads are covered.

Keywords:

Bioassays / Clinical assays / Paper-based microfluidic devices / Plastic substrates / Point-of-care / Thread
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1 Introduction

Using microfluidic devices to perform diagnostic assays is an exciting approach that has gained much attention in the past ten years [1, 2]. This increasing interest may be attributed to the fact that microfluidic devices are portable, require small sample and reagent volumes, and can conveniently integrate the necessary steps for rapid diagnostic assays. Thus, great effort and considerable resources have been focused on the development and evaluation of new substrates, microfabrication techniques, and detection methods to attain inexpensive, robust, disposable, and portable point-of-care (POC) diagnostic devices. Recently, microfluidic devices have emerged as a promising diagnostics solution to improve human health in resource-poor and remote settings [3]. In such environments, POC diagnostic devices could provide an adequate solution to be used even by untrained personnel under challenging environmental conditions and limited power.

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Abbreviations: AFP, α -fetoprotein; ALP, alkaline phosphatase; AuNP, gold nanoparticle; CEA, carcinoembryonic antigen; CRP, C-reactive protein; ECL, electrochemiluminescence; hCG, human chorionic gonadotropin; ITO, indium tin oxide; μ PAD, paper-based microfluidic devices; OTS, octadecyltrichlorosilane; PC, polycarbonate; POC, point-of-care; PSA, prostate-specific antigen; PT, polyester-toner; SAW, surface acoustic waves; SERS, surface-enhanced Raman spectroscopy

The cost of a POC diagnostic device is also critical and should be as low as possible, particularly if the microdevice is intended to be used in resource-poor settings [4]. Achieving such cost reduction using inexpensive materials together with cost-effective manufacturing process for mass production of POC microfluidic devices is possible. Despite the low cost, substrates must be biocompatible, easily functionalized, adequate for diagnostic detection methods, and non-biohazardous when disposed off.

Although silicon, and, to a greater extent, glass, have been primarily used for microfluidic device fabrication, the associated costs and required manufacturing processes are not practical for mass production of POC microfluidic devices for communities with limited resources. Currently, plastic and paper are considered among the most affordable, easily disposable, and versatile materials for large-scale fabrication of inexpensive microfluidic devices for diagnostic applications [3, 5–8].

This review covers the primary advances reported in works published from 2011 up to September 2013 regarding fabrication methods, designs, capabilities, and practical applications of microfluidic devices for diagnostic applications fabricated with low-cost materials, such as paper, plastic, and thread.

2 Paper-based microfluidic devices

Paper is affordable, abundant, disposable, and compatible with large-scale manufacturing processes for the production of microfluidic devices. Because of these unique features,

Colour Online: See the article online to view Figs. 1–3 and 5–11 in colour.

paper platforms have emerged as advantageous for POC diagnostic device fabrication [9]. Recent developments in paper-based microfluidic devices (μ PADs) for diagnostic applications are summarized in the following sections.

2.1 Fabrication techniques

The μ PADs are usually fabricated by patterning hydrophobic barriers in and on hydrophilic paper. Usually, photoresist and wax have been the preferred materials to create these hydrophobic regions in paper [3, 5]. The wax printing method [10] has become the most frequently used process for quickly producing μ PADs. In this technique, the microfluidic layout is printed onto a paper surface using a wax ink printer. The wax printed paper is then heated to melt the wax, which allows it to flow into the paper pores, generating hydrophobic walls within the paper. Some variations of this approach have also been reported by many authors. For instance, Dungchai et al. [11] used a screen-printing technique to deposit wax on a paper surface. In essence, this fabrication technique is similar to the wax printing method; however, the wax printer was replaced by a patterned screen. Songjaroen et al. [12] developed a wax-based fabrication method in which chromatographic paper was placed on a glass slide surface, and an iron template was kept attached to the paper surface by the attractive force of a permanent magnet. This paper was then dipped into melted wax, which penetrated the porous paper in the regions not covered by the iron template. The wax in this process was then cooled, the iron template was removed, and hydrophilic channels and detection zones were thus created on and in the paper. Zhong et al. [13] investigated the potential of different types of paper and wax to produce μ PADs. This particular group used printing paper, kitchen towels, napkins, and laboratory paper towels as microfluidic platforms. Wax from pencils, crayons, candles, and lipsticks were also used to generate hydrophobic barriers/walls on and in the paper substrates. In this work, the average pore diameter and the permeability of different papers were experimentally determined, and mechanics for fluid flow in each paper type was validated. Zhang and Zha [14] created hydrophilic channels on qualitative filter paper using a patterned copper sheet covered with paraffin film. The copper sheet was then placed in contact with the paper surface, both were heated, and the paraffin from the copper sheet diffused through the porous paper to generate patterned hydrophobic walls. Nie et al. [15] described a one-step fabrication process that used ink from a commercial permanent marker and a metallic (iron) pattern to plot microfluidic layouts onto chromatographic paper. This simple fabrication technique could produce μ PADs in only 1 minute.

Ge et al. [16] and Liu and Crooks [17] used the secular origami technique to fabricate three-dimensional (3D) μ PADs (Fig. 1). In this fabrication technique, a sheet of paper was initially patterned using photolithography and photoresist to create hydrophobic barriers. The patterned paper was folded in a specific sequence to build a 3D microfluidic struc-

ture. Using this origami technique, μ PADs with nine layers were produced without special tools or tape [17].

Tian et al. [18] demonstrated that a plasma treatment process could be used to recover wettability of paper modified with horseradish peroxidase and antibodies for blood typing. Bovine serum albumin (BSA) was used to preserve the activity of the biomolecules against plasma treatment. Lewis et al. [19] developed a fast fabrication process to produce numerous 3D μ PADs by gluing layers of wax-patterned paper with an adhesive spray, and then cutting to create individual microdevices. The authors demonstrated that the use of adhesive spray did not affect colorimetric assays for glucose and protein detection.

He et al. [20] demonstrated that octadecyltrichlorosilane (OTS) could be used for paper patterning. Chromatographic paper was made hydrophobic via immersion in an OTS/hexane solution. Hydrophilic channels and detection zones were obtained by degradation of OTS molecules upon exposure of the OTS paper to deep UV and ozone using a mask (photolithography). Glavan et al. [21] used an electronic craft-cutting/engraving tool to create microchannels in card-stock paper. By using engraving tips with different dimensions and controlling some parameters of the craft-cutter, microchannels with widths of 45–300 μ m and depths of 50–300 μ m were obtained. The patterned paper was silanized with alkyl or fluoroalkyl trichlorosilane to make it hydrophobic or omniphobic, respectively. These paper-based microdevices allowed for the creation of pressure-driven flows in open microchannels.

Schilling et al. [22] bonded layers of wax-patterned paper using laser-printed toner films that simultaneously acted as hydrophobic barriers and a thermal adhesive. The authors demonstrated that this paper and toner fabrication technique could be used to design 3D μ PADs for multiple diagnostic assays and for displaying encoded information by interpreting the pattern of the results.

Nie et al. [23] developed a one-step fabrication process by cutting paper with a commercial CO₂ laser cutting/engraving machine obtaining hollow microfluidic structures. Chitnis et al. [24] also used a CO₂ laser to fabricate μ PADs, but in a different manner, i.e., using the laser to create hydrophilic channels in surface-treated hydrophobic papers (parchment, wax, and palette papers). The laser power and scanning speed were optimized to remove the hydrophobic film on the paper surface without cutting the paper itself.

2.2 Detection methods

Development and improvement of μ PAD diagnostic detection methods remain active research fields. Electrochemical, electrochemiluminescence, and surface-enhanced Raman spectroscopy methods of detection have gained great attention in the past two years, although colorimetric detection methods continue to be used and developed. The subsections below describe the recent advances in detection techniques used in paper-based diagnostic devices.

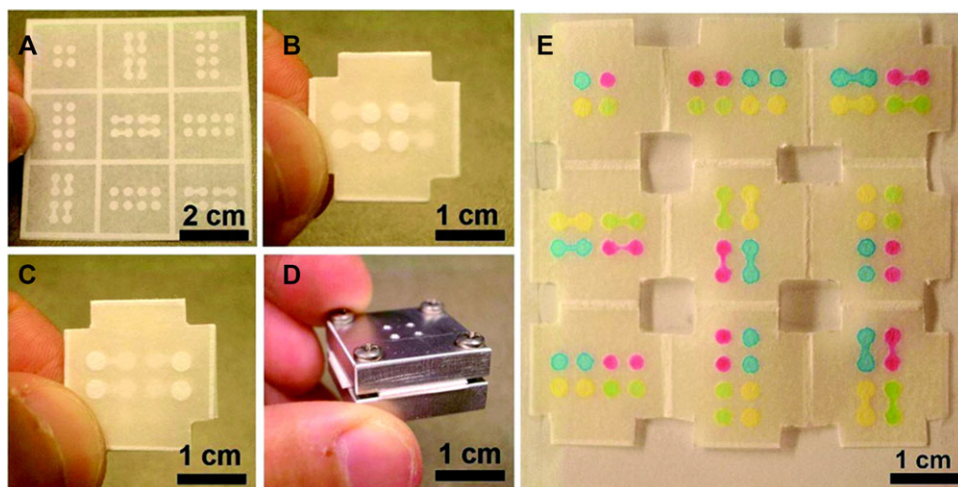


Figure 1. Fabrication process of a 3D μ PAD using an origami technique: (A) unfolded patterned chromatographic paper; (B) top and (C) bottom layers of folded patterned paper; (D) 3D μ PAD supported by an aluminum clamp; (E) unfolded origami μ PAD after wicking of colored solution. Reproduced with permission from [17].

2.2.1 Colorimetric detection method

Colorimetric detection has been the most common detection method used in μ PADs because of its simplicity in terms of implementation and use [3, 5]. This detection method is based on the color development caused by chemical reactions between the analytes and colorimetric reagents spotted in detection zones on the paper. In the period covered by this review, some authors have made remarkable advances in colorimetric detection in μ PAD for clinical assays. For instance, Ornatska et al. [25] detected glucose (colorimetric method) using ceria nanoparticles and glucose oxidase immobilized on paper after silanization with aminopropyltrimethoxysilane (APTS). A layer of chitosan was also used to stabilize the glucose oxidase. This ceria-based colorimetric detection method was able to detect glucose with a limit of detection (LOD) of 0.5 mM. Peng et al. [26] used the interaction of alkaline phosphatase (ALP) and colloidal calcium carbonate to increase the retention of ALP in filter paper after filtration. By using this pre-concentration strategy, a very low LOD (1.1 nM) was attained for paper-based colorimetric assay of ALP with a *p*-nitrophenyl phosphate substrate. Tseng et al. [27] described a photothermal process to produce metallic nanoparticles on paper for the colorimetric detection of cysteine. A thin film of gold or silver was deposited on paper, via a sputtering method, and a KrF excimer laser melted the metal film. During the cooling process, metal nanoparticles were generated due to surface tension.

2.2.2 Fluorescence detection method

Although fluorescence detection in μ PADs requires external instrumentation, it was demonstrated to provide good analytical performance [28]. Yuan et al. [29] performed fluorescence detection in μ PADs using a hybrid material synthesized by encapsulating CdTe quantum dots and enzymes with a film of poly(diallyldimethylammonium chloride). By using a fluorescence quenching effect, the hybrid material

containing glucose oxidase and tyrosinase demonstrated suitability for the detection of glucose and catechol, respectively. Yu et al. [30] modified cellulose paper with divinyl sulfone in order to covalently immobilize carbohydrates, proteins, and DNA on paper for applications in colorimetric and fluorimetric bioassays. Liang et al. [31] immobilized polystyrene microbeads functionalized with antibody for goat IgG on paper. In these experiments, cyanine dye (Cy3) and gold nanoparticles (AuNPs) conjugated with secondary antibody (anti-goat IgG) were used for sandwich-type fluorescence and colorimetric immunoassays, respectively, for the detection of IgG. Yildiz et al. [32] fabricated a PVDF porous membrane modified with poly(3-alkoxy-4-methylthiophene) that was used for fluorimetric detection of a microRNA sequence associated with lung cancer. Noor et al. [33] immobilized quantum dots on paper by imidazole ligands, and used them as donors for fluorescence resonance energy transfer (FRET). By using this paper-based FRET method, nucleic acid hybridization was detected with a LOD of 300 fmol.

2.2.3 Chemiluminescence detection method

Chemiluminescence detection relies on measuring the light emitted by certain chemical reactions. In μ PADs, this detection technique provides detectability comparable to fluorescence detection. Yu et al. [34] developed a multiplexed μ PAD for simultaneous determination of glucose and uric acid presence by respective reactions with glucose oxidase and uricase (urate oxidase). The hydrogen peroxide produced reacted with a rhodamine derivative to produce chemiluminescence emission. Wang et al. [35] used a wax screen-printing method [11] to fabricate μ PADs for a chemiluminescent ELISA. In these experiments, paper was pre-coated with chitosan to allow for covalent immobilization of antibodies for α -fetoprotein (AFP), cancer antigen 125 (CA125), and carcinoembryonic antigen (CEA). With these wax screen-printed μ PADs, a 4-iodophenol-enhanced luminol chemiluminescence assay

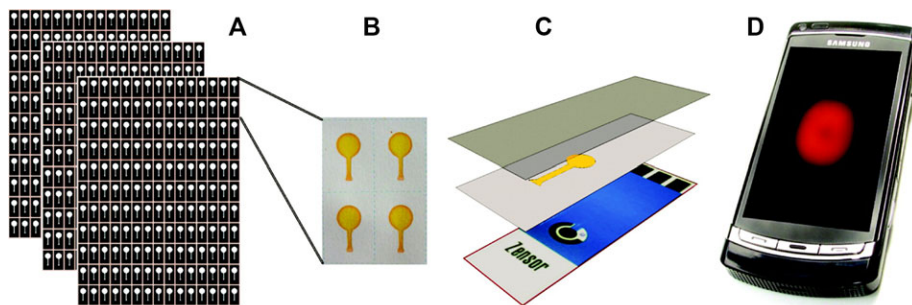


Figure 2. Fabrication of a μ PAD with ECL detection: (A) sheet of paper patterned with many microfluidic devices; (B) paper devices modified with luminophore reagent $\text{Ru}(\text{bpy})_3^{2+}$; (C) paper microfluidic is aligned to the face of a screen-printed electrode and laminated with transparent film; (D) a camera phone captures and analyzes the ECL emission. Reproduced with permission from [39].

was performed to detect AFP, CA125, and CEA in human serum. Wang et al. [36] used a periodate oxidation reaction to generate aldehyde groups on cellulose paper for covalent bonding of antibodies used in a chemiluminescence immunoassay. Using this approach, a μ PAD for CEA detection in human serum was developed. Wang et al. [37] described a paper-based chemiluminescence DNA biosensor employing *N,N'*-disuccinimidyl carbonate to immobilize captured DNA strands on paper and label carbon dots of nanoporous gold to improve the sensitivity.

2.2.4 Electrochemiluminescence detection method

In procedures based on electrochemiluminescence (ECL), a set of electrodes is used to trigger and control a chemiluminescence reaction involving a luminophore compound [38]. Thus, ECL detection combines electrochemical and luminescence techniques that can provide good selectivity and sensitivity for detection in μ PAD. Delaney et al. [39] described an ECL detection in μ PADs using tris(2,2'-bipyridyl)ruthenium(II) ($\text{Ru}(\text{bpy})_3^{2+}$) as a luminophore reagent. As shown in Fig. 2, these inkjet-printed μ PADs were laminated with screen-printed electrodes for ECL detection. ECL emission was detected using a photodetector and a mobile camera phone as an alternative detection method. Shi et al. [40] immobilized CdS quantum dots on a double-sided carbon adhesive tape supported by an indium tin oxide (ITO) glass that was used as a working electrode for ECL in μ PADs. This approach provided good stability for the quantum dots, and consequently improved the reproducibility of ECL emissions for H_2O_2 solutions. Ge et al. [41] incorporated eight screen-printed carbon working electrodes in a 3D μ PAD for a multiplexed ECL immunoassay detection. Yan et al. [42] used chitosan and glutaraldehyde cross-linking to immobilize the cancer biomarker CEA in a screen-printed carbon electrode that was used for ECL immunoassay in a 3D μ PAD.

Wang et al. [43] replaced the traditional potentiostat system with a 3 V lithium battery and a simple electronic circuit to provide a tunable voltage to trigger ECL immunoassay in μ PADs. Li et al. [44] also used the same battery-triggered ECL system with functionalized nanoporous silver for signal amplification and highly sensitive detection of tumor markers for prostate specific antigen (PSA) and CEA. Xu et al. [45,46] obtained highly sensitive solid-state ECL detection in μ PADs

using screen-printed carbon working electrodes modified with composite films of poly(sodium 4-styrenesulfonate) functionalized graphene/Nafion [45] and Fe_3O_4 nanocrystal clusters/graphene sheets [46]. This surface modification improved the immobilization and electron transfer of the luminophore agent $\text{Ru}(\text{bpy})_3^{2+}$. Yan et al. [47] immobilized oligonucleotides (aptamers) on a porous working electrode in order to increase selectivity of ECL detection in a 3D μ PAD fabricated by origami technique.

2.2.5 Electrochemical detection method

Electrochemical detection has demonstrated suitability for μ PAD [48–51] because it is easily miniaturized, can provide high sensitivity, and is more rugged than optical detections. In the period covered by this review, many advances were reported in this field in terms of implementation, electrode modification, and multiplex electrochemical detection. Liu and Crooks [52] described a μ PAD with an electrochemical sensor powered by an integrated metal/air battery that used the clinical sample (urine) as electrolyte. Transparent ITO electrodes were used in the sensor and in the battery assembly. The analytical signal for glucose and H_2O_2 detection was provided via color change of a Prussian blue spot on an ITO electrode, so that the assembly worked as an onboard electrochromic display. Rattanasarat et al. [53] concentrated and electrochemically detected dopamine in human serum using a three-layered μ PAD assembled with commercial disposable electrodes. In this work, modification of the surface of the paper with SDS pre-concentrated and increased the selectivity for dopamine detection.

Godino et al. [54] developed a disposable hybrid paper/polymer microfluidic device with an electrochemical cell. Combining wax printing with the use of a patterned adhesive stencil, hydrophobic features and electrodes were deposited in chromatographic paper. Using a pressure-sensitive adhesive, the paper with electrodes was then bonded to a patterned poly(methylmethacrylate) (PMMA) film. The hybrid microdevice was electrochemically characterized by cyclic voltammetry using 1 mM ferrocyanide. Santiago et al. [55] fabricated microelectrodes in a μ PAD by applying carbon paste on paper using a polyester (transparency film) stencil, generated by laser engraving. By using these carbon paste electrodes modified with cobalt phthalocyanine (catalyst) in a μ PAD, the authors were able to electrochemically measure cysteine.

Shiroma et al. [56] described a μ PAD that performed chromatographic separation and electrochemical detection of paracetamol and 4-aminophenol. This μ PAD comprised a hydrophilic separation channel and three gold electrodes, deposited by sputtering, for the amperometric detection of the analytes. Santhiago and Kubota [57] used a low-cost pencil graphite as an external working electrode for electrochemical detection in their μ PAD. Their reference and counter electrodes were screen-printed on paper with silver ink. Noiphung et al. [58] developed an electrochemical μ PAD that used two membranes to separate plasma from whole blood. The isolated plasma flowed to the detection zone in the μ PAD, which contained immobilized glucose oxidase. Hydrogen peroxidase generated by the enzymatic assay was detected using commercial screen-printed electrodes modified with Prussian blue. The electrochemical μ PADs were used for determination of glucose in human whole blood samples and the results were in good agreement with a reference spectrophotometric method.

Lu et al. [59] used an inkjet printing technique to fabricate electrodes on a coated paper substrate. Modification of the working electrode by electropolymerization allowed the paper-based three-electrode system to be used for pH and glucose determination. Ge et al. [60] developed a μ PAD that used an array of addressable screen-printed electrodes for amperometric immunoassays to detect tumor markers. In this work, carbon nanotubes and chitosan were used to immobilize the capture antibodies on paper. A secondary antibody and horseradish peroxidase were coupled to AuNPs/carbon nanotubes in a composite and employed as an electrochemical probe.

Wang et al. [61] used multi-walled carbon nanotubes to enhance electronic conductivity in an electrochemical immunodetection process performed with a 3D μ PAD. Antibodies for carcinoma antigen and CEA were immobilized in the paper by pre-coating it with chitosan and cross-linking with glutaraldehyde, allowing both tumor markers to be simultaneously detected. Zang et al. [62] described a 3D μ PAD that contained eight screen-printed working electrodes and was capable of performing multiplexed electrochemical immunoassays (Fig. 3).

Liu et al. [63] described a 3D μ PAD assembled by an origami method [17] to fabricate a concentration electrochemical cell to detect adenosine. Aptamers for adenosine immobilized in polystyrene microbeads facilitated high selectivity for the electrochemical detection. Sensitivity was enhanced by using an external capacitor that was charged by the potential generated in the electrochemical cell. The instantaneous current from the capacitor discharging was used as an analytical signal. Li et al. [64] developed an origami 3D μ PAD that employed a multiplexed electrochemical immunoassay to detect cancer biomarkers (CEA and AFP). A nanoporous silver (NPS) layer was applied to the paper fibers placed over the screen-printed working electrode (PWE). The completed NPS-PWE was then modified with capture antibodies. The amperometric response to the tumor biomarkers was enhanced via incubation with a secondary antibody coupled

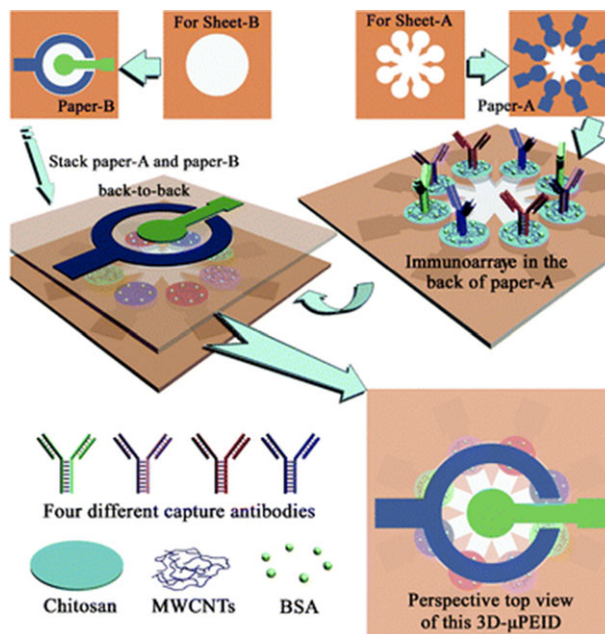


Figure 3. Schematic diagram of fabrication process of a 3D μ PAD with eight working electrodes for electrochemical immunoassay for detection of tumor markers. Reproduced with permission from [62].

to a nanocomposite containing nanoporous gold, chitosan, and absorbed metal ions (Cu^{2+} or Pb^{2+}). Lu et al. [59] described a folding 3D μ PAD for electrochemical DNA detection using a screen-printed working electrode modified with single-stranded capture DNA attached to an AuNP/graphene composite.

2.2.6 Surface-enhanced Raman spectroscopy detection method

Surface-enhanced Raman spectroscopy (SERS) is an attractive detection method for μ PADs because of its high sensitivity. Usually, metal nanoparticles are spotted onto the paper and the porous nature of the paper contributes to increase the amount of SERS nanoparticles deposited in the detection zone. Yu and White [65, 66] used an inkjet printing technique to deposit silver nanoparticles (Fig. 4) on μ PADs for SERS detection. By using these paper-based SERS microdevices, highly sensitive detection of Rhodamine 6G, malathion, heroin, and cocaine was attained. Ngo et al. [67] studied a dipping method for addition of AuNPs on a paper substrate for SERS detection. The compound 4-aminothiophenol (4-ATP) was employed as a Raman probe to monitor adsorption of AuNPs on the cellulose fibers. The concentration influence of nanoparticles and 4-ATP on SERS signal was also investigated. Chen et al. [68] performed an immunoassay for mouse IgG using magnetic beads capable of separation and SERS detection on filter paper modified with poly(vinyl pyrrolidone) (PVP) and silver colloid. Abbas et al. [69] developed a μ PAD that could separate, pre-concentrate, and detect analytes at a sub-attomolar level via SERS. The fabrication process for

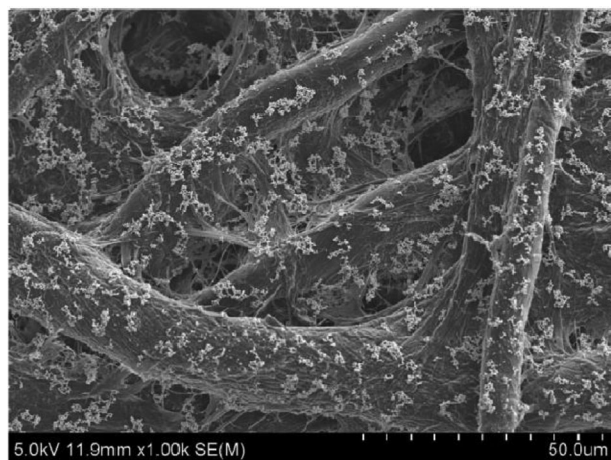


Figure 4. Scanning electron microscopy (SEM) image of cellulose paper modified with silver nanoparticles for SERS detection. Adapted with permission from [65].

this μ PAD involved cutting paper into a star-like shape. The authors demonstrated that cutting paper into this shape enhanced the capillary forces that, together with a polyelectrolyte coating, improved separation and concentration of analytes in the paper. Gold nanorods for SERS detection were added to the paper via a dipping method.

2.2.7 Other detection methods

This subsection describes certain reported detection methods used in μ PADs that employed particular approaches that do not fit well in any of the previously described detection methods. For instance, Lewis et al. [70] stacked layers of wax-patterned paper to produce 3D μ PADs to detect hydrogen peroxide with two different readout mechanisms: (i) the time the sample took to flow through the μ PAD, and (ii) the number of colored bars produced by the assay. For both designs, the hydrogen peroxide reacted with a hydrophobic compound deposited within the paper, producing hydrophilic spaces that changed the wettability of the paper and improved sample wicking. The higher the concentration of hydrogen peroxide solution, the lesser the flow-through time and higher the number of colored reaction zones (bars) (Fig. 5).

Tian et al. [71] immobilized gold nanorods functionalized with antibodies on filter paper to produce a bioplasmonic paper to be used as a localized surface plasmon resonance (LSPR) substrate. This paper-based LSPR substrate was able to detect a kidney cancer biomarker (aquaporin-1 protein) in artificial urine with an LOD of about 0.16 pM. Ge et al. [72] described a novel μ PAD that converted the light emitted by a chemiluminescence assay into an electric current (photocurrent) used to charge an integrated paper supercapacitor. The instantaneous current from the discharging capacitor was employed as an analytical signal. A chemiluminescence assay was performed with *N*-(aminobutyl)-*N*-(ethylisoluminol)-functionalized AuNPs and adenosine triphosphate (ATP) aptamer for molecular recognition of ATP. Wang et al. [73]

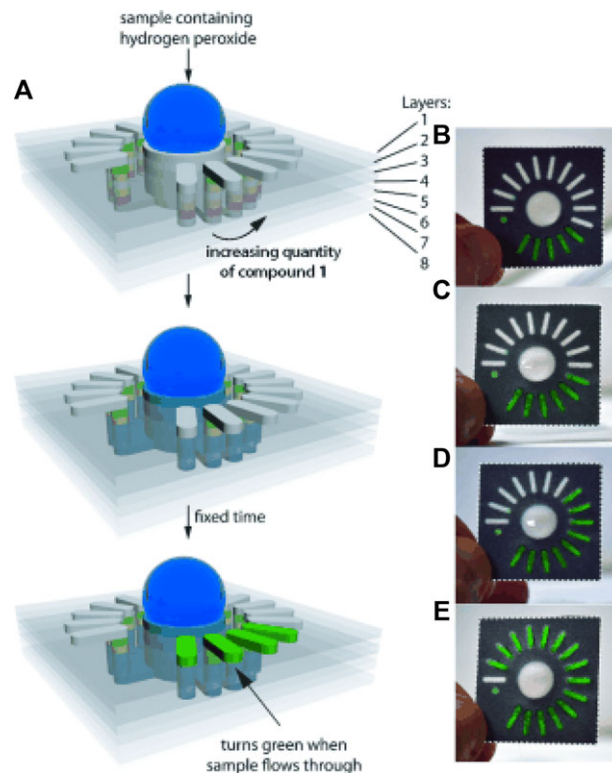


Figure 5. Diagram of 3D μ PAD and the assay procedure with readout using number of produced colored bars (test zones): (A) representation of the liquid flow in the 3D μ PAD; (B–E) readout after 10 minute assay with hydrogen peroxide solution at (B) 1 mM, (C) 35 mM, (D) 75 mM, and (E) 100 mM. Reproduced with permission from [70].

described a multiplexed μ PAD with a similar photoelectrochemical method to detect four cancer markers in human serum.

2.3 New functions and design

Remarkable improvements were observed in terms of capabilities in μ PADs with new insights into flow control, mixing, and self-powering. Fu et al. [74] developed a platform that is adaptable to a conventional paper-based lateral flow test to perform two-dimensional assays. The additional dimension can be used to carry out steps that enhance sensitivity. The authors demonstrated the applicability of the system by adding rinse and signal amplification steps for a commercial strip tests used to detect hormone chorionic gonadotropin (pregnancy test).

Hwang et al. [75] adapted a paper strip and a centrifugal microfluidic platform to demonstrate that centrifugal force could be used for active control flow rate and inversion of the flow direction in paper. Lutz et al. [76] designed an ingenious two-dimensional (2D) paper network that could be used to perform a programmable sequence of assay steps by controlling the arrival time and flow duration of all reagents in reaction zones (Fig. 6). The authors demonstrated that this

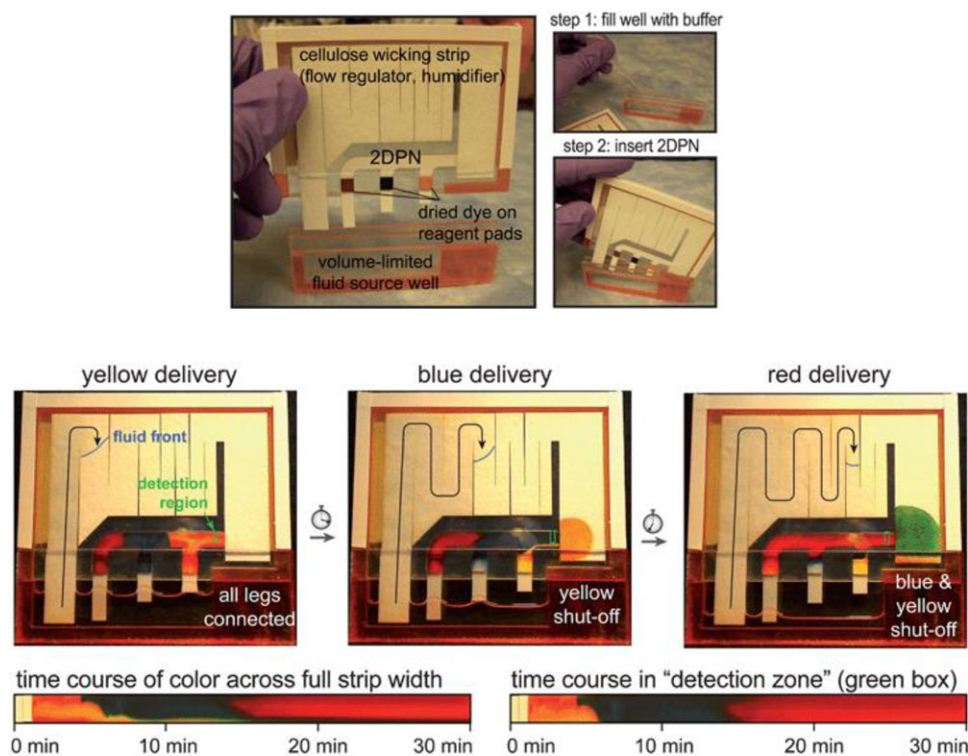


Figure 6. 2D paper networks with automated flow-delivery sequence for controlling the arrival time and flow duration of each reagent in the reaction zones. Adapted with permission from [76].

2D μ PAD could perform complex and automated multistep chemical assays. The authors also used experimental, theoretical, and computer-simulation studies to investigate flow behavior in this multistep μ PAD [77].

Schilling et al. [78] used laser-printed layers of toner, on both sides of a μ PAD, to fully enclose established microchannels and thus enhance the wicking rate of fluids, avoid contamination, and reduce evaporation of solution. Kwong and Gupta [79] integrated unit operations, such as separation of analytes and fluid manipulation on paper, by depositing functional polymers on paper via initiated chemical vapor deposition (iCVD). Poly(methacrylic acid) and poly(dimethylaminoethyl methacrylate) were used as ion-exchange coatings to separate analytes, while poly(*o*-nitrobenzyl methacrylate) was employed to produce a UV-responsive switch on the paper. Jahanshahi-Anbuhi et al. [80] demonstrated that sandwiching paper between flexible polyester films could enhance the liquid wicking speed by one order of magnitude due to deformation of the cover layer caused by the negative capillary pressure in the liquid. This approach was used to accelerate the liquid flow in a μ PAD and accelerate assays. Rezk et al. [81] demonstrated that surface acoustic waves (SAW) could enhance the mixing of solutions in a μ PAD. The authors found that 30 MHz acoustic waves could induce more uniform, reproducible, and faster mixing of two solutions compared to capillary-driven passive mixing.

Thom et al. [82] integrated galvanic cells, so-called "fluidic batteries," on a multilayer μ PAD to generate power when sample was added. The galvanic cells on paper were assembled with electrodes of Ag and Al, electrolytes (AgNO_3 and AlCl_3), and salt bridges containing NaNO_3 . By a serial and

parallel association of galvanic cells, the paper-based battery was able to power an LED for 8.2 minutes. In another article, Thom et al. [83] investigated several parameters, including layout configuration, electrolyte concentration, electrode material and size, electrical resistance of salt bridge, and the number of galvanic cells in serial and parallel association, that could be controlled to obtain predictable and tunable fluidic batteries on paper.

2.4 Diagnostic applications

In the period covered by this review, μ PADs have been developed for many diagnostic applications including blood typing and the detection of molecular biomarkers and microorganisms, such as bacteria, virus, and fungi. In the following sections, these applications are briefly summarized.

2.4.1 Blood typing

Al-Tamini et al. [84] developed a fast paper-based assay for the detection of human blood type (ABO and RhD) by modifying a paper surface with antibodies against red blood cells. Agglutinated blood was trapped on the paper fibers, and a solution of NaCl eluted non-agglutinated red blood cells in this process, which could assay 100 blood samples within 1 minute. Li et al. [85] developed an ingenious "writing" read-out method in a μ PAD for testing blood that uses haemagglutination reactions to form letters (A, B, or O) and symbols (– or +) to display the test results. Su et al. [86] studied the effect of weight, porosity, density, and composition of

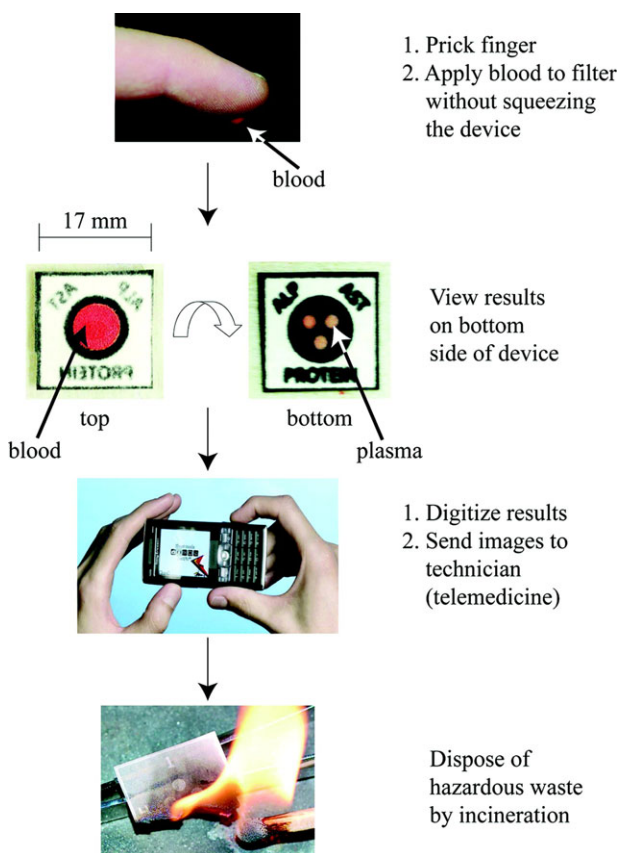


Figure 7. 3D μ PAD for colorimetric assay and parallel detections of alkaline phosphatase, aspartate aminotransferase (markers of liver function), and total serum protein in a drop of blood from a fingerstick. Reproduced with permission from [87].

commercial papers (filter papers, towel, and blotting paper) for blood type testing.

2.4.2 Detection of molecular biomarkers

Vella et al. [87] described a μ PAD that employed a blood drop sample to perform colorimetric assays for parallel detection of alkaline phosphatase, aspartate aminotransferase (enzymes that are markers of liver function), and total serum protein (Fig. 7). This μ PAD is comprised of a filter membrane for filtering red blood cell, a wax-patterned paper in which the colorimetric reagents were immobilized, and a plastic sheath to avoid evaporation of water from the sample. Pollock et al. [88, 89] described the development of a μ PAD with a design very close to that developed by Vella et al. [87] that was clinically tested for multiplexed liver functions assays.

Maattanen et al. [90] fabricated arrays of hydrophilic reaction zones on paper using inkjet [91] and flexographic [92] printing. This μ PAD was evaluated for colorimetric detection of glucose based on enzymatic reaction. Cha et al. [93] immobilized 2,4,6-tribromo-3-hydroxy benzoic acid (TBHBA) in cellulose paper strips to be used as a chromogenic reagent for the enzymatic detection of glucose with glucose oxidase and peroxidase. Lankelma et al. [94] described a paper-based

flow-injection analysis system for the electrochemical detection of glucose in urine. In this experiment, a urine sample was injected on a nitrocellulose membrane containing immobilized glucose oxidase. This membrane was in contact with a platinum working electrode, and a continuous flow of buffer solution was driven through the nitrocellulose membrane and a strip of paper via gravity and capillary wicking. The glucose concentration in the urine sample was obtained by amperometric detection of the hydrogen peroxide produced by the enzymatic glucose oxidation reaction. Tan et al. [95] also developed a flow-injection analysis system with a paper-based electrochemical bioassay based on glucose activity inhibition in a paper tool containing immobilized glucose oxidase. A cartridge tube was used to store the liquid reagents in a separated air space so that they could be delivered in a specific sequence for an electrochemical assay in the μ PAD. This flow-injection system detected silver ions in standard solutions, but the apparatus could be adapted for diagnostic applications.

Liu et al. [96] used the principle of SlipPad in their μ PAD by employing two adjacent wax-patterned fluidic layers with arrays of reservoirs to facilitate the wicking of samples, standard solutions, and reagents. Using this approach, high throughput and multiplexed assays for colorimetric and fluorescent detection of glucose and BSA could be performed. Chen et al. [97] used a 3D μ PAD with colorimetric bi-enzyme assay capacity to detect glucose and uric acid in human serum. By catalytically linking glucose oxidase or urate oxidase to horseradish peroxidase, the bioassay sensitivity was enhanced, reaching LOD values of 3.81×10^{-5} M and 4.31×10^{-5} M for glucose and uric acid, respectively.

Yang et al. [98] immobilized antibodies (Anti-A, B) on paper for agglutination of red blood cells and separation, by filtration, of serum from whole blood. Following this, the serum wicked to a paper test zone containing reagents for colorimetric assay. By using this μ PAD with serum separation, glucose could be detected in whole blood samples. Songjaroen et al. [99] also described a μ PAD for separation of plasma from whole blood by combining filter paper and a blood separation membrane patterned using a wax dipping method [12]. Different types of separation membranes were evaluated, and a colorimetric assay for protein detection was performed in separated plasma with precisions of 2.62% within a day ($n = 10$) and 5.48% between days ($n = 30$).

Rohrman and Richards-Kortum [100] developed a hybrid microfluidic device using layers of paper and plastic that could store and mix reagents to perform recombinase polymerase amplification of HIV DNA. The authors demonstrated that this microdevice could be coupled to commercial lateral flow strips to obtain a point-of-care HIV DNA test.

Lo et al. [101] performed an assay to diagnose dengue fever (specific to serotype-2) at the molecular level using a μ PAD and fluorescently based detection. Dengue virus serotype-2 RNA was transcribed reversely and then amplified via reverse transcription loop-mediated isothermal amplification. The amplified double-stranded DNA was mixed with commercially available fluorescent nucleic acid probes, and

then fluorescence detection was performed on the μ PAD. Wang et al. [102] also carried out the diagnosis of serotype-2 dengue fever through either paper-based indirect ELISA or lateral flow immunoassays. Both antigens and antibodies were physically adsorbed onto paper-based 96-well plates to perform ELISA for the detection of dengue virus nonstructural protein-1 antigens and dengue virus serotype-2 envelope proteins in either buffer solution or human serum. For the lateral flow immunoassays, AuNP-conjugated antibody (against dengue virus nonstructural protein-1 antigens) was immobilized on a sample/conjugation release pad as a captured antibody. For the reaction pad, another antibody (against dengue virus serotype-2 nonstructural protein-1 antigens) was immobilized as a test antibody. This lateral flow test was demonstrated to be appropriate as a screening test (yes or no) for the diagnosis of serotype-2 dengue fever.

Apilux et al. [103] developed a μ PAD that required a single sample application to perform sequential and automatic steps of an ELISA. The microfluidic layout was designed on a piece of nitrocellulose membrane patterned with an inkjet printing method. The reagents were spotted in proper locations on the microfluidic circuit for sequential multistep of ELISA. By using this automated ELISA μ PAD and a digital camera to measure color intensity, human chorionic gonadotropin (hCG) was detected in urine samples. Bai et al. [104] employed SiO_2 microbeads functionalized with amino/carboxyl groups to enhance protein adsorption in paper-based micro-zone plates for ELISA. By detecting goat anti-rabbit IgG the authors demonstrated that the microbeads allowed a higher level of antigen immobilization and decreased the background signal due to nonspecific adsorption.

Paper substrate has been used in ambient ionization for mass spectrometry analysis without requirement of sample preparation or separation. Epsy et al. [105] investigated the mechanism of paper spray ionization by using images and droplet-sized measurements. Ho et al. [106] described a paper-based microfluidic device that used a SAW for delivery and ionization of samples for mass spectrometry. By using this paper-based SAW ionization source, the authors detected (in the nM level) drugs in untreated physiological fluids (whole blood and plasma) and heavy metals in tap water samples. Paper spray ionization coupled with mass spectrometry has also been used for direct analysis of biological tissue [107], determination of amphetamine derivatives in saliva [108, 109], and determination of acylcarnitines [110], pharmaceutical, and therapeutic drugs [111–113] in whole blood spots.

2.4.3 Detection of pathogenic microorganisms

Li et al. [114] described a multiplexed and disc-shaped μ PAD with immobilized antibody-conjugated AuNPs for colorimetric detection of bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Jokerst et al. [115] described a μ PAD capable of detecting pathogenic bacteria (*Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*) by colorimetric assays involving characteristic enzymes produced by these bac-

teria and the respective chromogenic substrates. This μ PAD was able to detect pathogenic bacteria in food samples in a shorter time than that required by the standard method. Larson et al. [116] coated filter paper with polyelectrolyte layers to increase adsorption of virus (M13 bacteriophage) by electrostatic interaction. Colorimetric immunodetection of the adsorbed viruses was attained via an antibody/horseradish peroxidase conjugate and 3,3',5,5'-tetramethylbenzidine (TMB) substrate.

Miranda et al. [117] developed a paper-based immunosensor to detect Asian soybean rust caused by the fungus *Phakopsora pachyrhizi*. In this protocol, antigen was trapped in nitrocellulose paper and recognized by fluorescent nanoparticles labeled with polyclonal antibodies. This paper-based device was able to detect the soybean disease with 10-fold higher sensitivity than a commercial test kit.

Veigas et al. [118] reported on a μ PAD for colorimetric assay to detect tuberculosis causing agent *Mycobacterium tuberculosis*. The paper surface was modified with AuNPs functionalized with an oligonucleotide sequence for tuberculosis detection. The hybridization between the gold nanoprobe (red color) and the target DNA sequence of the pathogen agent avoided gold nanoparticle agglutination and no color changing was detected. If the DNA sequence was not present, the agglutinated nanoparticles turned blue. Tsai et al. [119] used a similar approach for tuberculosis diagnosis, but in their assay, unmodified AuNPs were mixed with single-stranded DNA (ssDNA), specific to the sequence of *M. tuberculosis* complex (MTBC), after a direct hybridization with double-stranded DNA (dsDNA) extracted from serum samples of patients with and without tuberculosis. In the absence of the targeted sequence, the ssDNA adsorbed onto the unmodified AuNPs, preventing their agglutination. Simultaneously, ssDNA sequences were hybridized with targeted dsDNA, the unprotected AuNPs aggregated, and a color change resulted (red to blue). By using this approach, 2.6 nM MTBC target sequences have been detected.

3 Plastic devices for clinical diagnostics

In addition to μ PADs, other low-cost microfluidic platforms have been successfully used for clinical diagnostics. The substrate materials employed include polyester-toner (PT), PDMS, PMMA, polycarbonate (PC), polyethylene (PE), polystyrene (PS), and cyclic olefin copolymer (COC). The following sections aim to present and discuss some remarkable applications for laser-printed, PDMS, and thermoplastic microfluidic devices and describe the latest advances in thread-based (fabric) devices.

3.1 Laser printed microfluidic devices

As described in a previously reported review article, toner and paper-based devices comprise the current generation of disposable microfluidic platforms [5]. Toner-based devices are fabricated by laser printing, a method that was proposed by

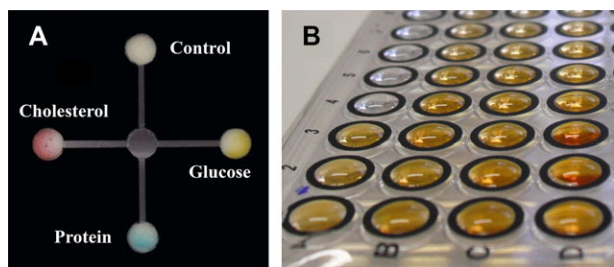


Figure 8. Examples of (A) capillary-driven multi-zone toner-based devices and (B) toner-based zones for clinical assays. Images were reprinted with permission from [123] and [124].

do Lago et al. [120] about 10 years ago. Toner-based devices are often printed on a polyester film surface with an office laser printer in a matter of minutes to generate PT devices. In the period covered by this review, different authors have demonstrated the feasibility of PT chips for application in clinical investigations.

Duarte et al. [121] used this platform for genetic analysis with the development and integration of dynamic solid phase DNA extraction and PCR amplification steps on PT devices. In their work, toner layers were printed onto two polyester films, which were then laser cut to define channels with depth on the order of hundreds of micrometers. The two intermediary layers were then laminated with two blank polyester pieces (base and cover) containing drilled holes to access the microfluidic channels. This multilayer PT device was able to recover ca. 65% of DNA from 0.6 μ L of sample blood. Furthermore, the authors also reported the successful amplification of the 520 bp fragment of the λ -phage genome. The same group described the separation of DNA fragments on a PT electrophoresis device [122]. The low electro-osmotic flow (EOF) magnitude from PT chips ensured five consecutive separations without replacement of the porous matrix.

De Souza et al. [123] developed toner-based microfluidic devices to perform clinical diagnostics with capillary action and colorimetric detection. The authors showed that detection zones could be integrated with microfluidic channels for quick distribution of the sample by capillary action. The spontaneous fluidic transport was achieved by adding an intermediary polyester film to increase channel depth and the aspect ratio of the channel. Colorimetric assays for glucose, protein, and cholesterol were successfully performed in artificial human serum samples using a desktop scanner (see Fig. 8A). The LOD values found for protein, cholesterol, and glucose were 8, 0.2, and 0.3 mg/mL, respectively. These values, associated with the dynamic range reported by the authors, enable this platform for clinical assays.

The toner-based platform has also been used by two different groups to perform immunoassays. First, Oliveira et al. [124] described the quick and simple fabrication of toner-based 96-microzone plates. Detection zones (wells) were created by printing a toner layer (ca. 5 μ m thick) that acted as a hydrophobic barrier to confine small volumes of sample

within test zones (see Fig. 8B). In this study, the authors have successfully demonstrated the use of a PT platform to detect dengue virus with a cell phone camera. Dengue virus was detected in human serum samples from infected patients based on capture ELISA of IgM antibody. IgM is a specific marker related to the primary infection of dengue. Most recently, Kim et al. [125] reported the use of PT microchips to perform immunoassays to detect C-reactive protein (CRP), a highly conserved plasma protein related to the inflammatory state. This protein was immobilized on the surface of silica microbeads and placed in the PT microchannel. A detection antibody with a fluorescent tag was then added onto the functionalized surface, generating a complex. After cleaving the protein-target complex, the fluorescent tags were analyzed by microchip electrophoresis. The time needed for the complete analysis to be carried out on a PT microchip was lower than 35 minutes. The dynamic range of the CRP in 10-fold diluted serum was 0.3–100 mg/L, and the LOD achieved was 0.3 mg/L, which demonstrated the possibility for quantitative analysis of CRP in serum in clinical trials.

3.2 PDMS platforms

PDMS is certainly the most popular polymeric material used in microfluidic applications. PDMS offers several advantages including high optical transparency (above 230 nm), compatibility for biological studies, low cost, and capacity for self-sealing against flat and smooth surfaces [126]. This substrate has been extensively employed in the fabrication of microfluidic devices focused on clinical applications. Examples of biomedical applications published between 2011 and 2013 include the detection of tumor markers [127], anticancer activity evaluation [128], the diagnostic of influenza virus [129], the detection of IgG in rat whole blood samples [130], and HIV-1 infection [131].

Zhu and Trau [127] presented a PDMS microfluidic microparticle array containing gel-based microstructures as a multiplex detection platform for two protein tumor markers, human chorionic gonadotropin (hCG) and PSA. In this microfluidic arrangement, spherical biofunctionalized polystyrene microbeads were incorporated on polyacrylamide gel microstructures (Fig. 9A). Both hCG and PSA were successfully detected based on a binding assay in serum samples with LOD below the cut-off values for cancer diagnosis. Ziłkowska et al. [128] developed a three-dimensional PDMS microfluidic chip with an integrated array of microwells for long-term tumor spheroid cultivation (see Fig. 9B–D) and anticancer drug activity evaluation. The authors cultured spheroids of HT-29 human carcinoma cells into the microfluidic chip during four weeks. A cytostatic drug (5-fluorouracil) was introduced and incubated on the PDMS chip to evaluate its cytotoxic effect on HT-29 cells. The authors observed cell death based on decreasing of the spheroid diameter. Wang et al. [129] identified multiple subtyping of influenza virus by an integrated PDMS microfluidic device. The proposed device was able to extract the viral RNA from clinical samples

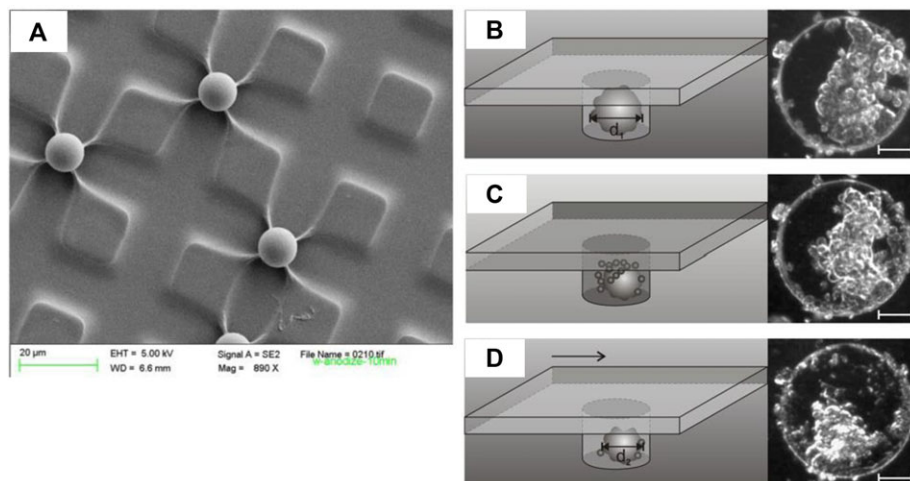


Figure 9. Examples of (A) microparticle array and (B) microwell-based drug activity effect integrated on PDMS microfluidic devices. In (A), the scanning electronic micrograph depicts the spherical streptavidin-coated polystyrene microbeads on a gel pad array. In (B–D), images represent the evaluation principle of a drug effect on spheroids which involves (B) measurement of initial spheroid diameter; (C), exposition to a drug, dead cells detachment, and; (D), medium flow, removal of dead cells and measurement of decreased spheroid diameter. Micrographs shown in (A) and (B–D) were reprinted with permission from [127] and [128], respectively.

by using specific nucleotide probes conjugated on the surface of magnetic beads. The extracted RNA was amplified by one-step reverse-transcription polymerase (RT-PCR), and the products were optically detected with a TaqMan fluorescence system. Experimental results showed that different subtypes of influenza viruses could be automatically detected within 110 minutes.

The rapid diagnosis of HIV-1 has been demonstrated by Wang et al. [131] using a microfluidic array equipped with a sample treatment system and a nucleic acid amplification stage. The authors developed a PDMS microfluidic chip integrated with functional analytical steps including cell lysis, extraction of DNA, PCR, and optical detection. The proposed device was able to detect DNA fragments from an HIV-infected Jurkat-T cell line within 95 minutes. The immunoassay-based detection of IgG in rat whole blood samples was reported by Chen et al. [130] using a PDMS-cellulose composite film. This multilayer microfluidic device was able to isolate plasma from raw samples based on the cross-flow principle.

Another remarkable biomedical application associated with the PDMS platform was reported by Cira et al. [132], who demonstrated the self-loading capability of a microfluidic device to determine the minimum inhibitory concentration (MIC) of antibiotics against bacteria. The growth of bacteria into microfluidic chambers in the presence of a pH indicator produced a colorimetric change that could be visually detected using ambient light. Based on this principle, the authors measured the MIC of vancomycin, tetracycline, and kanamycin against *Enterococcus faecalis* 1131, *Proteus mirabilis* HI4320, *Klebsiella pneumoniae*, and *E. coli* MG1655.

3.3 Thermoplastic devices

Besides paper, toner, and PDMS devices, thermoplastic substrates have also been used in clinical applications in the last years. Song et al. [133] developed PC chips for rapid detection of influenza A/H1N1 virus in human clinical specimens. PC chips were fabricated via a multilayer injection molding technique, so three plastic layers were used to con-

struct the inlet, outlet, fluidic channel, and reaction chambers with dimensions of approximately $72 \times 25 \times 1.5$ mm. The reaction volume for the fluidic flow chip was 15 mL for 6 channels (Fig. 10A). The efficiency of these plastic devices was confirmed using novel primer sets specifically targeted to the hemagglutinin (HA) gene of influenza A/H1N1 and clinical specimens (Fig. 10B). Eighty-five human clinical swab samples were tested using real-time PCR. The results demonstrated 100% sensitivity and specificity, showing 72 positive and 13 negative cases. The PC platform was also investigated by Cooney et al. [134], who developed a disposable and valveless flow cell that supports PCR coupled with microarray hybridization in the same chamber. The assembled device confined liquid to a reaction chamber while undergoing thermocycling until additional wash buffer advanced the reagent to the waste chamber. Based on this approach, the authors achieved 300 copies of bacterial DNA without need of disassembly or specialized instrumentation.

Chou et al. [135] used a hot embossing technique for fabrication of through-hole microarrays in low-density polyethylene (Fig. 10C–E). The embossing mold was replicated from an anisotropically etched silicon wafer to an aluminum epoxy crating. The clinical capacity of the proposed device was demonstrated by measuring the essential inflammatory biomarker CRP. Based on the performance recorded, the plastic device has exhibited great compatibility with real-world clinical measurements in the context of point-of-care testing.

Segato et al. [136] interconnected PMMA microfluidic devices to assemble a capillary electrophoresis system with capacitively coupled contactless conductivity detection (C4D) [137]. This modular fabrication method was demonstrated to be easy, fast, versatile, and inexpensive. These attributes make this approach suitable for the development of microfluidic devices for clinical assays in low-resource communities.

3.4 Thread-based microfluidics

In addition to the disposable platforms mentioned earlier, thread has also appeared as an alternative substrate for use in

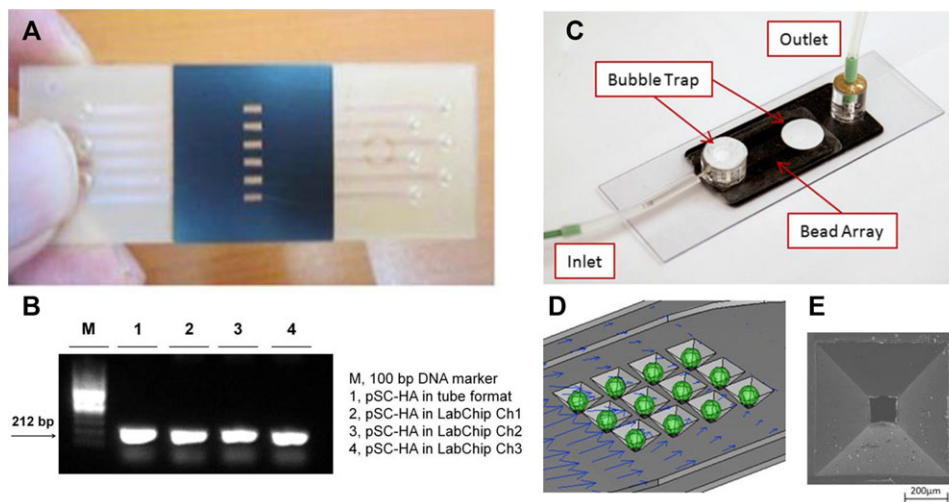


Figure 10. Examples of (A, B) polycarbonate and (C–E) polyethylene microfluidic devices for clinical applications. Images shown in (A, B) and (C–E) were reprinted with permission from [133] and [135], respectively.

microfluidic applications. Like paper-based devices, thread is porous, inexpensive, and globally available. The surface nature of the thread allows fluidic transport via capillary action, i.e., without the need of external forces or pumps [7]. A few research groups have demonstrated the capacity for threads to be used in immunochromatographic assays [138], chemical synthesis and sensing [139], and electrophoretic separations coupled to electrochemical detection [140].

Immunochromatographic assays on threads (ICAT) were reported by Zhou et al. [138]. The authors used cotton threads and nylon fiber bundles in place of pads and membranes. This ICAT is a sandwich assay performed on a cotton thread knotted to a nylon fiber bundle, both of which are previously coated with recognition antibodies against one target analyte (Figs. 11A and B). The assay results become visible to the eye within a few minutes and are quantified using a flatbed scanner. The cotton threads (made of twisted cellulose fibers) were rendered hydrophilic by plasma treatment to allow spontaneous wicking of aqueous solutions. Using lateral flow, the authors demonstrated multiplex measurements of CRP, leptin, and osteopontin with LOD values in the pM range.

Banerjee et al. [139] proposed chemical syntheses and sensing using threads as microchannels. The authors have demonstrated the synthesis of brown ferric hydroxide in a Y-geometry thread reactor (see Fig. 11C) by passive mixing of ferric chloride and ammonium hydroxide. The synthesis efficiency on threads was ca. 84%. In addition to synthesis, colorimetric assays for BSA and glucose were successfully performed in blood plasma.

More recently, Wei et al. [140] described the first use of a polyester thread-based microfluidic system for electrophoretic separation coupled with amperometric detection. The design of the thread-based electrophoresis device was similar to the structure of a traditional musical instrument called a dulcimer. The strings were used as the sample routes, and the protruding nuts were used as electrical contacts (see Fig. 11D). The authors reported that oxygen plasma-treatment improved wettability and surface quality (Fig. 11E) of the threads. In addition, the measured electrical currents on

plasma-treated threads were 10 times greater than currents on native threads. Amperometric detection was performed using a conventional three-electrode cell integrated with a gold decoupler. The authors achieved separations of anions (Br^- , Cl^- , and I^- , see Fig. 11F) and catecholamines (catechol and dopamine) within 5 minutes, with LOD estimated at ca. 0.3 mM.

4 Commercialization of low-cost microfluidic devices for clinical diagnostics

Among the technologies described in this review, several of them have the potential to generate commercial products that could provide inexpensive, fast, portable, and reliable diagnostics, particularly in resource-poor and remote settings, where there is a high demand for low-cost and easy-to-use diagnostic devices. In a critical review published in 2012 by Chin and colleagues [4], the authors have listed the main companies responsible by the current scenery regarded to the commercialization of POC diagnostic devices.

The use of μ PADs for clinical assays has received great attention for commercialization because of the remarkable advantages of using paper as a microfluidic platform. Governmental and nongovernmental organizations, such as the Sentinel Bioactive Paper Network, the Bioresource Processing Research Institute of Australia (Biopria), the Program for Appropriate Technology in Health (PATH), Diagnostic for All (DFA), and the Bill & Melinda Gates Foundation have played an important role in the development and commercialization of μ PADs for diagnostic applications in resource-poor settings. For instance, the μ PAD described in this review for liver function testing [87] is in an advanced stage of development for mass production by the non-profit enterprise DFA. A μ PAD for blood typing test also has been considered for commercialization by Biopria.

In comparison with μ PADs, the use of plastic microfluidic devices for clinical purpose is more mature and for this

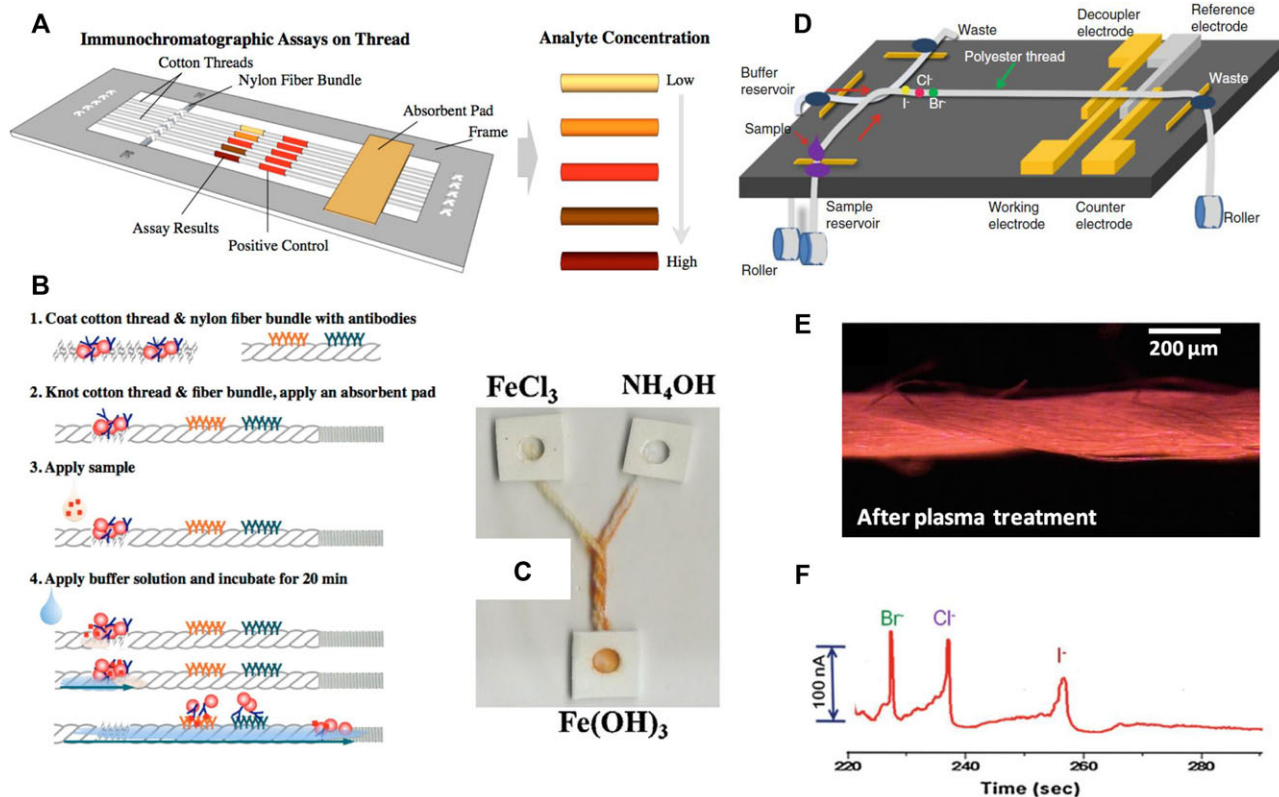


Figure 11. Examples of thread-based devices and microfluidic applications: (A) device for immunochromatographic assays; (B) operation scheme of a bioassay on a thread; (C) device for chemical synthesis; (D) device for electrophoresis coupled with amperometric detection; (E) example of a polyester thread after plasma treatment and, (F) typical electropherogram recorded on a thread. Images were reprinted with permission from [138, 139], and [140].

reason there are more companies commercializing POC devices in plastic platforms. In addition, the fabrication of plastic devices is often carried out by well-established technologies such as injection-molding and hot embossing. This feature enables the integration of different fluidic components for sample pretreatment, volume control, sample mixing and signal detection. Consequently, plastic platforms have allowed the sample-in-answer-out capability, which is desirable for POC diagnostics. Different companies have commercialized polymeric devices for clinical diagnostics, where the sample including whole blood, tear, and urine can be handled under centrifugal, capillary, mechanical or gravitational forces. In the products available in the market, the signal is processed by absorbance, colorimetric, electrochemical or fluorescence detection. The pioneering companies include Abaxis (<http://www.abaxis.com/>), Alere (<http://alere-technologies.com/>), Focus Diagnostics (<http://www.focusdx.com/>), Micronics (<https://www.micronics.net/>), Mbio Diagnostics (<http://mbiodx.com/>), TearLab (<http://www.tearlab.com/>) and Zyomyx (<http://www.zyomyx.com/>) [4]. In addition to μ PADs and polymeric devices, toner-based platforms have also demonstrated great potential to be inserted in the market, especially where the availability of funds is quite limited [5].

Despite these examples, the commercialization of low-cost microfluidic devices for diagnostics is still in an embry-

onic stage. This scenario could be improved if the academic researchers and the diagnostic industry worked closer. The path from proof of concept to commercial product is, naturally, long. Before going to market, a product typically must be improved in such a way as to allow for mass production while providing suitable feasibility and reliability for its diagnostic application. Moreover, approval of a diagnostic device by the regulatory agencies can require a large number of clinical tests. Therefore, much of this work could be better done outside the academy if the diagnostic industry could be persuaded to turn the academic prototypes in real products.

5 Concluding remarks

In the period covered by this review, a remarkable increasing trend in the use of low-cost substrates to develop microfluidic devices dedicated to diagnostic and clinical assays has been observed. Paper substrate has dominated this field, especially because of its unique features for producing POC diagnostic devices for resource-poor settings. Nevertheless, contributions from plastic platforms should not be disregarded. The development of diagnostic microfluidic devices produced on affordable platforms has been enhanced in many ways by new and enhanced detection approaches, integration and

automation of analytical steps, inclusion of functionalities (self-power and easy readout, for example), and applications close to real-life scenarios. Certainly many of the microfluidic devices described in this review have the potential to improve life quality by providing inexpensive, rapid, portable, and reliable diagnostics. The remaining bottleneck in this process is launching and streamlining the translation of academic research to commercial product.

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