Biosensors and Bioelectronics 99 (2018) 77–84

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Sliding-strip microfluidic device enables ELISA on paper

Mohit S. Verma[a], Maria-Nefeli Tsaloglou[a,d], Tyler Sisley[a], Dionysios Christodouleas[a], Austin Chen[a], Jonathan Milette[a], George M. Whitesides[a,b,c,*]

[a] Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA
[b] Wyss Institute for Biologically Inspired Engineering, Harvard University, 60 Oxford Street, Cambridge, MA 02138, USA
[c] Kavli Institute for Bionano Science and Technology, Harvard University, 29 Oxford Street, Cambridge, MA 02138, USA
[d] Diagnostics for All, 4 Technology Way, Salem, MA 02138, USA

ARTICLE INFO

Keywords:
Resource-limited
C-reactive protein
Blood
Sepsis
Inflammatory bowel diseases
Receiver operator characteristics

ABSTRACT

This article describes a 3D microfluidic paper-based analytical device that can be used to conduct an enzyme-linked immunosorbent assay (ELISA). The device comprises two parts: a sliding strip (which contains the active sensing area) and a structure surrounding the sliding strip (which holds stored reagents—buffers, antibodies, and enzymatic substrate—and distributes fluid). Running an ELISA involves adding sample (e.g. blood) and water, moving the sliding strip at scheduled times, and analyzing the resulting color in the sensing area visually or using a flatbed scanner. We demonstrate that this device can be used to detect C-reactive protein (CRP)—a biomarker for neonatal sepsis, pelvic inflammatory disease, and inflammatory bowel diseases—at a concentration range of 1–100 ng/mL in 1000-fold diluted blood (1–100 μg/mL in undiluted blood). The accuracy of the device (as characterized by the area under the receiver operator characteristics curve) is 89% and 83% for cut-offs of 10 ng/mL (for neonatal sepsis and pelvic inflammatory disease) and 30 ng/mL (for inflammatory bowel diseases) CRP in 1000-fold diluted blood respectively. In resource-limited settings, the device can be used as a part of a kit (containing the device, a fixed-volume capillary, a pre-filled tube, a syringe, and a dropper); this kit would cost ~ $0.50 when produced in large scale (> 100,000 devices/week). This kit has the technical characteristics to be employed as a pre-screening tool, when combined with other data such as patient history and clinical signs.

1. Introduction

Microfluidic paper-based analytical devices (μPADs) (Li et al., 2008; Martinez et al., 2007, 2008b) are valuable tools for satisfying the World Health Organization's ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users) (Kettler et al., 2004) criteria for diagnostic devices in developing countries (Cate et al., 2015; Mao and Huang, 2012; Yetisen et al., 2013). They are, however, typically limited to single-step biochemical assays (i.e. a single step of mixing samples and reagents), and thus are unable to perform complex assays—such as enzyme-linked immunosorbent assays (ELISAs)—that require mixing of multiple reagents and removal of excess reagents in an ordered sequence. Here, we circumvent this limitation of μPADs by incorporating a sliding strip (Connelly et al., 2015; Liu et al., 2011) into the device that allows switching among fluidic paths, and makes it possible to contact the sample and the reagents required for a bioassay—capture antibodies, enzyme-linked detection antibodies, and substrate for enzyme—in a timed sequence, and only in the correct order. This project is intended primarily as a demonstration of principle of a sliding-strip device for immunoassays and its accuracy is still short of that required for clinical analysis. It is, however, sufficient for a screening or pre-screening assay.

ELISA is one of the most common methods for detecting and quantifying biomarkers (both proteins and small molecules) (Engvall et al., 1971; Engvall and Perlmann, 1971; Lequin, 2005). As used in a conventional 96-well format, ELISA is not suitable for resource-limited environments because it requires: i) trained personnel, ii) expensive analytical instruments (e.g. a microplate reader), and iii) multiple steps of mixing reagents and washing (these steps are difficult to adapt to a simple and portable device to be used by healthcare workers with limited experience). Multi-well plate formats also assume the need for multiple assays, and are designed for high-volume laboratories; they are thus often inappropriate for single-patient assays at the point of care, or in low-volume clinics. μPADs have the potential to overcome many of these drawbacks because they are: i) lightweight (a few grams),

* Corresponding author at: Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA.
E-mail address: gwhitesides@gmwgroup.harvard.edu (G.M. Whitesides).

http://dx.doi.org/10.1016/j.bios.2017.07.034
Received 18 April 2017; Received in revised form 12 July 2017; Accepted 12 July 2017
Available online 19 July 2017
0956-5663/ © 2017 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/BY/4.0/).

ii) easily fabricated from paper, double-sided adhesive tape, and hydrophobic film, and iii) easily disposed of by incineration (Cate et al., 2015; Mao and Huang, 2012; Martinez et al., 2010b; Yetisen et al., 2013). They operate without the need for external power or equipment (e.g., pumps). In particular, μPADs with complex, 3D microfluidic channels (3D μPADs (Martinez et al., 2010a; Martinez et al., 2008b)) are capable of distributing small quantities (~ 20–100 μL in current designs) of samples from a single inlet into a large array of test zones (in principle, up to thousands (Martinez et al., 2008b)), and permit the multiplexing of array-based assays. They are also suitable for use with single patients at the point of care. We (Cheng et al., 2010; Connelly et al., 2015; Martinez et al., 2007, 2010a, 2008b; Pollock et al., 2012; Vella et al., 2012) and others (Abe et al., 2010; Ali et al., 2009; Dungchai et al., 2009; Fu et al., 2010; Khan et al., 2010; Li et al., 2008, 2010b; Liu et al., 2013; Noh and Phillips, 2010; Struss et al., 2010; Toley et al., 2013; Yu et al., 2011) have developed μPADs for diagnostic applications, but these devices were generally not equipped to perform ELISA—except for some demonstrations where reagents were simply added to paper instead of to a 96-well plate (Cheng et al., 2010; Hsu et al., 2014). Even in these examples of μPADs for ELISA, the complexity of the assay is maintained because the user is required to dispense multiple reagents (antibodies and substrate for the enzyme).

Here, we describe a portable device for ELISA that contains stored reagents—capture antibody, detection antibody, substrate, and buffers—in isolated zones and requires only the addition of sample and water to complete an assay. The reagents are brought into contact with each other by moving the sliding strip manually to different zones. Ismagilov *et al.* have developed a conceptually similar method for manipulating fluids in glass/plastic microfluidic devices (called "SlipChip" (Begolo et al., 2013, 2014; Du et al., 2009; Li et al., 2010a; Liu et al., 2010; Ma et al., 2014; Shen et al., 2010a, 2010b, 2011)), where two plates with patterned micro-wells and channels slide relative to one another to form different fluidic pathways and bring reagents in and out of contact (Begolo et al., 2013, 2014; Du et al., 2009; Li et al., 2010a; Liu et al., 2010; Ma et al., 2014; Shen et al., 2010a, 2010b, 2011). We have also developed a similar method for manipulating fluids using a "paper machine" for molecular diagnostics (loop-mediated isothermal amplification reaction for *Escherichia coli malB* gene), where a magnetic strip moves between layers of magnets, and paper is used as the active reaction matrix (Connelly et al., 2015). We compare our "sliding strip" methodology to Ismagilov's SlipChip and Connelly's paper machine in the Results and discussion section under the title "comparison to similar devices."

2. Materials and methods

2.1. Materials and equipment

Please see Supplementary information for details on suppliers for materials and equipment.

2.2. Fabrication

Please see Supplementary information for details on the fabrication of the device (Fig. 1).

2.3. Detection

The sample was prepared by mixing 1 μL sheep blood, 1 μL of solution of C-reactive protein (CRP) (at concentrations of 1, 20, 40, 60, 80, and 100 μg/mL), and 998 μL 1% w/v bovine serum albumin (BSA) in phosphate buffered saline (PBS, pH 7.4) to simulate a 1000-fold diluted blood sample. The 998 μL of 1% w/v BSA was measured using a micropipette and pre-aliquoted in tubes, while the 1 μL blood and CRP were measured using fixed-volume capillaries. The capillaries were placed in the tube containing BSA and the tubes were shaken to mix the liquids.

While running the assay, 100 μL of sample (as prepared above) was added to the first hole using a micropipette and allowed to wick into the device and incubated for 30 min at room temperature. Water (100 μL) was added to the first hole to wash off excess sample. The sliding strip was pulled to the second position and 150 μL of water was added to the second hole to elute the detection antibody and to wash off excess antibodies. The sliding strip was pulled to the third position and 150 μL of water was added to the third hole to dissolve the stored 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) substrate and to wash off excess substrate. The sliding strip was pulled out of the dock and allowed to dry for 30 min under ambient conditions before imaging using a desktop scanner (Epson J251A) to obtain 48-bit Red, Green, and Blue (RGB) images (48-bit images provide a larger dynamic range for a concentration-dependent response than 24-bit images).

When emulating resource-limited settings, the sample (100 μL) was added using a disposable syringe (instead of a micropipette) and water (two drops) was added using a plastic dropper (instead of a micropipette).

2.4. Analysis

Once the images were collected from the color scanner, they were inverted and the RGB color values of a circle with a diameter of 25 pixels in the center of the test zone in the sensing area were measured (similar to an approach we have described previously (Christodouleas et al., 2015)). The color intensity from each of the R, G, and B channels was averaged to obtain the average intensity. The average color intensity of the control zone was used as background and subtracted from the value of the test zone. All the measurements were performed using National Institutes of Health (NIH) ImageJ. The data presented in Fig. 2 are from two different experiments (each experiment included n = 3–4 devices for each concentration tested) and thus, pooled standard deviations (for each concentration) were used as an estimate of the standard deviation of the mean from the two experiments.

The receiver operator characteristics (ROC) curve was obtained by varying the cut-off for average color intensity from 5000 to 45,000 AU in increments of 1000 AU and then counting the true positive and true negative results to obtain the specificity and sensitivity, for each cut-off of 10 ng/mL and 30 ng/mL. The figures were plotted using OriginLabs Origin®.

3. Results and discussion

3.1. Design and operation of the sliding-strip 3D μPAD

The sliding-strip 3D μPAD comprises two principal parts: a sliding strip (fabricated in nitrocellulose, chromatography paper, and PET film) and a functional "dock"—a structure that surrounds a channel for the sliding strip, and that also distributes fluids (Fig. 1). The sliding strip contains the sensing area (which is a piece of wax-patterned nitrocellulose containing two zones: one is a control zone without capture antibody, and the other is a test zone with immobilized capture antibody) attached to a piece of wax-patterned chromatography paper (which is attached to a poly(ethylene terephthalate) (PET) film (transparency), to make handling more convenient, and to smooth the sliding of the strip). The sliding strip is labeled with the numbers 1, 2, and 3, and three alignment marks to guide the user during the three steps of operation of the device, and to ensure that the sensing area is aligned with the reagent areas of the functional dock (Fig. 1A). The sensing area has yellow wax circles (Fig. S4) around the active zone to provide better contrast than the black surroundings (black was used as the color for printing because preliminary experiments demonstrated that black wax, when printed, provides a better hydrophobic barrier

Download English Version:

https://daneshyari.com/en/article/5031315

Download Persian Version:

https://daneshyari.com/article/5031315

Daneshyari.com