FABRICATION OF CAPILLARY-DRIVEN TONER-BASED MICROFLUIDIC DEVICES FOR CLINICAL DIAGNOSTICS WITH COLORIMETRIC DETECTION

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ABSTRACT

The fabrication of toner-based microfluidic devices to perform clinical diagnostics with capillary force and colorimetric detection is described in this work. Microfluidic devices were fabricated by a direct-printing technology. The printed layout and its mirror image were aligned with an intermediary cut-through polyester film and then thermally laminated together at 150 ºC at 60 cm/min to obtain a channel with *ca.* 100-µm depth. Colorimetric assays for glucose and total protein were successfully performed using a desktop scanner. The limit of detection (LD) for glucose and protein was *ca.* 1 mM and 3 mM, respectively. For protein assays, the standard relative deviation (RSD) for zone-to-zone comparison was below 5%.

KEYWORDS: Microfluidics devices, Colorimetric detection, bioassays

INTRODUCTION

The development of microfluidic devices for clinical diagnostics has exhibited an impressive growth in the last years. Recent reports have focused on the fabrication of low cost devices to be used in point-of-care (POC) diagnostics and developing countries where the application of financial funding is quite limited [1]. In this scenario, toner and paper substrates have appeared as promising and disposable platforms for microfluidic applications [2]. The availability of non- or minimally instrumented microfluidic devices is well-suited to be used in these locations in association with low cost devices [3]. Capillary-driven microfluidic devices can meet these requirements once they are efficient, fast and, most importantly, they do not need external power equipments [4]. The spontaneous motion of a liquid inside microchannels towards functionalized surfaces or reaction chambers is one of the key advantages to obtain a non-instrumented device for a rapid clinical diagnostic. For this reason, capillary-driven microfluidics have received special attention mainly because they can be combined with emerging platforms, including paper and toner-based devices, for bioanalytical applications or POC diagnostics.

EXPERIMENTAL

Toner-based microfluidic devices were fabricated (see Figure 1) by a direct-printing process [5]. First, the desirable layout and its mirror image were drawn in Corel Draw software and printed on a polyester film using a laser printer with 1200-dpi resolution (Hewlett Packard model 1102w). A cut-through polyester film was inserted between both printed images in order to enhance the channel depth and assure the fluidic transport by capillary force. The three polyester pieces were aligned and after thermally laminated at 150 ºC under a rate of 60 cm/min. The layout of the proposed device (35 mm × 35 mm) consisted of four test zones interconnected by microfluidic channels and one central inlet zone to sample distribution (Figure 1C). All channels were 10-mm long, 1-mm wide and *ca.* 100-µm deep.

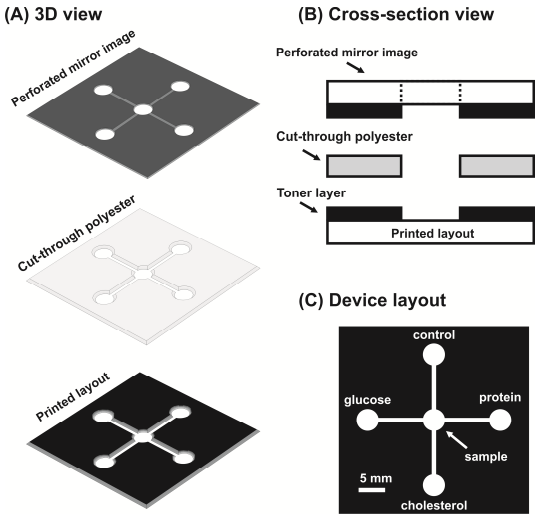

Figure 1. Scheme of the toner-based devices fabrication process in (A) 3D and (B) cross section view and (C) example of a toner-based device for clinical assays.

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In order to avoid sample leakage, a cellulose paste (1:4, cellulose/water) was added to all four test zones. The addition of cellulose paste ensures that the color reagent does not enter inside channel. In this case, the development of color occurs just in the test zones after adding sample in the zone inlet. Colorimetric detection was performed using a scanner mode of multifunction printer (Hewllet-Packard, F4280). The images were captured and converted to gray scale or CMYK. The glucose assay was based on the oxidation of glucose to gluconic acid and hydrogen peroxide and the subsequent reduction of hydrogen peroxide and oxidation of iodide to iodine [6]. For the total protein assay, test zones were spotted with 6 µL of a 10 mM brilliant blue G solution followed by 20 min of drying.

RESULTS AND DISCUSSION

Preliminary tests were performed to evaluate the flow rate in the channel. The influence of the channel width on the flow rate magnitude was also investigated. For this study, channels with width ranging from 0.5 to 1.0 mm were produced. Figure 2A shows that lower flow rates were found to wider channels. As it can be seen in Figure 2B, the higher the sample volume, the greater flow rate magnitude.

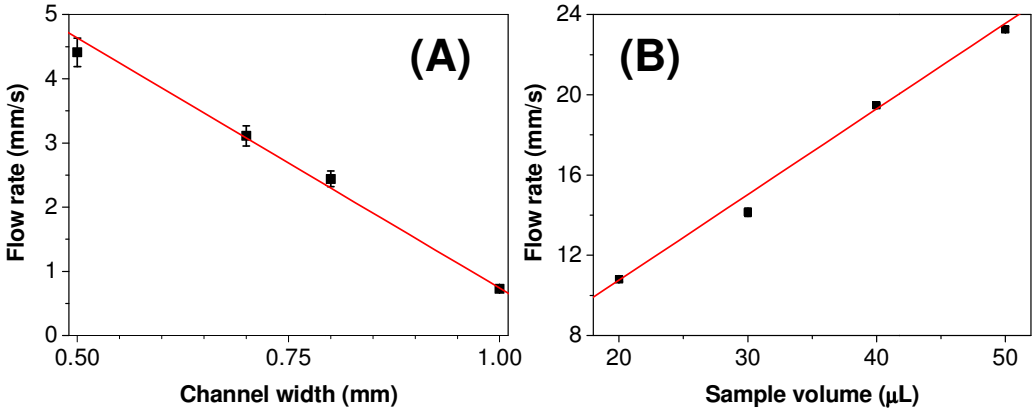

Figure 2. Effect of (A) channel width (B) sample volume on the flow rate induced by capillary force on toner-based microfluidic devices. In (A), channel width was 0.5 mm; in (B), the sample volume added to central zone was 10 µL.

Bioassays for analyzing glucose and total proteins were successfully performed on these microfluidic platforms (Figure 3). Toner-based device has exhibited a good correlation for concentrations of protein (0-40 mM) and glucose (0-20 mM), as depicted in Figure 3E (for glucose only). The limit of detection (LD) for glucose and protein was *ca.* 1 mM and 3 mM, respectively. For protein assays, the standard relative deviation (RSD) for zone-to-zone comparison was below 5%. Even with a low cost per device (around $0.05), the proposed device can be reusable. Consecutive protein assays (n=3) revealed that mean color intensity decreases around 35% (data not shown). Current experiments are being performed to quantify the amount of glucose and proteins in artificial serum sample.

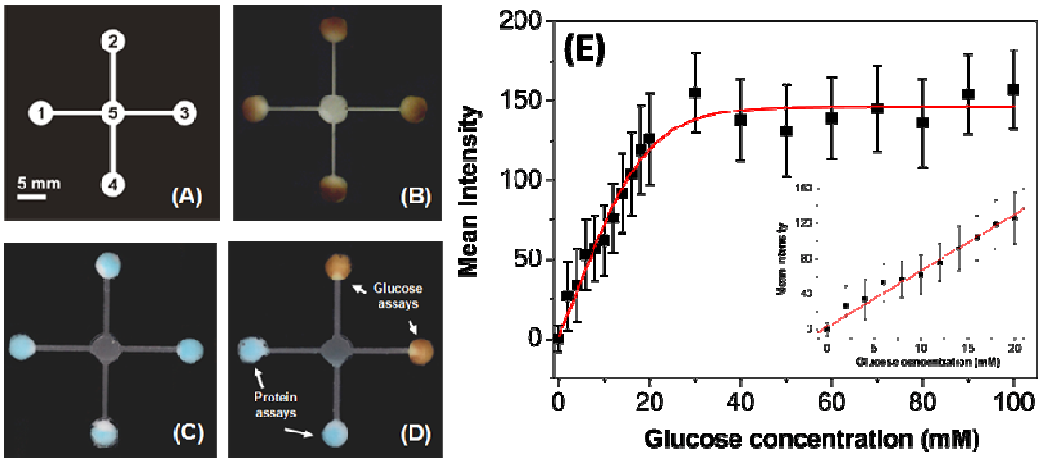

Figure 3. Representation of the (A) device layout and examples of bioassays for (B) glucose, (C) total protein, (D) glucose plus protein together on the same device and (E) calibration curve for glucose assays.

The lifetime of the devices has been evaluated over five consecutive days, storage at 10 ºC. In this preliminary test it has been observed that the color intensity decreases around 70% for five days. The shelf life for these assays has been estimated to be 3 days. To overcome this problem, all assays are being currently tested in the presence of threalose dealing to extend this short lifetime. In addition, the stability has been investigated in different storage temperatures to demonstrate the feasibility of using the proposed device in developing countries.

CONCLUSIONS

It has been demonstrated that toner-based microfluidic devices can be used to perform clinically relevant assays with capillary force and colorimetric measurements. Tests for glucose and total proteins were successfully performed. One of the main advantages is that the toner-based technology does not need either photolithographic steps or thermal treatment. Furthermore, a single A4-size polyester sheet has been used to print thirty five devices with dimensions of 35 mm x 35 mm each. The cost of a single device has been estimated to be *ca.* 0.10 cents of dollar (including the costs of polyester film, toner layer the also the cut-through polyester which was provided by a specialized local service). This low cost stimulates the use these proposed microfluidic devices in regions where the financial resources are very limited. Recents advances in our research group have successfully showed the use of other equipments to perform colorimetric detection such as mobile phone cameras, microscopes and conventional cameras. Besides total proteins and glucose assays, the proposed device has been explored to perform independently cholesterol, bovine serum albumine and triglycerides assays. Current experiments are focused on the fabrication of a more complex device to perform simultaneously all assays mentioned above. Furthermore, some additional advantages have been found including the excellent chip-to-chip reproducibility (RSD < 5%, n = 4) as well as the possibility of reuse a single chip. We believe this technology can spread out the insertion of toner-based technologies into different fields including clinical, genetic and biological applications. We believed that the proposed devices are an alternative tool to be applied in diagnostics of infectious diseases.

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