Sensors & Actuators: B. Chemical 273 (2018) 536–542

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

A hybrid paper-based microfluidic platform toward veterinary P-ELISA

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ARTICLE INFO

Keywords:
P-ELISA
Paper microfluidics
serum
Laser micromachining
Lamination
Point-of-care

ABSTRACT

This study describes a novel fabrication method to produce a hybrid paper-based microfluidic platform, termed Multi-Pad Paper Plate (MP^3) for transfer and optimisation of enzyme-linked immunoassays (ELISA) on paper (P-ELISA). The new fabrication technique is based on a combination of laser micromachining of paper and packaging through thermal lamination. This method is simple, rapid and of high-resolution and can produce robust, versatile and low-cost devices, compatible with a standard 96-well microplate format. The MP^3 was used to transfer a sandwich ELISA for detection of bovine haptoglobin (Hp), a marker of inflammation in animals allowing optimisation of the assay in the new format. Using the MP^3, a standard curve was generated and the limit of detection achieved was 0.73 µg/ml. The optimised protocol was also applied to the detection of Hp in bovine serum, demonstrating the possibility of using this platform with biological samples. The new platform allowed for a reduction of 93% in time and of 88% in cost for performing the assay and represents a valid alternative to other commonly used device fabrication methods, especially in the context of low-resource settings. It has the potential to translate similar assays into P-ELISA and offers a starting point for achieving animal-side testing.

1. Introduction

One of the most promising technologies that have recently been applied within point-of-care (POC) diagnostics is microfluidics [1]. At present, two main types of microfluidic systems are used in the diagnostic field: micro total analysis systems (µTAS) and microfluidic paper-based analytical devices (µPADs). The latter, in particular, represent an appealing application for low resource settings, due to their low-cost and ease of fabrication. Paper is cheap, easy to source, biodegradable and simple to chemically modify [2]. Furthermore, POC devices made from paper have the considerable advantage of not requiring external power sources, whilst fabrication techniques and machinery for production are usually cheaper than those required for other materials [3]. Paper represents an excellent medium for diagnostic testing, because of its high surface to volume ratio, which allows reagents to be concentrated and also enables more rapid reaction times [4].

Within the field of µPADs, an interesting application has recently been demonstrated in the translation of ELISA onto paper (P-ELISA) [4–8]. P-ELISA combines the sensitivity and specificity of ELISA with the intrinsic low cost and ease-of-use of paper-based platforms [9]. There are increasing numbers of studies reporting the use of paper ELISA as a valid alternative to the conventional format [6,7,10]. The most commonly used methods for fabrication of these platforms are photolithography [10] and wax printing techniques [6,8,11]. The main drawbacks are the numerous steps involved for production of the final device [4], the cost of the use of photoresist [12], poor channel resolution [12] and the lack of resistance to sustain high temperature. To overcome these limitations a new concept of low-cost device has also been introduced, called paper hybrid microfluidic platforms [13]. A combination of paper and other materials, like PMMA [14], PDMS [15], cotton [16] or glass [17] are combined to fully exploit the properties of each material [13], reducing the limitations and drawbacks of the previous techniques. However, the current fabrication methods are still limited by the speed of prototype devices and high cost for scalable manufacturing.

Within veterinary diagnostics, the use of haptoglobin (Hp) as a marker of disease status has recently attracted considerable attention [18–22]. Hp is classified as a serum biomarker, one of a number of acute phase proteins which increases rapidly following infection or inflammation, providing a quantification of tissue damage in a diseased animal [23]. Hp is normally present in the serum of healthy cattle in concentrations < 0.02 mg/ml [24], but a level rise above 25% of the normal circulating level is usually indicative of a diseased status [25]. Measurement of Hp in bovine serum is currently available with

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https://doi.org/10.1016/j.snb.2018.06.075
Received 26 March 2018; Received in revised form 31 May 2018; Accepted 15 June 2018
Available online 18 June 2018

laboratory-based assays, either through a colorimetric reaction based on peroxidase activity of the haptoglobin-haemoglobin complex or with a commercially available ELISA kit [26]. Furthermore, Hp standards are commercially available making this assay the ideal candidate for transfer to the P-ELISA system. It is also a very relevant assay for investigation into future POC or "animal-side" diagnostics as the possibility of detecting the level of Hp at the POC, will allow for disease to be diagnosed in a timely and cost-effective manner, improving overall animal health and welfare [27].

This study describes a novel fabrication method for realisation of a hybrid paper-based microfluidic platform, termed multi-pad paper plate (MP^3), which can produce a device compatible with standard 96-well microplate format allowing rapid and low-cost translation and optimisation of laboratory-based ELISA assay into P-ELISA. The model used for the translation and optimisation of a laboratory-based assay using this platform is reported and is based on the development of a standard curve for detection of haptoglobin. The optimised protocol was further tested by analysing bovine serum samples with known Hp concentrations and by comparing results with the existing laboratory-based ELISA.

2. Experimental

2.1. MP^3 design and fabrication

The MP^3 was fabricated using a laser micromachining of paper and thermal lamination technique. A 3D mechanical computer-aided design (CAD) (SolidWorks 2012, USA) was used to design both the paper device and the plastic lamination used for device packaging (Fig. 1). Whatman Grade 1 (WHT 1) cellulose chromatography paper (Sigma-Aldrich, UK) and 75μ gloss laminating pouches (Staples, UK) were cut using a class 2 CO_2 laser (Speedy 300 Laser Engraver - Trotec). The optimised settings for cutting both materials were: power 4%, speed 0.4, PPI/Hz 1000, passes 1, air assist on. These settings were chosen as the best combination for fast and precise production, while avoiding material burning. A range of settings were evaluated to achieve the high resolution required, while maintaining fast production (minimum time possible for fabrication) and ease of removal of devices (both paper and lamination sheets not requiring manual trimming or tearing). Increasing power settings to above 4% (i.e. 0.16 W) was associated with burning (darkening) of the paper edges, while below the 4% threshold there was incomplete cutting of the material. When the speed was increased to 0.6, paper had to be manually detached from the main sheet, with high risk of tearing the microchannels (0.5 mm) and making the device not usable.

Paper and lamination sheets were cut in the desired format and then laminated to produce the final device. The MP^3consists of 96 circular paper pads of 6 mm diameter connected by a channel of 0.5 mm width and 3 mm in length (Fig. 1A) and a complementary lamination sheet of 96 circular holes of 4 mm diameter (Fig. 1B), assembled in the pre-cut lamination sheet (Fig. 1C) and laminated at 110 °C at the lowest speed setting (Fig. 1D) using a roll laminator (GBC Catena 35 Roll Laminator). After lamination, the laminated MP^3 was placed back into the laser and the connecting microchannels between pads were severed by the laser cutter (Fig. 1E), making each pad completely independent from the others. During this final step of fabrication, plate information including numbers, letters and other information were cut or engraved on the final device. Optimisation was also carried out for this second cutting process. To keep the same power and speed settings already optimised, a second cut (passes 2) was necessary to completely severe the channels without requiring additional manual handling.

The final device was tested with food colour (Morrison Natural Food Colouring Blue) for determination of the optimum volume of liquid to allow complete wetting of each pad (Fig. 1F). Alterations to the CAD

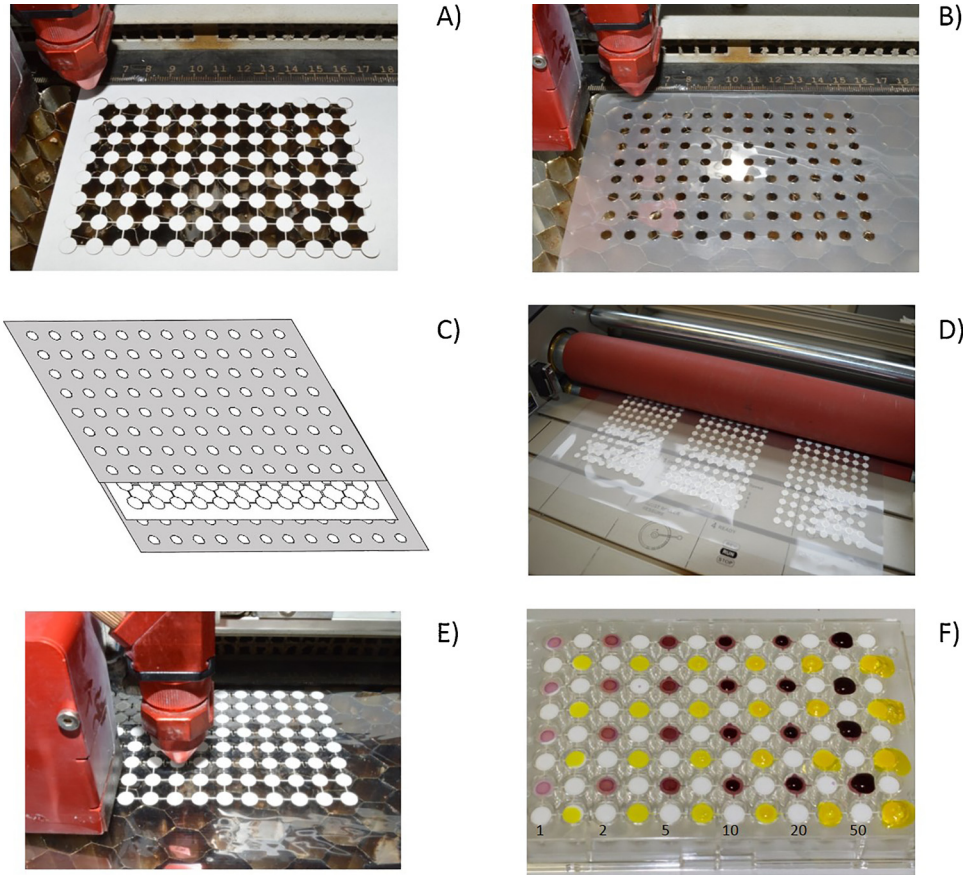

Fig. 1. Schematic of the fabrication of the MP^3. A) Paper cut with a laser cutter. B) Laminating pouches cut with a laser cutter. C) Assembly of device in the pre-cut lamination sheets. D) Lamination of the device. E) Severing of interconnecting paper channels by laser cutter. F) Application of two different food colours (red and yellow) to the device with no evidence of cross-contamination between pads. Numbers represent volume of fluids applied (μl).