Wendell Karlos Tomazelli Coltro[1,2]
Dosil Pereira de Jesus[2,3]
José Alberto Fracassi da Silva[2,3]
Claudimir Lucio do Lago[2,4]
Emanuel Carrilho[2,5]

[1]Instituto de Química, Universidade Federal de Goiás, Goiânia-GO, Brazil
[2]Instituto Nacional de Ciência e Tecnologia em Bioanalítica, Campinas, São Paulo, Brazil
[3]Instituto de Química, Universidade Estadual de Campinas, Campinas, São Paulo, Brazil
[4]Instituto de Química, Universidade de São Paulo, São Paulo, Brazil
[5]Instituto de Química de São Carlos, Universidade de São Paulo, São Carlos, Brazil

Received February 5, 2010
Revised April 11, 2010
Accepted April 14, 2010

Review

Toner and paper-based fabrication techniques for microfluidic applications

The interest in low-cost microfluidic platforms as well as emerging microfabrication techniques has increased considerably over the last years. Toner- and paper-based techniques have appeared as two of the most promising platforms for the production of disposable devices for on-chip applications. This review focuses on recent advances in the fabrication techniques and in the analytical/bioanalytical applications of toner and paper-based devices. The discussion is divided in two parts dealing with (i) toner and (ii) paper devices. Examples of miniaturized devices fabricated by using direct-printing or toner transfer masking in polyester-toner, glass, PDMS as well as conductive platforms as recordable compact disks and printed circuit board are presented. The construction and the use of paper-based devices for off-site diagnosis and bioassays are also described to cover this emerging platform for low-cost diagnostics.

Keywords:
Lab-on-a-chip / Microfluidic devices / Paper microfluidic devices / Polyester-toner / Toner transfer masking DOI 10.1002/elps.201000063

1 Introduction

The insertion of microfluidic devices into analytical and bioanalytical chemistry has received much attention in the last two decades. An exponential increase in the number of publications has been observed since the concept of micro total analysis systems (μTAS) was first reported [1]. Several review papers have detailed all the history about the beginning of the development [2, 3] as well as the recent advances in this field [2–6]. The fast development of microfluidic devices is attributed to their attractive advantages including (i) reduced volume consumption, (ii) low waste production, and (iii) great device portability; besides, the possibility of integration of many analytical procedures on a single device is highly desired [2, 4].

Photolithographic processes were the foundation of this field and have been extensively used to fabricate microfluidic devices [2, 7, 8]. However, this fabrication process is laborious, time consuming, and present a high cost *per* device [8]. Furthermore, these technologies require sophisticated instrumentation located in expensive cleanrooms that are not always readily accessible for researchers. As a result, the introduction of new types of substrate materials and emerging fabrication techniques has offered an alternative way for rapid prototyping of disposable devices [9, 10]. Consequently, there is a significant interest in the development and use of polymers as substrates for microchip fabrication. A benefit of most polymeric devices is that they can be fabricated without cleanroom environment in a faster and cheaper way than glass devices [10–13].

In this direction, toner and paper-based devices appear as two promising substrate materials for microfluidic applications. Toner is a complex powder composite used in laser printers to form an image on polyester film or wax paper. In this process, the toner starts off as a powder, becomes a fluid, and ends up as a solid structure bonded to the transparency sheet or to the wax paper surface [9]. Toner-based devices can be fabricated direct or indirectly using polyester, glass, elastomeric (PDMS or polyurethane), or conductive substrates [9]. Recently, paper-based devices were reported by Whitesides's group [14] as a very inexpensive platform for microfluidics. These devices stand out as a new class of point-of-care diagnostic devices that are inexpensive, easy to use, lightweight to transport, and compatible with biological samples. The advantages of paper-based devices bring new application capabilities for use in locations with resource-limited settings as well as in emergency situations, military field

Correspondence: Professor Wendell Karlos Tomazelli Coltro, Instituto de Química, Universidade Federal de Goiás, Campus Samambaia, P. O. Box 131, 74001-970, Goiânia-Go, Brazil
E-mail: wendell@quimica.ufg.br
Fax: +55-62-3521-1167

Abbreviations: μPAD, microfluidic paper-based analytical devices; **3-D,** three-dimensional; **C^{4}D,** capacitively coupled contactless conductivity detection; **CD-R,** recordable compact disks; **DMF,** digital microfluidics; **DTL,** double toner layer; **FLASH,** fast lithographic activation of sheets; **NC,** nitrocellulose; **NF,** nanofissures; **PCB,** printed circuit board; **PET,** polyethylene terephthalate; **PT,** polyester-toner; **STL,** single toner layer

assignments, home healthcare, and developing countries [15, 16].

This review describes some detailed examples of toner and paper-mediated fabrication techniques for microfluidic applications. The main goal of this review is to convey the feasibility of fabricating analytical microdevices, using readily accessible instrumentation (printers and laminator) and substrate materials (transparency films, toner, and papers) without spending a large amount of time and not requiring cleanroom facilities.

2 Toner-based devices

2.1 Direct fabrication techniques

Toner was first used in microfabrication science in 2001, when Tan *et al.* [17] introduced a simple method for the fabrication of PDMS microchips by using a photocopying machine to make a master on polyester film. This master was employed to produce PDMS channels using replica molding process in less than 1.5 h. Two years later, do Lago *et al.* [18] demonstrated that microfluidic devices can be directly formed on transparency films combining laser printing and laminating steps. Instead of a photocopying machine, a laser printer was used to transfer a toner layer on a transparency film. The microfluidic network is defined by the white regions of the drawing, which is interpreted by the printer as an instruction to avoid the deposition of toner particles. The sealing of the microfluidic channels is provided by a lamination step. This direct-printing process makes possible the production of tens of devices on a single transparency sheet (letter/A4 size) in matter of minutes.

The direct-printing technology [18] does not require any sophisticated instrumentation. Basically, it needs a computer equipped with graphic software and an office laser printer. Some accessories easily found such as a laminator and a paper driller are also required. In this low-cost technology, the desired layout of a device can be drawn in any graphic software and then be sent to the laser printer, which selectively deposits a toner layer on a polyester film (Fig. 1I). After printing, the toner layer (thickness ~7 μm) defines not only the channel wall but also the channel depth (Fig. 1II).

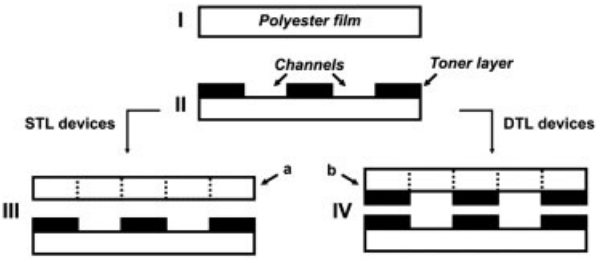

Figure 1. Direct-printing process of PT devices with STL and DTL structures. I, polyester film; II, polyester film with a deposited toner layer (printed channels); III, alignment of the printed channels against a perforated polyester piece (a), and; IV, alignment of the printed channels against a mirror image of the layout (b). The laminating step (for bonding the channels) is not presented in this figure.

As shown in Fig. 1, the printed layout can be laminated against a perforated blank polyester piece (Fig. 1III) or a mirror image of the device (Fig. 1IV). In this case, devices with a single toner layer (STL) or double toner layer (DTL) are obtained, respectively. The depths of the channel for STL and DTL devices are approximately 6 and 12 μm, respectively.

2.1.1 Polyester-toner electrophoresis devices

When compared with other popular microfluidic platforms – such as glass, PDMS (native and plasma oxidized) as well as PDMS/glass – polyester-toner (PT) electrophoresis devices exhibit the lowest EOF magnitude [19]. The EOF in PT devices is generated by a complex structure including the toner walls (polystyrene, polyacrylate, and iron oxide) and the polyester film (polyethylene terephthalate, PET). The PET film is coated with a thin layer of a neutral polymeric base containing silica (~0.6%) [20], which is responsible for the low EOF observed on PT devices [19, 21]. Besides these noticeable differences, PT chips offer advantages over conventional fluidic platforms. PT devices are thin and lightweight, and require only low-cost consumables [19]. For this reason, the interest for this substrate material for microfluidics has increased in recent years.

PT devices with STL or DTL structures have been used as electrophoresis chips by different groups. Electrophoresis microchips with end-channel amperometric detection using gold [21], pencil graphite [20], and Pt electrodes [22, 23] have been described to detect anionic species, such as iodide and ascorbate [21], neurotransmitters [20, 23, 24], and pharmaceutical compounds such as *p*-aminophenol and acetaminophen [22]. By manipulating the EOF magnitude, Yu *et al.* [23] have shown that the separation efficiency of neurotransmitters can be improved up to ~30 000 plates/m in a low-resolution PT chip.

Capacitively coupled contactless conductivity detection (C^4D) has also been used to monitor electrophoretic separation on PT devices. Copper adhesive tape was first used by do Lago *et al.* [18] to make electrodes for C^4D on PT devices. This approach is easy and inexpensive, but it requires skillful handling and lacks repeatability, because of the inconsistency of size and geometry of the cell for different prototypes. In order to improve the chip-to-chip repeatability, ensuring the production of electrodes for C^4D with the same dimensions (width and gap), aluminum (Al) electrodes were sputtered and integrated with printed channels [25]. To provide this integration, a perforated polyester sheet containing microfabricated electrodes (Fig. 2A) was aligned to another polyester film comprising printed channels (Fig. 2B) and then laminated at 120°C to obtain the final device for C^4D measurements (Fig. 2C) [25].

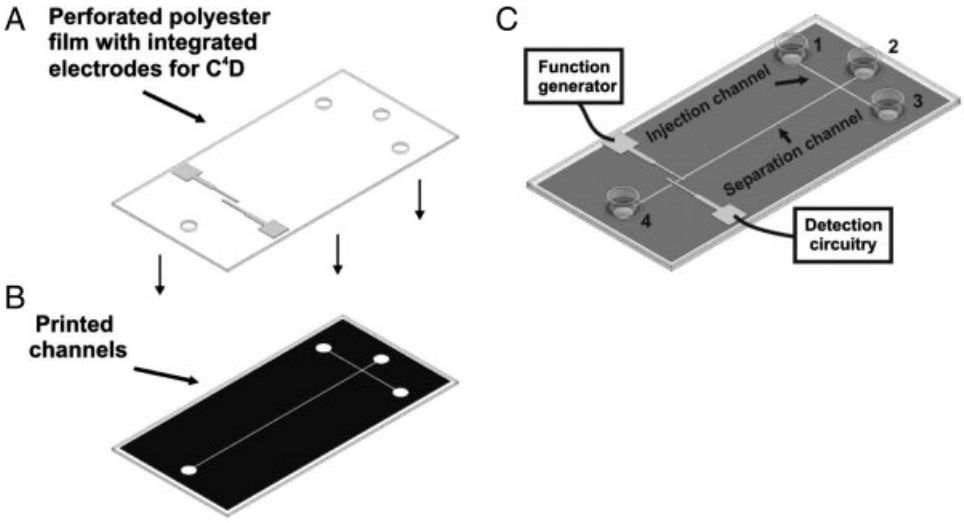

Figure 2. Generic scheme of the integration of electrodes to printed channels by thermal lamination. The devices (A), (B), and (C) represent, respectively, the polyester with the microfabricated electrodes, the printed channels, and the final device with electrodes for C^4D. Image (C) is reprinted from [25] with permission.

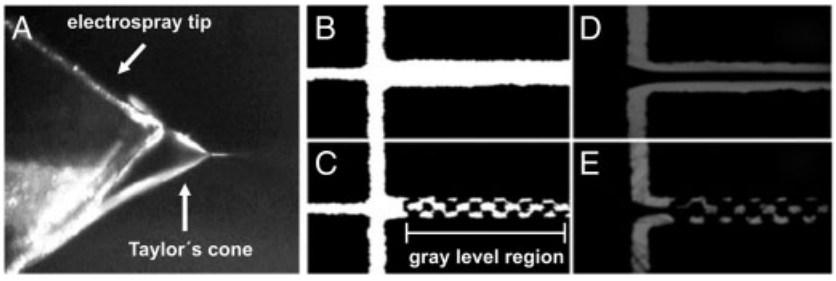

Figure 3. Examples of electrospray (A) and micromixer (B–E) devices produced by direct-printing process. (B) Typical microchannels defined in PT platform; (C) PT channel with a gray level applied on separation channel; (D) and (E) fluorescence images showing the flow behavior in the absence and presence of a gray level, respectively. Image (A) and images (B–E) are reprinted from [18, 26] with permission, respectively.

2.1.2 Electrospray and mixing devices

Electrospray and micromixer devices have also been reported by using the direct-printing process. The generation of Taylor's cone from a PT microchannel outlet was shown by do Lago and *et al.* [18]. The electrospray phenomenon on the PT device was observed for a drop of solution hanging at the wedge-cut tip (Fig. 3A). For a 1 mmol/L KCl solution, a stable Taylor's cone was obtained with a current of 170 and 240 nA for 150- and 200-μm wide channels, respectively. The stability of the electrospray obtained in such device was demonstrated and it is thus feasible. Reports using such interface systematically are addressed in the literature and eventually, some applications will appear soon.

As discussed in the previous reports [18–21], when standard laser printers are used with resolution lower than 1200 dpi, the definition of the channel is not as good as those ones obtained by photolithographic processes. The toner layer roughness and the presence of toner particles (~7 μm) in microchannels is a limitation associated with the direct-printing process. Liu *et al.* [26] exploited the presence of toner particles inside microchannel to create passive micromixers. The size and shape were determined by a gray level created in the drawing program. Using the toner patches as obstacles in the flow path, advective mixing was evaluated by ranging the gray level from 0 to 90%. For a gray level of 0%, no toner particle (or toner array) is observed in the microchannel after printing step (Fig. 3B). For a channel with gray level of 70%, for example, periodic and separated arrays (Fig. 3C) can be obtained inside the microchannel. If no mixing elements exist in the microchannel, a typical laminar flow is observed (Fig. 3D) because the Reynolds number is low and mixing process does not occur. On the other hand, the mixture of the fluids happens in the microchannel with printed elements (Fig. 3E). The gray level applied during the printing significantly affects the efficiency of mixing.

2.1.3 Microreactor, concentrator, and purification devices

In addition to the mentioned examples, Liu *et al.* described a PT device for performing enzymatic reactions [27]. Strong nonspecific adsorption of proteins was utilized to effectively immobilize enzymes onto the microchannel wall, forming an integrated on-column enzyme microreactor in a microchip. The properties of the enzyme microreactor were evaluated by using the oxidation of glucose catalyzed by glucose oxidase as a model. The product of this reaction (H_2O_2) was electrochemically detected in an end-channel mode using a Pt electrode.

Recently, toner-based devices have been proposed as microfluidic platform for rapid protein concentration and purification [28]. Electrokinetic concentration of fluorescein-labeled dog serum albumin (FITC-DSA) was successfully demonstrated by using two printed V-shaped microchannels in mirror image orientation (Fig. 4A) separated by a 100 μm wide gap (Fig. 4B). Under the application of electric field, nanofissures (NF) were formed in the toner layer by electric breakdown at the junction toner gap (Fig. 4C). The lower (I) and the upper (II) channels were filled with protein and phosphate buffer, respectively. During the course of protein concentration, voltage was applied to the reservoir "a," whereas the reservoir "c" was grounded (Fig. 4A). Concentration of negatively charged proteins was observed at the anode side of the NF upon application of an electric field across this junction, as shown in the fluorescence images recorded after 1.5 (Fig. 4D) and 5.5 min (Fig. 4E) of applying electric field. Besides the concentration phenomenon, the protein purification step was also confirmed in the device proposed by Yu *et al.* [28].

2.2 Indirect fabrication techniques

The major advantage of the laser-printing process is that it is not limited to produce microchips directly on the PT platform. As discussed in the previous section, PT devices are fabricated directly by a laser-printing step on a polyester film followed by a thermal lamination. Toner-based or toner-mediated devices can also be produced by an indirect method. Some substrate materials such as glass and PDMS cannot be placed directly in a tray of conventional laser printers. For this purpose, a toner layer (containing a negative image of the desired layout) is first printed on a wax paper sheet. This wax paper has a smooth and low-adherence surface, which allows the toner layer to be transferred to a higher affinity surface by heating [29].

Since 2004, a series of analytical devices fabricated on glass, elastomeric, or even conductive substrates have been reported [8]. Printed circuit board (PCB) and recordable compact disks (CD-R) have been used as low-cost conductive substrates for producing electrodes or devices for digital microfluidics (DMF). Examples of these applications are presented and discussed, according to the substrate material in the following sections.

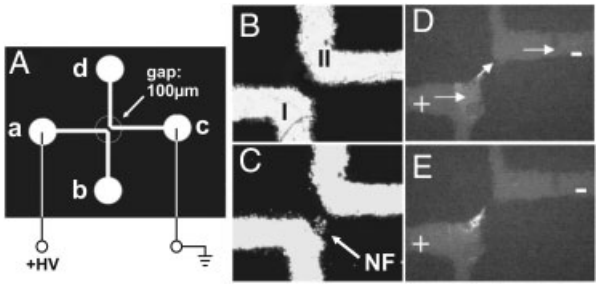

Figure 4. Layout and images of a PT device for protein concentration and purification. (A) Device layout in a V-shaped format with a 100 μm gap for NF generation; (B) and (C) photographs of the junction gap before and after electric breakdown, respectively. (D) and (E) Fluorescence images taken after applying voltage of 80 V between the two microchannels for 1.5 and 5.5 min, respectively. Images (A–E) are reprinted from [28] with permission.

2.2.1 Glass–toner and glass–glass devices

Although PT is a suitable platform for microfluidic applications, the chemistry of the resulting inner surface is still not well understood and requires further systematic investigation. As an alternative, the polyester can be replaced by a glass plate as proposed by do Lago *et al.* [29]. In this case, because glass cannot be directly printed, the toner is initially printed on a wax paper and then thermally transferred onto the glass surface.

Additionally, multiple toner layers can be sequentially transferred onto glass surface increasing the aspect ratio. The sealing of these channels is provided by using a thermal press, in which a glass cover binds to the upper toner layer under heating and pressure. Examples of devices with up to five toner layers were reported [29]. By using this glass–toner platform, a microchip for free-flow electrophoresis was developed showing promising results [30]. The proposed device did not employ a narrow channel to isolate the separation channel from the side reservoirs, where the potential separation was applied. For this purpose, a cross-linked polyacrylamide gel was used to decrease the voltage drop.

The toner layer on the glass surface was also evaluated as a mask in chemical etching process when exposed to hydrofluoric acid solution [31]. It was found that the toner presents a good resistance in HF solution. The resistance time is directly proportional to the number of toner layers. When using a 25% HF solution under continuous stirring, the resistance time of a mask with two-toner layers was around 7 min after a baking at 120°C/20 min. Under these conditions, 35 μm deep and 200 μm wide channels could be obtained in *ca.* 10 min at very low cost. C^4D and fluorescence detection were successfully performed in these devices during the electrophoretic separation of inorganic cationic species and fluorescently labeled amino acids [31].

2.2.2 Masters for soft lithography

PDMS is the most popular elastomeric substrate for microfluidics [10]. The production of PDMS device is based on soft lithography and it requires a high-relief master for prototyping. These masters are conventionally fabricated in SU-8 or electroplated metal by using photolithography. The versatility of the direct-printing process allows it to produce masters or molds for rapid prototyping of polymeric devices. Two different groups [32, 33] demonstrated that the printing of a toner layer on the transparency could be used as the positive relief of the masters. Examples of PDMS electrophoresis devices were successfully obtained and integrated to electrochemical detection of electroactive biological molecules and nonelectroactive inorganic ions [33].

A similar approach was presented by Vullev *et al.* [34], who constructed a continuous-flow PDMS device (Fig. 5A) for the detection of bacterial spores on the basis of enhancement of the emission of terbium (III) ions. Hong *et al.* [35] employed this nonlithographic process for the fabrication of arrays of microelectrodes on smooth substrates. A sequence of print-and-peel procedures allowed the fabrication of capacitance microsensors assembled with a microfluidic network (Fig. 5B). Further details about the development and advances of the print-and-peel technique for microfluidics can be found in a well-detailed review published recently [36].

Abdelgawad *et al.* [37] reported the printing of masters on demand for soft lithography. PDMS channels were produced by replica molding on masters formed by laser printing on PCB substrates. Channels were fabricated as narrow as 100 μm with heights ranging from 9 to 70 μm

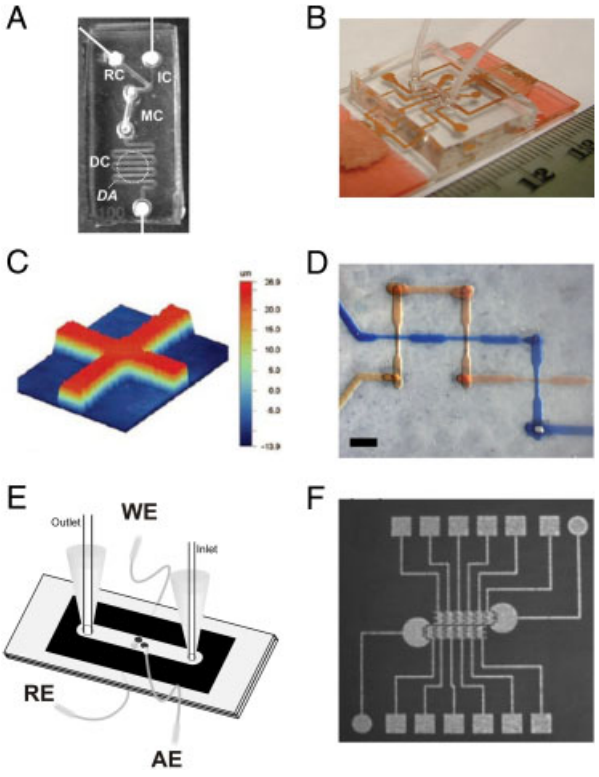

Figure 5. Toner-based devices produced by indirect approaches. (A) PDMS device for continuous flow; (B) capacitance cell fabricated by a print-and-peel process; (C) 3-D profilometric scan of a resulting master produced on a copper sheet; (D) image of crossing channels in a 3-D channel network made using toner transfer masking and multi-layer soft lithography; (E) three-electrode electrochemical microfluidic cell; (F) closeup image of a DMF device fabricated by using laser printing on copper sheets. In (A), the labels IC, RC, and MC mean the injection channel, reagent channel, and mixing chamber, respectively. The detection channel as well as the detection area is labeled as DC and DA, respectively. In (E), WE, RE, and AE represent working, reference, and auxiliary electrodes, respectively. Images (A), (B), (C), (D), (E), and (F) are reprinted with permission from [34, 35, 37, 38, 52, 57], respectively.

(Fig. 5C). Using devices produced by printed masters, the authors demonstrated electrophoretic separations, culture, and analysis of primary mammalian cells. Recently, by using toner transfer masking Easley *et al.* [38] described the fabrication of multilayer PDMS devices with three-dimensional (3-D) channel network (Fig. 5D). Wang *et al.* [39] have also made PDMS devices by toner transfer masking. The proposed micro/nanofluidic device comprised an enzymatic chamber and a nanogap for enzyme concentration.

Alternative technologies to produce masters for soft lithography have been reported by different groups. McDonald *et al.* [40] described the use of a solid-object printer to make thermoplastic masters for PDMS molding. This technique was able to print features with dimensions larger than 250 μm. Despite their versatility, solid-object printer technique is considerably expensive. Kaigala *et al.* [41] reported the use of a wax printer to fabricate high-resolution PDMS-based microfluidic devices. In this approach, the wax printer was able to print a high-relief design on a transparency film. After a thermal treatment, the printed features were shown to be smoother than those using toner particles as molds. PDMS channels produced using wax-printed masters were able to perform sizing of DNA fragments with no apparent loss of resolution when compared with glass chips with similar dimensions. On the other hand, Grimes and *et al.* [42] introduced a method of printing microfluidic networks of channels onto commercially available thermoplastic ''Shrinky-Dinks'' in a standard laser printer. Upon thermal relaxation of the prestressed polymer, the printed features shrink isotropically in plane in *ca.* 60% from the original size. Besides, Yuen and Goral [43] presented the prototyping of flexible microfluidic devices by using a desktop digital craft cutter. 3-D microfluidic channels wider than 200 μm were produced using double-side pressure-sensitive adhesive tape and transparency film.

2.2.3 Electrodes on compact disks and copper substrates

The preparation of electrodes (for electrochemical sensing or DMF) can also benefit from direct-printing processes. Simultaneously to the spread of laser printing technology, Daniel and Gutz [44] described an innovative method to manufacture gold microelectrodes using commercial CD-R as low-cost metal nanolayer [45, 46]. In this method, initially the direct-printing process is used to print the electrode design over a wax paper. Afterward, the printed image is thermally transferred (using a thermal press) onto the gold layer of a CD-R, from which the protective film must be previously removed with nitric acid. Following the toner transference, the wax paper is easily removed from CD-R surface and the toner-free gold areas are etched with an iodide/iodine solution. After toner removal, Au-CDtrodes are obtained [44].

This process was replicated by Richter *et al.* [47] for the production of electrodes for amperometric detection in CE.

These Au-CDtrodes were used first in a home-made CE system [47], and later applied to microchip electrophoresis [19]. The use of these Au-CDtrodes in both CE and microchip electrophoresis is attractive because the electrode replacement can be made quickly, requiring only a small adjustment between the electrode and the capillary/channel extremity. As reported by Lowinsohn *et al.* [48], the electrochemical behavior of these disposable Au-CDtrodes yielded relative standard deviation below 1% over ten different Au-CDtrodes.

The fabrication of microfluidic flow cells with interdigitated array of Au-CDtrodes was also reported by Daniel and Gutz [49]. The microfluidic channel was prepared by thermal transference of three toner layers over the polycarbonate CD base, with the bonding provided by a second polycarbonate slice. The same authors reported later [50] the integration of a microreactor for TiO_2-assisted photocatalysis in a microfluidic electrochemical cell constructed by combining CDtrodes and toner thermal transference. The technology involving CDtrodes and toner masks has been explored to create disposable electrochemical microcell containing working, reference, and auxiliary electrodes on a single device [51]. Silver epoxy was deposited on one of the gold bands, which was satisfactorily used as reference electrode. The integration of this 3-electrode electrochemical cell to microfluidic channels (Fig. 5E) has also been reported recently [52]. Generator-collector electrochemical microdevices [53–55] and new procedures for flow rate control and sample injection in microfluidic channels [56] are some additional examples of recent publications using toner-based techniques.

Abdelgawad and Wheeler [57, 58] reported the use of toner masks for rapid prototyping of DMF devices. For their purpose, a toner layer was printed directly onto the copper surface of a flexible PCB by using a laser printer. The pattern served as a mask for copper etching, after which the devices were coated with a dielectric layer and used for DMF (Fig. 5F). A similar approach has been reported to fabricate electrodes on a PCB piece for C^4D measurements [59]. The simultaneous determination of ionic and electroactive species was illustrated by the separation of peroxynitrite degradation products, *i.e.* NO_3^- and NO_2^-, using hybrid PDMS/glass chips with dual contactless conductivity and amperometric detection [59].

Overall, as presented and discussed in the previous subheadings, it was possible to note that microfluidic devices can be produced in different platforms using direct or indirect toner-mediated technologies. In these examples, the devices were fabricated without cleanroom facilities with low-cost instrumentation and consumables that provide noticeable advantages over photolithographic processes, as summarized in Table 1. The main limitation is related to the channel dimensions (width/depth).

3 Paper-based microfluidic devices

Since the last century, paper – generally made of cellulose fibers – has been used as platform for chromatographic separations [60], chemical [61, 62], and biochemical tests [63, 64]. Recently, however, Whitesides and co-workers [14, 15, 65–67] demonstrated the advantages of using this

Table 1. Description of the main advantages, required instrumentation, and limiting dimensions for the toner-based devices

Toner-based devices (references)	Advantages	Required instrumentation/consumables	Limiting dimensions
PT [18–28]	Direct-printing of microfluidic channels on polyester films in less than 5 min	Laser printer, laminator, paper driller, trans parency, toner cartridge	150 ± 12 μm width 6 ± 1 μm depth (STL) 12 ± 2 μm depth (DTL)
Glass–toner [29, 30]	Multiple toner layers can be transferred to glass surface increasing the aspect ratio	Laser printer, heat press, glass slides, wax paper sheet, toner cartridge	100 μm width (STL) 11 ± 3 μm depth (STL)
Glass–glass [31]	Glass channels are etched using toner layers as etching masks, *i.e.* without any photolithographic step	Laser printer, heat press, glass slides, wax paper sheet, toner cartridge, wet chemical etching solution	200 μm (DTL) 35 μm depth (DTL) 60 μm width
Masters for PDMS molding [32–36]	Disposable toner masters for PDMS molding can be prepared in matter of minutes	Laser printer, transparency, toner cartridge, Sylgard 184	10 μm depth
Toner masks for sputtering [25]	Electrodes can be fabricated using toner masks for metal deposition	Laser printer, transparency, toner cartridge	100 μm width
Gold electrodes in CD-Rs [21, 44–56]	*ca.* 50 electrodes can be fabricated from one CD with area defined by toner masks reducing the cost/electrode (cents/dollars)	Laser printer, heat press, wax paper sheet, toner cartridge, CD-R	100 μm width
Copper electrodes in PCB for C^4D or DMF [57–59]	Copper electrodes are produced on PCB using toner layers as metal etching masks	Laser printer, heat press, wax paper sheet, toner cartridge, PCB substrates	50 μm gap 300 μm width

substrate for fabrication of microfluidic devices that allow conducting multiples, simultaneous, and independent assays (multiplexed detection). For this purpose, the main attractive features of the paper are its low cost, high abundance, elevated porosity, great biodegradability, and excellent chemical compatibility with many applications. Moreover, paper can be patterned by traditional photolithography processes, or by alternatives methods for mass production of microfluidic paper-based analytical devices (μPAD). Introduction of solutions into μPADs is easily attained by capillary action without external – and/or complex – pumping system. Chemical modification of paper surface with appropriated reagents allows the development of μPADs for simple and fast colorimetric detection of many analytes. The next sections of this review describe the main fabrication techniques, some detection methods, and the main applications for microfluidic devices using paper as substrate. This emerging new (or at least renewed) platform carries a great potential to provide near ''zero-cost'' diagnostics [16].

3.1 Fabrication techniques

In the first approach to fabricate a μPAD, Whitesides and coworkers [14] applied SU-8 photoresist onto chromatographic paper that was subsequently patterned with millimeter-size channels by using conventional photolithography processes (Fig. 6A). The surface of the patterned paper was treated with oxygen plasma in order to restore the paper hydrophilicity that was affected, probably by photoresist residues. After fabricating the devices, the reagents for the bioassays were spotted in their respective test zones (Fig. 6B).

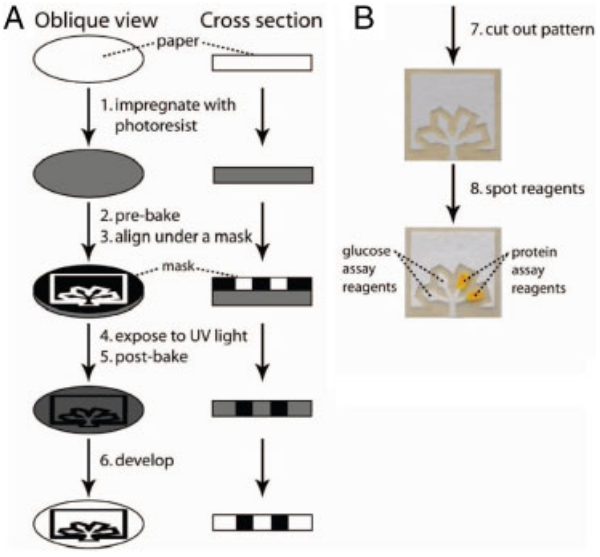

Figure 6. Scheme of the main steps for fabrication of μPADs. (A) Photolithography procedure for SU8-photoresist patterning onto paper substrate; (B) chemical derivatization of the patterned paper for colorimetric detection. Figure adapted from [15] with permission.

A year later, the same research group proposed an ingenious microfabrication method [66] that used an *x*–*y* desktop plotter with a modified pen that allowed direct printing of polymeric barriers onto paper substrate. Solutions of PDMS, polystyrene, and others polymers were evaluated onto different kinds of paper such as newsprint, paper towels, and filter paper. The polymer solutions were able to penetrate the paper fibers and generate hydrophobic barriers that avoided leakage of aqueous solutions between channels. PDMS polymer was the preferable choice because this material is well established for microfluidic devices. Moreover, it was found the high-PDMS flexibility provides to the μPAD a higher mechanical resistance than that offered by SU-8. The use of PDMS barriers to define microchannels in paper substrates does not require clean-room facilities or UV radiation exposition; consequently, it is less expensive than a photolithographic process.

Whiteside's group also introduced a low-cost version of the herein-described photolithographic method that was called fast lithographic activation of sheets (FLASH) [67]. In the FLASH method, initially the paper substrate is impregnated with a home-made, low-cost, photoresist derived from SU-8 resin. In the sequence, the photoresist-impregnated paper is kept together between a transparency film and a black-colored paper. An inkjet printer, a photocopying machine, or even a black pen can be used to print the microfluidic layout on the side of the transparency film. This printed film acts as a photomask during the subsequent exposition of the FLASH paper to UV or sunlight radiation. The transparency film and the black paper are then removed from the FLASH paper and the photoresist-impregnated paper is washed with acetone and isopropanol solution. The soluble photoresist (unpolymerized) is dissolved during the washing step and consequently the microfluidic layout is patterned on the paper. With the FLASH method, microfluidic devices could be produced in less than 30 min at considerably lower costs than that using conventional photolithography.

The Harvard group also demonstrated that 3-D microfluidic devices with high fluidic complexity and analytical capabilities could be easily obtained by the intercalation of SU-8 patterned paper with double-sided adhesive tape (Fig. 7A) [65]. In these 3-D microfluidic devices, the fluids can move along the hydrophilic channels (horizontal) and between the layers (vertical) by passing through holes made in the adhesive tape (Figs. 7B and C). This fluidic system has four channels (with 800 μm wide and *ca.* 5 cm long each) that cross one another multiple times in a basket-weave pattern, and include eight connections between the top and the bottom layers of paper.

Naturally, soon after this new use of paper has been demonstrated, other research groups also proposed fabrication technologies for μPADs. For instance, Li *et al.* [68] used plasma treatment to pattern microfluidic structures onto previously hydrophobized paper. The paper was made hydrophobic after soaking it in a solution of alkyl ketene dimmer, followed by a heating treatment. Patterned

stainless steel sheets were used as masks – stencils – during the plasma treatment. In this way, the regions of the paper exposed to the plasma become hydrophilic defining the microfluidic pathway. Similarly to the PDMS printing technique [66], the plasma method does not significantly affect the flexibility of the paper. This technology was applied to fabricate microfluidic devices containing channels and other elements such as switches, filters, and separators.

Fenton *et al.* [69] used a computer-controlled cutting plotter to precisely cut chromatographic paper and nitrocellulose (NC) membranes in suitable shapes to conduct microfluidic experiments. The shaped paper can readily be used in the assays, or it can be covered (one, or even both sides) with a polyester adhesive tape containing inlet vias to introduce sample solutions. The same group contributed to the rational design of μPAD to present a quasi-stationary continuous flow of liquid throughout the whole extension of the paper channel [70].

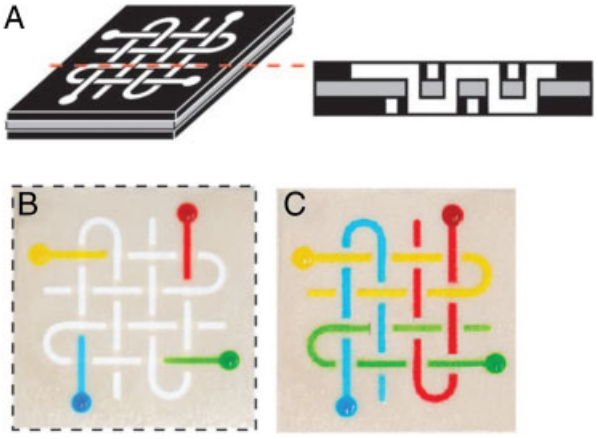

Figure 7. Presentation of 3-D μPADs. (A) Final device and its cross-section view; (B) and (C) photographs taken 10 s (B) and 4 min (C) after adding red, yellow, green, and blue dyes. In (A), the black and gray colors indicate the photoresist and tape layers, respectively. All reprinted from [65] with permission.

In two independent works [71, 72], wax printing was used as the simplest and fastest technique to pattern paper for microfluidic applications. In this method, initially the fluidic layout is printed onto chromatographic or filter paper with a commercial wax ink printer. In the next step, the wax-printed paper is heated in order to melt the wax that consequently diffused (vertical and horizontal) by capillarity through the porous paper, generating hydrophobic barriers across the paper (Fig. 8A). A set of microfluidic devices can be fabricated in less than 5 min. The smallest width of a channel produced by this process was found to be around 550 μm [72], that is quite higher than those usually obtained by photolithographic methods. Nevertheless, the simplicity, low cost, and speed of the wax printing method make it very attractive for mass production of μPAD. Moreover, researchers without access to photolithographic facilities can use this technique for rapid development of new prototypes of μPADs. Examples of μPAD for detecting total protein, cholesterol, and glucose in biological fluids (Fig. 8B) as well as 384-zone paper plates (Fig. 8C) were successfully demonstrated by using wax-printing process. The use of wax printer was expanded recently to cover applications using NC membranes as porous media to production of μPADs [73]. One advantage of using NC membrane is that the

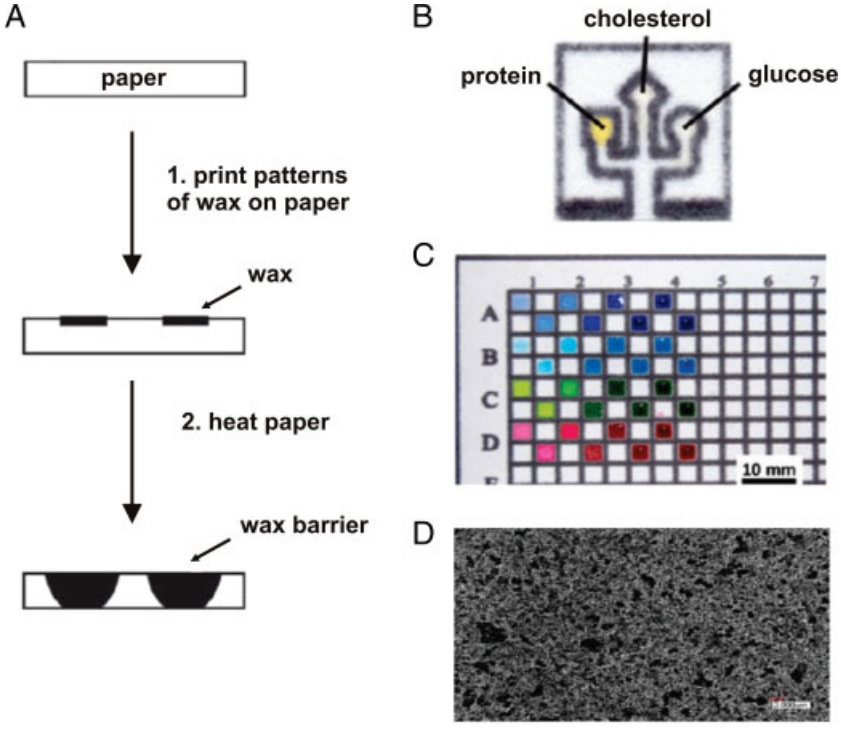

Figure 8. Fabrication technique based on wax printing and resulting μPADs. (A) Wax printing process; (B) μPAD for detecting total protein, cholesterol, and glucose in biological fluids; (C) example of a 384-zone paper plate, and (D) NC membrane surface profile showing the pore size uniformity before wax printing. (A), (B), (C), and (D) are reprinted from [16, 72, 73], respectively, with permission.

average pore size is quite small (0.45 μm) and uniform (Fig. 8D). For this reason, the wax penetration process during baking is much slower and can be controlled more precisely. Examples of protein immobilization and sample purification were carried out on the wax-patterned NC membrane [73].

3.2 Detection methods

Most of the presented μPADs have been evaluated for colorimetric bioassays [14, 15, 65–69, 74, 75]. Detection areas are created by chemical modification of the paper surface by deposition of reagents such as enzymes [14, 15, 65–69], acid–base indicators [66, 68, 69], dyes [14, 15, 75], gold nanoparticles [74], *etc.* When the sample solution reaches the detection zone, a chemical reaction between the target compound and the immobilized reagents is carried out and a color is developed. The intensity of this color can be used for quantitative analysis and the specificity of the reactions guarantees good detection selectivity. Multiple detection zones for different compounds can be implemented in a same microfluidic device. For instance, 1024 detection zones were designed in a unique 3-D μPAD [65]. Whitesides and co-workers used this multiplex analysis capability for simultaneous qualitative and quantitative determination of glucose and proteins (bovine serum albumin) [14, 15, 66] in a single device. The use of instrumental detection techniques in μPAD can be considered a new trend in this field. Cell-phone cameras and scanners [15] are relatively inexpensive off-the-shelf solutions to provide quantitative chemical information by reflectance with very minor imaging processing. This last step just requires the transmission of the images to a computer and their conversion to a grayscale for further quantitative analysis. Whitesides and co-workers developed a portable and low-cost apparatus to measure light transmittance [76] through μPAD dedicated to bioassays. Quantitative analysis of protein in artificial urine was shown on μPAD (Figs. 9A and B) by using a transmittance colorimeter (Fig. 9C). Vykoukal *et al.* [77] also proposed a dedicated low-cost image sensor array for low-cost diagnostic analysis base on complementary metal-oxide semiconductor image sensor obtaining great correlation ($R^2 = 1$) for glucose concentration.

The quantitative correlation of chemical composition to the color intensity is a very active topic as well. Carrilho *et al.* [78] briefly compared the quantitative correlation of concentration of chemicals with their absorbance using a microplate reader as spectrophotometer. Further, the absorbance was compared with reflectance, which was obtained with a simple flatbed scanner. The image from the scanner was minimally processed by the conversion of the colors into gray scale. Obviously, this simple approach was only proposed to demonstrate the feasibility of the quantitative analysis in paper-based assays. On the other hand, color analysis can be very sophisticated. For example, many color spaces are available for standardizing color such as RGB – red, green, and blue; CMYK – cyan, magenta, yellow, and black; CIE L*a*b; and HSV – hue, saturation, and value (see the review of Cheng *et al.* [79] for comprehensive mathematical derivation for all of them). The later color space (HSV) was particularly evaluated in quantitative analytical processing by Cantrell *et al.* [80] and they found that the *H*-parameter was the most robust for quantitative determination when color change was involved in the chemical analysis.

Electrochemical detection on paper devices (Fig. 9D) has been successfully explored by different research groups for the detection of glucose, lactate, uric acid [81, 82], heavy metals [83], and ascorbic acid [82] in model solutions and biological samples. Although these instrumental detections increase the complexity and costs of the devices, the sensitivity, selectivity, and precision are

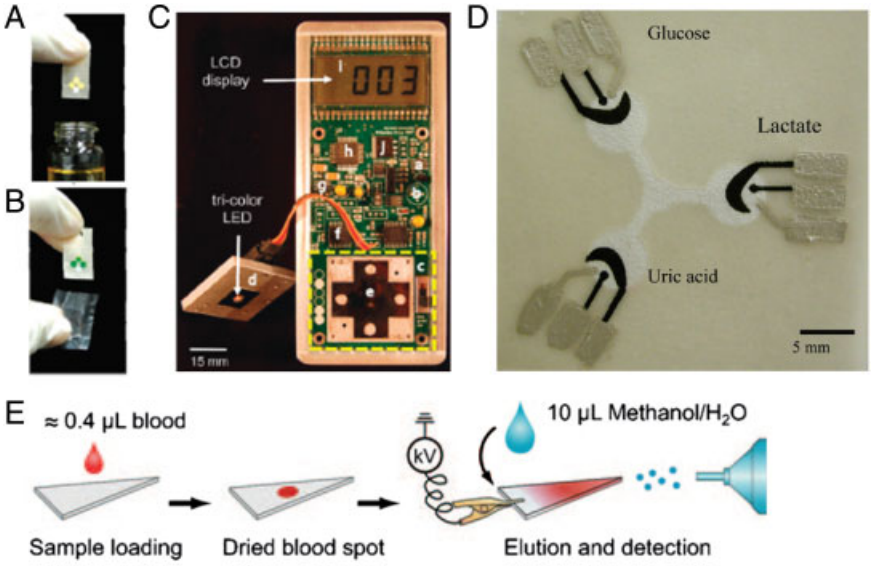

Figure 9. Examples of detection modes for bioassays on paper devices. (A) μPAD spotted with reagents for detecting protein in urine; (B) μPAD after colorimetric assay; (C) photograph of the colorimeter; (D) μPAD with three test zones containing electrodes for electrochemical detection of glucose, lactate, and uric acid; (E) paper spray for direct analysis of complex mixtures using MS. (A–C), (D), and (E) are adapted from [76, 81, 84], respectively, with permission.

significantly improved when compared with the visual colorimetric detection.

MS could be considered the ultimate detection system for any analytical platform. Cooks and co-workers demonstrated that paper tips could successfully replace nanotip injectors (nanospray) for the direct analysis of complex mixtures, such as whole blood (Fig. 9E) [84]. MS is not, however, ideally aligned with the concepts of (i) low cost, (ii) easy-to-use, and (iii) portable assays to be carried out on the field as paper-based assays. Nevertheless, the authors properly addressed that with the current miniaturization of MS [85], resulting in evolution of hand-held mass spectrometers; it will be possible that clinical applications can be carried in hospitals or in modern medical practice offices. We foresee that the combination of μPAD (both 2-D and 3-D systems) with portable MS will be a powerful combination with countless applications. Preliminary results shown by Carrilho *et al.* [86] demonstrated that μPADs are compatible with matrix-assisted laser desorption ionization time-of-flight MS on the analysis of proteins and peptides.

3.3 Applications

The applications of μPADs have been frequently addressed to bioassays. In a recent work [78], paper was patterned using photolithography and dilute photoresist to generate 96 and 384 microzones plates as an alternative for the conventional multiwell plates made of polymeric materials. The paper-made device was considered less expensive and provided a better sensitivity for fluorescence detection. Additionally, it is possible to add function to these paper plates if interconnecting hydrophilic channels are fabricated within the microzones. Such functionality is not easily attained in plastic microplates.

Rapid diagnostic tests for people in underdeveloped regions worldwide are considered the promising application for the μPADs [16, 87]. This statement is supported by the facts that paper is inexpensive, accessible, absorbent, disposable, and easy to modify chemically. Moreover, most of the used microfabrication technologies practically do not need complex and expensive instrumentation. Hence, this substrate allows an inexpensive mass production of simple and portable microfluidic devices that do not require use of pipettes, pumps, or electric energy for conducting diagnostic assays. The features of these devices are suitable for use in remote settings by nontrained personal. As an example of this capability, Whitesides's group demonstrated that a digital camera, a portable scanner, or a camera phone can be used to digitalize the colored images of detection zones on paper devices, after a diagnostic bioassay [15]. The respective electronic file is used for a more accurate quantitative analysis. Sending the image of the device through the cell phone in real time to a specialist for data analysis and interpretation uses the telemedicine concept. If required, the specialist off-site indicates a suitable medical treatment for the patient by returning a message to the field agent.

Certainly, the applicability of the μPAD is not restricted to diagnostic assays. It is believed [16, 87] that these devices can also be explored for homeland security, environmental monitoring, food safety, and other point-of-care applications. Regardless the application, further development of functional or active papers is required. Bioactive paper is a field as new and promising as the μPAD field itself. Bioactive paper includes a range of potential paper-based materials that can perform analytical functions normally reserved for multiwell plates in the laboratory or for portable electronic devices [88]. The development of one is highly bound to the development of the other, and a detailed coverage of this field would result in a review on its own right. For an in-depth review of the current progress in bioactive paper and a good coverage of the basics of paper, refer to review of Pelton [88] and other studies from the Sentinel Bioactive Paper Network (http://www.bioactivepaper.ca/).

4 Concluding remarks

As presented in this review, toner and paper-based techniques – albeit different approaches to assays – have shown potential to become powerful technologies for both rapid prototyping and low-cost massive production of microfluidic devices. The production of microdevices is advantageous because the techniques used in the fabrication do not require sophisticated instrumentation or cleanroom facilities. In addition, the consumables employed in both platforms (transparency, wax paper, CD-R, PCB, toner, paper, wax ink, *etc.*) are very inexpensive and ubiquitous.

Future researches with toner-based devices should include the investigation of high-resolution laser printers to produce narrower and better-defined microchannels aiming to improve the analytical performance of such devices. We believe that the chemistry involved in the toner composition can also be evaluated to a better understanding of the EOF generation. Furthermore, the use of toner chips for the separation of proteins, peptides, DNA fragments, as well as the integration of other analytical procedures on a single device is straightforward and can provide a significant advance to the next generation of chips.

As well as toner chips, paper is a very promising platform with several capabilities for bioanalytical assays. In addition to colorimetric bioassays [14, 15, 65–69, 74, 75], the recent integration of electrochemical detection on μPMDs [78–80] opens new application possibilities for this substrate. μPADs with electrochemical detection can be perfectly applied in areas such as neurochemistry, immunoassays, clinical diagnostics, and environmental assays. We can expect a rapid and large expansion of this field in the incoming years because of the great support from public–private partnerships [88], not for profit institutions (http://www.dfa.org/), and philanthropic foundations (http://www.gatesfoundation.org/Pages/home.aspx) that are currently dedicated to low-cost diagnostics to improve the quality of life of billions of people.

The authors gratefully acknowledge financial support from CNPq and FAPESP. Professor Carol Collins is also thanked for the manuscript revision and language assistance.

The authors have declared no conflict of interest.

5 References

[1] Manz, A., Graber, N., Widmer, H. M., *Sens. Actuat. B* 1990, *1*, 244–248.

[2] Reyes, D. R., Iossifidis, D., Auroux, P. A., Manz, A., *Anal. Chem.* 2002, *74*, 2623–2636.

[3] Auroux, P. A., Iossifidis, D., Reyes, D. R., Manz, A., *Anal. Chem.* 2002, *74*, 2637–2652.

[4] Vilkner, T., Janasek, D., Manz, A., *Anal. Chem.* 2004, *76*, 3373–3385.

[5] Dittrich, P. S., Tachikawa, K., Manz, A., *Anal. Chem.* 2006, *78*, 3887–3907.

[6] West, J., Becker, M., Tombrink, S., Manz, A., *Anal. Chem.* 2008, *80*, 4403–4419.

[7] Dolnik, V., Liu, S. R., Jovanovich, S., *Electrophoresis* 2000, *21*, 41–54.

[8] Castano-Alvarez, M., Ayuso, D. F. P., Granda, M. G., Fernandez-Abedul, M. T., Garcia, J. R., Costa-Garcia, A., *Sens. Actuat. B.* 2008, *130*, 436–448.

[9] Coltro, W. K. T., Carrilho, E., in: Landers, J. P. (Ed.), *Handbook of Capillary and Microchip Electrophoresis and Associated Microtechniques*, 3rd Edn, CRC Press, New York 2007, pp. 1169–1184.

[10] Fiorini, G. S., Chiu, D. T., *Biotechniques* 2005, *38*, 429–446.

[11] Duffy, D. C., McDonald, J. C., Schueller, O. J. A., Whitesides, G. M., *Anal. Chem.* 1998, *70*, 4974–4984.

[12] Becker, H., Gartner, C., *Electrophoresis* 2000, *21*, 12–26.

[13] Becker, H., Gartner, C., *Anal. Bioanal. Chem.* 2008, *390*, 89–111.

[14] Martinez, A. W., Phillips, S. T., Butte, M. J., Whitesides, G. M., *Angew. Chem. Int. Ed.* 2007, *46*, 1318–1320.

[15] Martinez, A. W., Phillips, S. T., Carrilho, E., Thomas III, S. W., Sindi, H., Whitesides, G. M., *Anal. Chem.* 2008, *80*, 3699–3707.

[16] Martinez, A. W., Phillips, S. T., Whitesides, G. M., Carrilho, E., *Anal. Chem.* 2010, *82*, 3–10.

[17] Tan, A., Rodgers, K., Murrihy, J. P., O'Mathuna, C., Glennon, J. D., *Lab Chip* 2001, *1*, 7–9.

[18] do Lago, C. L., Silva, H. D. T., Neves, C. A., Brito-Neto, J. G. A., da Silva, J. A. F., *Anal. Chem.* 2003, *75*, 3853–3858.

[19] Coltro, W. K. T., Lunte, S. M., Carrilho, E., *Electrophoresis* 2008, *29*, 4928–4937.

[20] He, F. Y., Liu, A. L., Yuan, J. H., Coltro, W. K. T., Carrilho, E., Xia, X. H., *Anal. Bioanal. Chem.* 2005, *382*, 192–197.

[21] Coltro, W. K. T., da Silva, J. A. F., Silva, H. D. T., Richter, E. M., Furlan, R., Angnes, L., do Lago, C. L., Mazo, L. H., Carrilho, E., *Electrophoresis* 2004, *25*, 3832–3839.

[22] Liu, A. L., He, F. Y., Hu, Y. L., Xia, X. H., *Talanta* 2006, *68*, 1303–1308.

[23] Yu, H., He, F. Y., Lu, Y., Hu, Y. L., Zhong, H. Z., Xia, X. H., *Talanta* 2008, *75*, 43–48.

[24] Lu, Y., Hu, Y. L., Xia, X. H., *Talanta* 2009, *79*, 1270–1275.

[25] Coltro, W. K. T., da Silva, J. A. F., Carrilho, E., *Electrophoresis* 2008, *29*, 2260–2265.

[26] Liu, A. L., He, F. Y., Wang, K., Zhou, T., Lu, Y., Xia, X. H., *Lab Chip* 2005, *5*, 974–978.

[27] Liu, A. L., Zhou, T., He, F. Y., Xu, J. J., Lu, Y., Chen, H. Y., Xia, X. H., *Lab Chip* 2006, *6*, 811–818.

[28] Yu, H., Lu, L., Zhou, Y. G., Wang, F. B., He, F. Y., Xia, X. H., *Lab Chip* 2008, *8*, 1496–1501.

[29] do Lago, C. L., Neves, C. A., de Jesus, D. P., Silva, H. D. T., Brito-Neto, J. G. A., da Silva, J. A. F., *Electrophoresis* 2004, *25*, 3825–3831.

[30] de Jesus, D. P., Blanes, L., do Lago, C. L., *Electrophoresis* 2006, *27*, 4935–4942.

[31] Coltro, W. K. T., Piccin, E., da Silva, J. A. F., do Lago, C. L., Carrilho, E., *Lab Chip* 2007, *7*, 931–934.

[32] Branham, M. L., Tay, R. T. S., Schoonover, C., Davis, P. S., Allen, S. D., Shyy, W., *J. Mater. Res.* 2002, *17*, 1559–1562.

[33] Bao, N., Zhang, Q., Xu, J. J., Chen, H. Y., *J. Chromatogr. A* 2005, *1089*, 270–275.

[34] Vullev, V. I., Wan, J., Heinrich, V., Landsman, P., Bower, P. E., Xia, B., Millare, B., Jones, G., *J. Am. Chem. Soc.* 2006, *128*, 16062–16072.

[35] Hong, C., Bao, D., Thomas, M. S., Clift, J. M., Vullev, V. I., *Langmuir* 2008, *24*, 8439–8442.

[36] Thomas, M. S., Millare, B., Clift, J. M., Bao, D., Hong, C., Vullev, V. I., *Ann. Biomed. Eng.* 2010, *38*, 21–32.

[37] Abdelgawad, M., Watson, M. W. L., Young, E. W. K., Mudrik, J. M., Ungrin, M. D., Wheeler, A. R., *Lab Chip* 2008, *8*, 1379–1385.

[38] Easley, C. J., Benninger, R. K. P., Shaver, J. H., Head, W. S., Piston, D. W., *Lab Chip* 2009, *9*, 1119–1127.

[39] Wang, C., Li, S. J., Wu, Z. Q., Xu, J. J., Chen, H. Y, Xia, X. H., *Lab Chip* 2010, *10*, 639–646.

[40] McDonald, J. C., Chabinyc, M. L., Metallo, S. J., Anderson, J. R., Stroock, A. D., Whitesides, G. M., *Anal. Chem.* 2002, *74*, 1537–1545.

[41] Kaigala, G. V., Ho, S., Penterman, R., Backhouse, C. J., *Lab Chip* 2007, *7*, 384–387.

[42] Grimes, A., Brealauer, D. N., Long, M., Pegan, J., Lee, L. P., Hine, M., *Lab Chip* 2008, *8*, 170–172.

[43] Yuen, P. K., Goral, V. N., *Lab Chip* 2010, *10*, 384–387.

[44] Daniel, D., Gutz, I. G. R., *Electrochem. Commun.* 2003, *5*, 782–786.

[45] Angnes, L., Richter, E. M., Augelli, M. A., Kume, G. H., *Anal. Chem.* 2000, *72*, 5503–5506.

[46] Richter, E. M., de Jesus, D. P., Neves, C. A., Lago, C. L., Angnes, L., *Quim. Nova* 2003, *26*, 839–843.

[47] Richter, E. M., da Silva, J. A. F., Gutz, I. G. R., do Lago, C. L., Angnes, L., *Electrophoresis* 2004, *25*, 2965–2969.

[48] Lowinsohn, D., Richter, E. M., Angnes, L., Bertotti, M., *Electroanalysis* 2006, *18*, 89–94.

[49] Daniel, D., Gutz, I. G. R., *Talanta* 2005, *68*, 429–436.

[50] Daniel, D., Gutz, I. G. R., *Electrochem. Commun.* 2007, *9*, 522–528.

[51] Ferreira, H. E. A., Daniel, D., Bertotti, M., Richter, E. M., *J. Braz. Chem. Soc.* 2008, *19*, 1538–1545.

[52] Silva, R. A. B., Almeida, E. G. N., Rabelo, A. C., Silva, A. T. C., Ferreira, H. E. A., Richter, E. M., *J. Braz. Chem. Soc.* 2009, *20*, 1235–1241.

[53] Ferreira, T. L., Paixao, T. R. L. C., Richter, E. M., El Seoud, O. A., Bertotti, M., *J. Phys. Chem. B* 2007, *111*, 12478–12484.

[54] Paixao, T. R. L. C., Richter, E. M., Brito-Neto, J. G. A., Bertotti, M., *J. Electroanal. Chem.* 2006, *596*, 101–108.

[55] Paixao, T. R. L. C., Richter, E. M., Brito-Neto, J. G. A., Bertotti, M., *Electrochem. Commun.* 2006, *8*, 9–14.

[56] Daniel, D., Gutz, I. G. R., *Anal. Chim. Acta* 2006, *571*, 218–227.

[57] Abdelgawad, M., Wheeler, A. R., *Adv. Mater.* 2007, *19*, 133–137.

[58] Abdelgawad, M., Wheeler, A. R., *Microfluid. Nanofluid.* 2008, *4*, 349–355.

[59] Vázquez, M., Frankenfeld, C., Coltro, W. K. T., Carrilho, E., Diamond, D., Lunte, S. M., *Analyst* 2010, *135*, 96–103.

[60] Clegg, D. L., *Anal. Chem.* 1950, *22*, 48–59.

[61] Dickinson, D., Harris, F. J. T., *Analyst* 1950, *75*, 687–688.

[62] Qureshi, S. Z., Ahmad, S. T., Haque, S., *Talanta* 1990, *37*, 763–765.

[63] Comer, J. P., *Anal. Chem.* 1956, *28*, 1748–1750.

[64] Rorem, E. S., Lewis, J. C., *Anal. Biochem.* 1962, *3*, 230–235.

[65] Martinez, A. W., Phillips, S. T., Whitesides, G. M., *Proc. Natl. Acad. Sci. USA* 2008, *105*, 19606–19611.

[66] Bruzewicz, D. A., Reches, M., Whitesides, G. M., *Anal. Chem.* 2008, *80*, 3387–3392.

[67] Martinez, A. W., Phillips, S. T., Wiley, B. J., Gupta, M., Whitesides, G. M., *Lab Chip* 2008, *8*, 2146–2150.

[68] Li, X., Tian, J., Nguyen, T., Shen, W., *Anal. Chem.* 2008, *80*, 9131–9134.

[69] Fenton, E. M., Mascarenas, M. R., López, G. P., Sibbett, S. S., *Appl. Mater. Interface* 2009, *1*, 124–129.

[70] Mendez, S., Fenton, E. M., Gallegos, G. R., Petsev, D. N., Sibbettt, S. S., Stone, H. A., Zhang, Y., López, G. P., *Langmuir* 2010, *26*, 1380–1385.

[71] Lu, Y., Shi, W., Jiang, L., Qin, J., Lin, B., *Electrophoresis* 2009, *30*, 1497–1500.

[72] Carrilho, E., Martinez, A. W., Whitesides, G. M., *Anal. Chem.* 2009, *81*, 7091–7095.

[73] Lu, Y., Shi, W., Qin, J., Lin, B., *Anal. Chem.* 2010, *82*, 329–335.

[74] Zhao, W., Ali, M. M., Aguirre, S. D., Brook, M. A., Li, Y., *Anal. Chem.* 2008, *80*, 8431–8437.

[75] Li, X., Tian, J., Shen, W., *Anal. Bioanal. Chem.* 2010, *396*, 495–501.

[76] Ellerbee, A. K., Phillips, S. T., Siegel, A. C., Mirica, K. A., Martinez, A. W., Striehl, P., Jain, N., Prentis, M., Whitesides, G. M., *Anal. Chem.* 2009, *81*, 8447–8452.

[77] Vykoukal, D. M., Stone, G. P., Gascoyne, P. R. C., Alt, E. U., Vykoukal, J., *Angew. Chem. Int. Ed.* 2009, *48*, 7649–7654.

[78] Carrilho, E., Phillips, S. T., Vella, S. J., Martinez, A. W., Whitesides, G. M., *Anal. Chem.* 2009, *81*, 5990–5998.

[79] Cheng, H. D., Jiang, X. H., Sun, Y., Wang, J., *Pattern Recogn.* 2001, *34*, 2259–2281.

[80] Cantrell, K., Erenas, M. M., Orbe-Payá, I., OCapitán-Vallvey, L. F., *Anal. Chem.* 2010, *82*, 531–542.

[81] Dungchai, W., Chailapakul, O., Henry, C. S., *Anal. Chem.* 2009, *81*, 5821–5826.

[82] Carvalhal, R. F., Kfouri, M. S., Piazzeta, M. H. O., Gobbi, A. L., Kubota, L. T., *Anal. Chem.* 2010, *82*, 1162–1165.

[83] Nie, Z., Nijhuis, C. A., Gong, J., Chen, X., Kumachev, A., Martinez, A. W., Narovlyansky, M., Whitesides, G. M., *Lab Chip* 2010, *10*, 477–483.

[84] Wang, H., Liu, J., Cooks, R. G., Ouyang, Z., *Angew. Chem. Int. Ed.* 2010, *49*, 877–880.

[85] Gao, L., Sugiarto, A., Harper, J. D., Cooks, R. G., Ouyang, Z., *Anal. Chem.* 2008, *80*, 7198–7205.

[86] Carrilho, E., Laromaine, A., Dillon, S. T., Shaw, B. T., Benassi, M., Cooks, R. G., Lieberman, T., Whitesides, G. M. *3rd Symposium of the Brazilian Society for Mass Spectrometry*, Campinas, Brazil 2009, pp. 12–15.

[87] Zhao, W., van den Berg, A., *Lab Chip* 2008, *8*, 1988–1991.

[88] Pelton, R., *Trends Anal. Chem.* 2009, *28*, 925–942.

