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Finger-Powered Microfluidic Electrochemical Assay for Point-of-Care Testing

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***Abstract*— This paper presents a finger-powered microfluidic electrochemical assay for rapid measurements of protein biomarkers. This device employs a valveless, piston-based pumping mechanism which utilizes a human finger for the actuation force. Liquids are driven inside microchannels by pressing on pistons which generate pressure-driven flows. Reagents are preloaded in microwells allowing for the entire testing process to be completed on-chip. For proof-of-concept, this device was used to detect *Plasmodium falciparum* histidine-rich protein-2 (*Pf*HRP2) in human plasma samples using a mobile phone biosensing platform. Using this device, *Pf*HRP2 was detected from 0.1 to 20 µg/mL with high specificity and each measurement could be completed in ≤ 6 min. Based on its simplicity, portability and good analytical performance, this platform is promising for point-of-care testing, particularly in remote and resource-limited regions.**

INTRODUCTION

Many diagnostic assays rely on the detection of protein biomarkers in bodily fluids, such as blood or plasma. Lateral flow assays (LFAs) are rapid, easy to use and cheap, but can only offer qualitative measurements. The gold standard method for protein quantification is enzyme-linked immunosorbent assay (ELISA) which offers high sensitivity and relatively low cost-per-sample. However, ELISA is laborious, time consuming and requires specialized equipment, making it impractical for use in remote or resource-limited settings. Towards this end, researchers have been developing portable biosensing platforms for diagnostic testing which can provide similar detection performance as laboratory-based techniques [1, 2].

Mobile phones have been increasingly utilized for diagnostic testing due to their portability, broad accessibility and multi-functionality [3, 4]. Additionally, mobile phones offer a simple user interface, data processing and wireless transmission capabilities, which are useful features for point-of-care testing. Mobile phone biosensing platforms have been demonstrated employing various detection techniques including microscopy [5], colorimetry [6-8], fluorimetry [9, 10], electrical impedance spectroscopy [11] and electrochemistry [12, 13]. Of these, electrochemical sensing offers several advantages for analytical measurements including high sensitivity, good reproducibility and rapid results [14].

In order to automate sample handling, many mobile phone-based biosensors utilize microfluidic chips which employ various means for pumping including capillary flow [13], electrolytic pumping [15] and pipetting [16]. Recently, finger-powered microdevices have been demonstrated for electricity-free operation based on various mechanisms such as push-and-release action [17] and vacuum activation [18]. While functional, these systems typically rely on elastomer valves which suffer from poor reliability or require complicated microfabrication. To address these issues, we have developed a valveless, piston-based micropump which is robust and can easily be incorporated into plastic microfluidic devices. This unique pumping mechanism is integrated with an electrochemical sensor for quantitative measurements of protein biomarkers. All of the reagents required for the assay are stored on-chip, thereby simplifying the testing process. This chip is coupled with a custom detection circuit and a smartphone for rapid, high sensitivity measurements of protein biomarkers in human samples.

MATERIALS AND METHODS

Microfluidic chip

1.5 mm-thick poly(methyl methacrylate) (PMMA) and rubber gaskets were obtained from McMaster-Carr (Elmhurst, IL). Prior to fabrication, PMMA was rinsed with 2-propanol, deionized (DI) water and dried with purified N_2 gas. Capillary tubes were purchased from Sigma (St. Louis, MO) and double-sided adhesive film was purchased from Adhesive Research (Glen Rock, PA).

Proteins, chemicals and reagents

Anti-*Pf*HRP2 mouse IgM (primary) and horseradish peroxidase (Px)-conjugated anti-*Pf*HRP2 mouse IgG (secondary) were obtained from Immunology Consultants Laboratory (Portland, OR). Recombinant *Pf*HRP2, *P. falciparum* lactate dehydrogenase (*Pf*LDH) and dengue virus type 2 (DEN-2) were purchased from CTK Biotech (San Diego, CA). Bovine serum albumin (BSA), phosphate buffer solution (PBS), PBS-casein, hydrogen peroxide (H_2O_2), 4-aminopyridine (4AP), dimethyl sulfoxide (DMSO), Traut's Reagent (2-iminothiolane) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma. DI water was generated using a Thermo Scientific Smart2Pure water purification system (Tustin, CA). Human plasma was purchased from Bioreclamation (Westbury, NY).

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Electrochemical instrumentation

Microcontroller LaunchPad and data converter chip were purchased from Texas Instruments (Dallas, TX). Amplifiers and channel multiplexers were purchased from Analog Devices (Norwood, MA). Discrete components and miscellaneous parts were purchased from Digi-Key (Thirf River Falls, MN). For our prototype device, we used a Nexus 5 smartphone running on Android 5.0.2 OS.

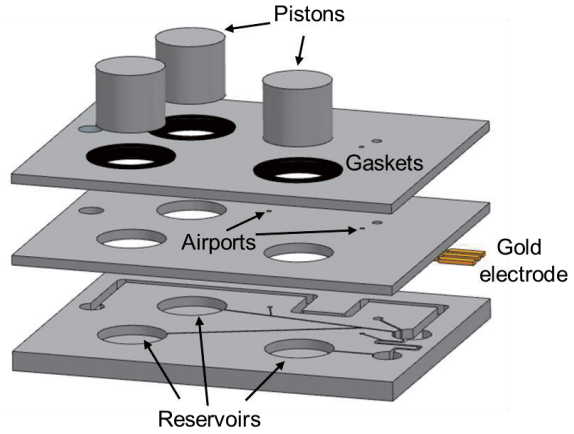

Figure 1: Exploded view of the microfluidic chip with relevant regions labelled.

Fabrication of the microfluidic chip

The microfluidic chip consists of three layers of PMMA, as shown in Fig. 1. The microfluidic network was designed using AutoCAD software and etched into the PMMA using a CO_2 laser cutter (Universal Laser Systems, Scottsdale, AZ). The bottom layer consists of three reservoirs (7 mm in diameter and 2 mm deep) for storing liquid reagents. The electrochemical sensor, consisting of a working electrode (WE), reference electrode (RE) and counter electrode (CE), was deposited on the back side of middle layer through a shadow mask. Chromium and gold were sequentially evaporated with thicknesses of 150 nm and 600 nm, repectivelly using a thermal evaporator (Jefferson Hills, PA). The middle and top layers incorporate through-holes for the pistons, capillary tube inlet and air ports. Rubber gaskets are installed between the middle and top layer, and all three layers are bonded together using double-sided adhesive. The pistons were laser cut from 3-mm-thick PMMA and inserted in each hole after the reagents were dispensed in the reserviors.

Characterization of piston actuation force

Measurements of the piston actuation force were performed using an M&A Instruments digital push-pull force gauge (Arcadia, CA). The force gage was mounted on a vertical stage and positioned so that the compression rod was aligned with the piston of the chip (Fig. 2). The gauge was connected to a PC and measurements were recorded using the manufactures' software. Measurements were performed using straight microchannels having various widths filled with colored dye.

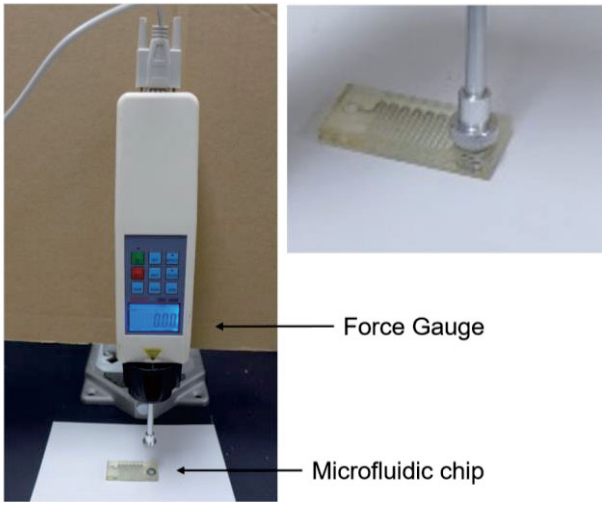

Figure 2: Photograph of experimental setup for piston actuation force measurements. The compression rod is aligned with the piston and manually depressed, and the resulting data is transmitted to a PC. Inset shows a close-up view of the compression rod pressing the piston during a measurement.

Sensor preparation

Thiolated antibody solution was prepared by combining 990 µL of PBS (EDTA) containing of 100 µg/mL anti-*Pf*HRP2 IgM with 10 µL of DMSO (1 mg/mL) in Traut's Reagent, incubating at 25ºC, and centrifugation at 12,000 rpm for 30 min to remove residual DMSO. Precipitated antibody was reconstitute using PBS (pH 7.4) and incubated on the sensor for 1 hr followed by rinsing with PBS and drying with N_2 gas. The antibody-immobilized sensor was incubated with 20 µL of PBS-casein for 30 min at 25ºC to block the surface from nonspecific binding. The sensor was then rinsed with PBS, dried using N_2 gas, and stored at 4ºC for up to 2 weeks prior to measurements.

Measurement procedures

For assay specificity measurements, antigens (*Pf*LDH, DEN-2 and *Pf*HRP2) were diluted to 10 µg/mL in PBS. For assay sensitivity measurements, *Pf*HRP2 was spiked in human plasma at varying concentrations from 0.1 µ/mL to 20 µg/mL. 80 µL of PBS containing 10 µg/mL of anti-*Pf*HRP2 IgG, PBS and 4AP/H_2O_2 substrate were dispensed into reservoirs R1, R2 an R3, respectively (Fig. 5a) using a pipette. To initiate the assay, 20 µL of sample is dispensed into the chip using a capillary tube plunger. Next, a 400 mV electrical field is applied to the sensor for 3 min to facilitate protein recognition and binding. Px-conjugated secondary antibody is delivered to the sensor by depressing piston R1. A second electrical field (200 mV) is applied for 1.5 min, followed by a rinsing step to remove unbound antibodies by pressing piston R2. Piston R3 is pressed to deliver the substrate solution, followed by amperometry at a bias potential of -150 mV for 1 min. The entire detection process is completed in ≤ 6 min. Each measurement was performed at room temperature using a new chip.

Results and Discussion

System layout

A photograph of our mobile phone biosensing platform is shown in Fig. 3. Amperometric measurements and signal processing are carried out by a custom detection circuit, named aMEASURE [19] (Fig. 3b). This circuit is comprised of a custom analog readout board and a microcontroller, which provides a physical/electrical interface between microfluidic chip and the mobile phone. The circuit can support a current signal from 0.94 nA to 2.4 mA, and automatically adapts to response current levels. The circuit consumes very low power (90 mA) and can be powered for up to 7 hr using the mobile phone battery. The microfluidic chip (Fig. 3a) plugs into aMEASURE via an edgeboard connector. Step-by-step instructions for each measurement and test results are displayed on the phone though a custom Android app.

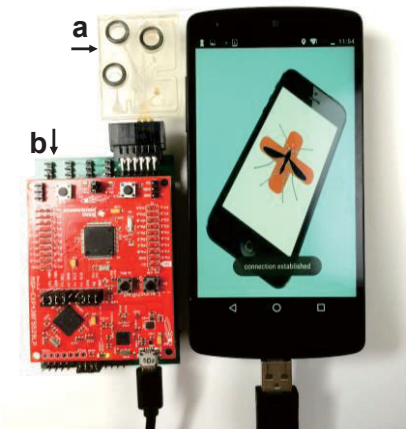

Figure 3: Photograph of our mobile phone biosensing platform. The microfluidic chip (a) directly plugs into aMEASURE (b), which is connected to the phone via the microUSB port using a USB OTG adapter.

Electrochemical detection scheme

A schematic of our *Pf*HRP2 assay is shown in Fig. 4. Thiolated anti-*Pf*HRP2 IgM is immobilized on the WE (Fig. 4a). In the absence of *Pf*HRP2 antigen, the Px-conjugated secondary antibody is washed away during the rinsing step and a negligible signal is generated. However, when *Pf*HRP2 antigen is present in the sample, it binds to sensor surface (Fig. 4b) allowing surface immobilization of the Px-conjugated secondary antibody (Fig. 4c). Upon application of a bias potential, an electrochemical reaction takes place between Px and 4AP/H_2O_2, resulting in a high signal (Fig. 4d). Based on this scheme, the concentration of *Pf*HRP2 in the sample is proportional to the current generated during the electrochemical reaction.

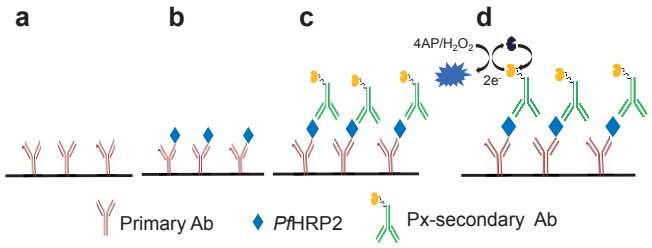

Figure 4: Schematic of our *Pf*HRP2 electrochemical assay. (a) The sensor is immobilized with thiolated anti-*Pf*HRP2 IgM antibody (b) If present, *Pf*HRP2 binds to the primary antibody. (c) Px-conjugated secondary antibody is immobilized on the sensor via binding to *Pf*HRP2, forming a sandwich structure. (d) Px reacts with 4AP/H_2O_2 generating a current that is proportional to the *Pf*HRP2 concentration.

Microfluidic chip design

Our microfluidic chip consists of major components: reagent storage reservoirs, finger-actuated pistons, microchannel network and an electrochemical sensor (Fig. 5). The pistons are secured in place via rubber gaskets which effectively seals off the reagents from the environment. Due to the mild hydrophobicity of native PMMA, the liquid reagents remain contained in the reservoirs until piston actuation. Two small air ports are placed downstream of the reservoirs to allow air exhaustion during actuation, thereby preventing bubble generation inside the fluidic network. The arrangement of the sample inlet, microchannels and reagent reservoirs were designed to facilitate the assay protocol and enhance the detection performance. The capillary tube inlet is connected to the sensing region via a separate channel to minimize diffusion between the sample and reagents. Separate channels were employed for the reporter solution (R1) and substrate (R3) to prevent them from mixing, which would result in a high background signal. However, merging the microchannels for the PBS wash buffer (R2) and substrate (R3) did not have a significant impact on the background signal and was adopted to minimize the chip size. Based on these design strategies, our microfluidic chip does not require any additional fluidic components, such as valves or diodes.

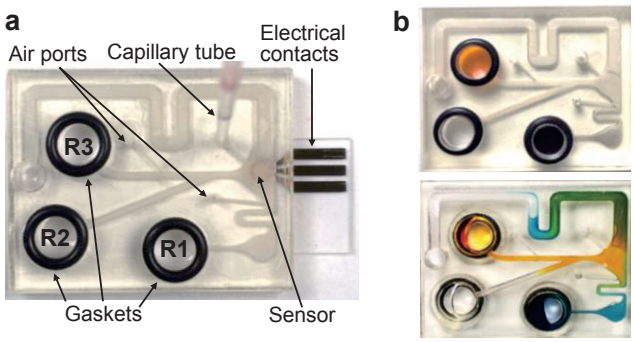

Figure 5: (a) Photograph of the assembled microfluidic chip with relevant regions labelled. Reservoirs R1, R2 and R3 are preloaded with a reporter solution, wash buffer and 4AP/H_2O_2 substrate, respectively. The dimensions of the chip are 40 mm × 30 mm. (b) Microfluidic chip filled with colored dye before (upper) and after (lower) piston actuation.

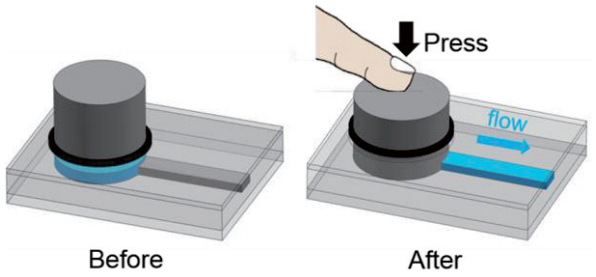

Figure 6: Schematic of the piston-based microfluidic pump before (left) and after (right) actuation.

Pumping mechanism and performance

A schematic of the pumping mechanism is shown in Fig. 6. Depressing the piston generates a uniform pressure on the liquid inside the reservoir, which drives it through the channel. The tight clearance between the piston and gasket prevents leaking and backflow after activation, which can be observed by using colored dyes (Fig. 5b). To characterize the effectiveness of this pumping mechanism for transporting liquids inside microchannels, we measured the pressing force required for various channel widths. As expected, the pressing force is proportional to the channel width since the fluidic resistance is a function of the channel size (Fig. 7) [20]. Specifically, larger pressing forces are required to drive liquids in smaller channels. However, the smallest channel that was tested (100 µm) requires ~18 N of force to generate flow, which is well within the capability of the average human [21]. Additionally, we observed that the rate at which the piston was pressed had a negligible impact on the flow performance. These results demonstrate that our finger-powered pumping mechanism is a simple and effective method for generating driving liquids in microfluidic devices.

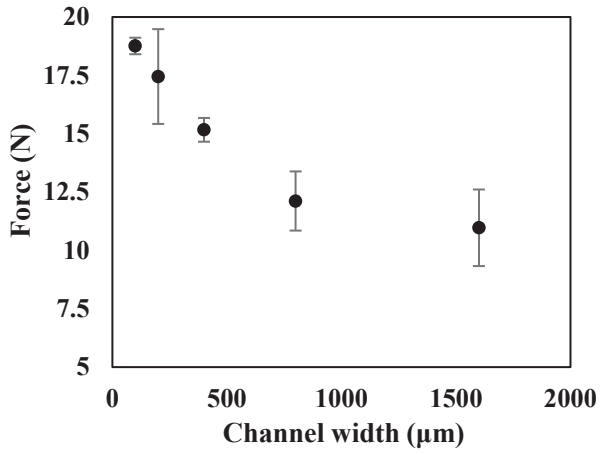

Figure 7: Pressing force required to pump water in straight microchannels of varying widths. Each data point represents of the mean ± standard deviation (SD) of three separate measurements.

PfHRP2 assay performance

We first evaluated the specificity of our *Pf*HRP2 assay by performing measurements using irrelevant targets including dengue virus type 2 (DEN-2) and *Pf*LDH, which is another malaria biomarker. As shown in Fig. 8, the sample containing *Pf*HRP2 generated a high signal-to-background ratio (SBR) of ~16 whereas the irrelevant targets generated low SBRs similar to PBS sample (blank control). These results suggest that the primary and secondary antibodies used in this assay specifically bind to only *Pf*HRP2 thereby minimizing the likelihood of false positive measurements.

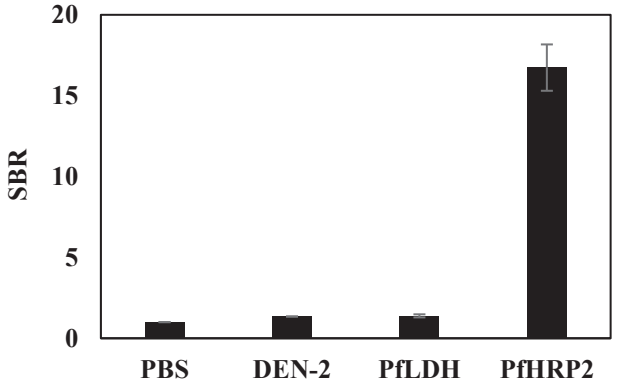

Figure 8: Specificity of *Pf*HRP2 assay. Measurements were performed using DEN-2, *Pf*LDH and *Pf*HRP2 in PBS at a concentration of 10 µg/mL, and PBS as a blank control. Each bar represents of the mean ± SD of three separate measurements.

Next, experiments were performed to measure *Pf*HRP2 in human plasma samples. As shown in Fig. 9, amperometric signals were clearly distinguishable at all of the tested concentrations with relatively smooth response profiles and minimal noise. In addition, this assay can detect a wide range of concentrations (0.1 µg/mL - 20 µg/mL), encompassing the clinical levels found in patients with *P. falciparum* infection [22], without sample dilution or concentration. Furthermore, each measurements is completed in ≤ 6 min, thus offering a rapid result for point-of-care testing.

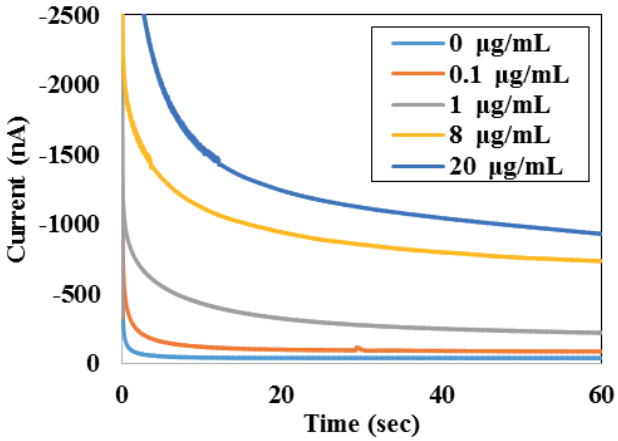

Figure 9: Amperometric signals of *Pf*HRP2 diluted in human plasma. Measurements were performed at a bias potential of -150 mV.

CONCLUSIONS

We have demonstrated a finger-powered microfluidic chip for rapid electrochemical measurements of protein biomarkers using a mobile phone biosensor platform. Reagents are stored on-chip and fluid transport is accomplished via a unique piston-based pumping mechanism. This electricity-free actuation mechanism is coupled with a high sensitivity, high selectivity electrochemical assay which offers rapid measurements and a wide detection range. For proof-of-concept, this platform was used for quantitative measurements of *Pf*HRP2 which could be detected from 0.1 µg/mL – 20 µg/mL in human plasma samples. Each measurement is completed in ≤ 6 min which is 3× faster than our previously reported mobile phone electrochemical biosensor [13]. Owing to its portability, simplicity and good analytical performance, this platform has the potential to improve point-of-care testing worldwide, particularly in remote and resource-limited settings.

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