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An integrated and automated electronic system for point-of-care protein testing

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***Abstract*— Protein testing in blood is important for clinical analysis. Traditional blood tests are performed in centralized laboratories and are slow to provide results. In contrast, point-of-care devices deliver rapid results in non-laboratory settings, allowing timely analysis and in turn reducing healthcare costs. Successful point-of-care platforms require seamless integration of chemical assays, fluid management and signal readout. In this regard, we present an integrated, compact and automated electronic system for point-of-care protein sensing. The system comprises of a microfluidics-based electrochemical biosensor, amperometry circuitry and automated microfluidic fluid handling circuitry. This platform utilizes magnetic microbeads to expedite an electronic enzyme-linked immunosorbent assay, and microfluidics to manage small volumes and automate assay operations. A commercial single-chip potentiostat is utilized for amperometry measurements and microfluidics control. Using this all-electrical system, we demonstrate an integrated and automated assay for human interleukin-6.**

I. Introduction

Measuring protein biomarkers in blood using immunoassays is important in various aspects of biomedicine, including clinical diagnosis and monitoring [1, 2]. Traditional blood tests are performed in centralized laboratories by large and expensive laboratory analyzers. Typically, turnaround times from blood draw to assay results are long (~ hours – days), which can increase patient visits and healthcare costs, and worsen healthcare outcomes. Alternatively, point-of-care (PoC) protein testing provides results quickly (~ min) and in non-laboratory settings. Therefore, PoC devices have been attracting considerable interest. While maintaining sensitivity, accuracy and specificity of laboratory tets, PoC protein testing devices should also be compact, low-cost and easy to use, and need small volume of samples [3, 4]. Because optical systems are expensive and challenging to miniaturize, optical protein tests performed in central laboratories cannot be directly translated into PoC settings.

Electrically mediated systems have a natural advantage for these metrics as they can reduce system complexity and leverage microelectronics' size and cost scaling. For sensing, electrochemical biosensors, which detect biological events through electrical measurements (e.g., amperometry), are appealing and have been seen commercial success (e.g., glucose sensing [5]). In concert, considerable efforts have been undertaken to develop miniaturized and portable electrochemical readout systems [6, 7]. Besides assay chemistry and signal readout, equally important to system design is fluid control functionality, as most immunoassays involve fluid operations such as diluting, washing and mixing. Microfluidics is ideally suitable for automated handling of small biofluid volumes (~ µL). Importantly, multilayer soft lithographic systems can implement fluid handling steps using electronic interfaces [8].

We have previously reported a multiplexed bead-based electronic enzyme-linked immunosorbent (ELISA) using a single-chip potentiostat, which features off-chip analyte capture using magnetic micro-beads and on-chip detection of magnetic beads using electrochemical measurement [9]. Here, we present an integrated and automated electronically controlled PoC system. While lab-on-chip ELISAs have been developed by taking advantage of microfluidics, most of those systems either rely on optical readout (which is expensive and challenging to miniaturize) [10] or need manual interventions [11]. Our system is automated, all-electrical and thus can be readily miniaturized and scaled up. Using this compact electronic system, we demonstrate a 30-mintue PoC detection of human interleukin-6 (IL-6), which has multiple indications in clinical diagnosis.

II. System design and implementation

A. System and circuitry design

Clinically useful PoC devices require seamless integration of biochemical assays, fluid handling and signal readout. Our platform integrates a bead-based electronic ELISA biosensor, an amperometry readout circuitry and an automated microfluidic fluid management circuitry into one system [Fig. 1 (a)]. Performing amperometry measurement and controlling microfluidic fluid management, the single-chip potentiostat (ADUCM350 from Analog Devices, Inc) is the heart of this all-electrical system [Fig. 1(b)]. Interfaced with two 8-bit shift registers, the microcontroller can control up to 16 solenoid valves. The use of shift registers allows for the operation of multiple solenoids simultaneously, which enables complex fluid management. For example, to realize peristaltic pumping, at least three solenoids are needed [12]. The solenoids valves are connected to microfluidic valves and can be programmed to achieve fluid management such as peristaltic pumping, mixing and valving.

Through a 16-channel single-pole-single-throw analog multiplexer, the microcontroller can alternate amperometry measurement across up to 16 electrochemical cells. The biosensor is connected to the amperometry circuitry through a card edge connector.

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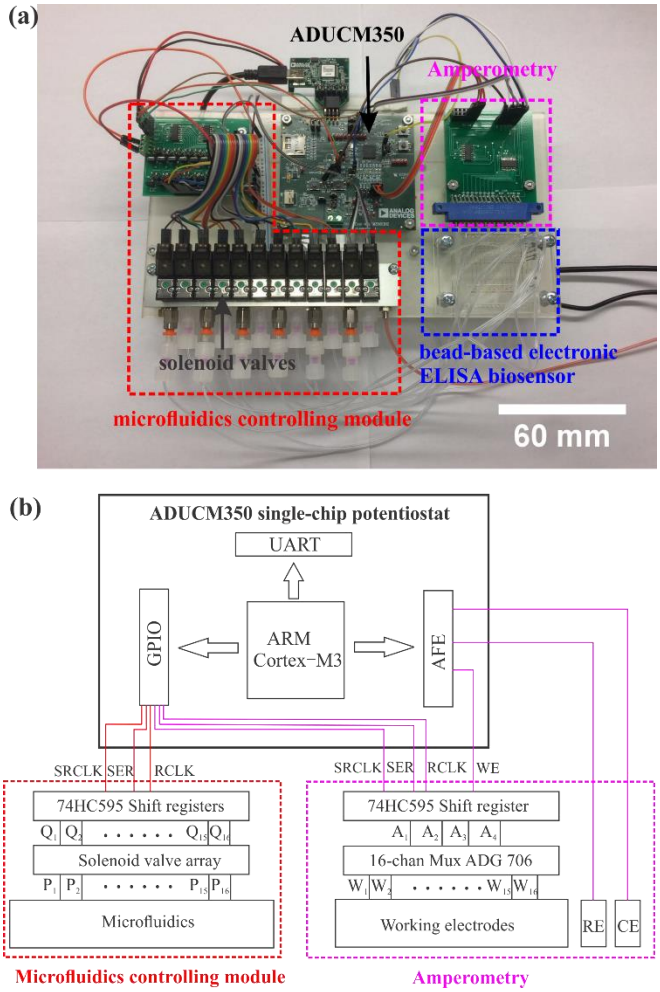

Figure 1. All-electrical PoC system. (a) Image of the implemented system. (b) Schematic system-level overview.

B. *Microfluidics-based electrochemical biosensor*

We previously developed a multiplexed bead-based electronic ELISA.[9] As illustrated in Fig. 2(a), the assay consists of three steps. First, magnetic beads (Dynabeads MyOne Streptavidin T1, Thermo Fisher Scientific) that are loaded with antibodies and enzymes (horseradish peroxidase) are mixed with samples to capture protein biomarkers. These beads are then sent to interact with electrodes coated with antibodies. In this step, the beads that capture protein molecules will attach to the electrode surface and remain after a washing step. During the readout stage, a substrate (mixture of 3,3',5,5'-Tetramethylbenzidine and hydrogen peroxide) is introduced to generate current.

Here, we present a microfluidics-based electrochemical biosensor to integrate antigen capture and bead detection together and automate the assay. The biosensor comprises three electrodes (gold working electrode, gold counter electrode and Ag/AgCl reference electrode) and a microfluidic channel. To improve assay throughput and enable multiplexed measurement, we created a device comprising eight parallel sensors [Fig. 2(b)]. Electrodes are fabricated by patterning 15 nm Ti and 200 nm Au on a Pyrex wafer through standard photolithography and lift-off techniques. The microfluidic channels consist of two layers: control layer (at the top) and flow layer (at the bottom). Both channels have a rectangular cross-section and a height of 70 μm. The width of flow channel is 600 μm.

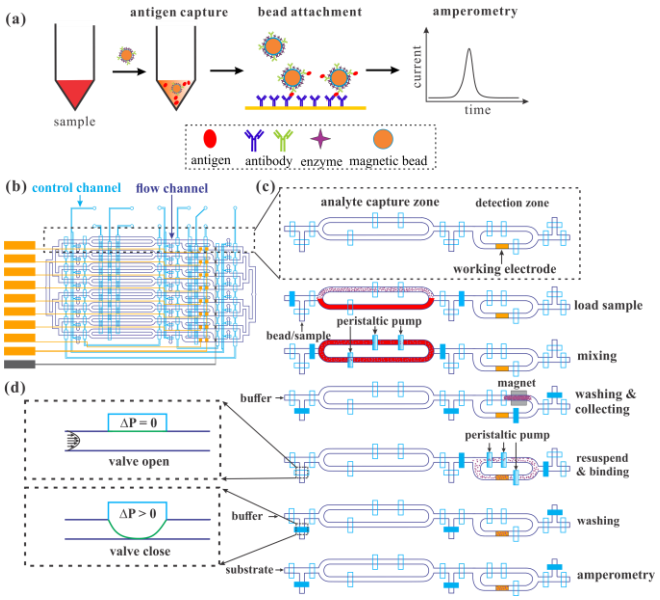

Figure 2. Schematic illustration of the microfluidic and electrochemical biosensor. (a) Schematic illustrating the bead-based electronic ELISA. (b) Layout of the biosensor. (c) Workflow of the automated bead-based electronic ELISA. (d) Diagram of operation of microfluidic valves.

The microfluidics primarily consists of an analyte capture zone (total volume of 1.07 μL) and a detection zone (total volume of 0.47 μL) [Fig. 2(c)], each of which features a rotary mixer and microfluidic valves. Using the programmable fluid control module to actuate those valves, we are able to realize every step of the bead-based electronic ELISA on this biosensor. The assay begins with loading sample and magnetic beads. The rotary peristaltic pump drives solution to circulate in the analyte capture zone, mixing beads with samples and enhancing analyte binding onto beads. The beads are then moved into the detection zone by a flow and concentrated by a magnet. The rotary pump in the detection zone brings the beads into solution uniformly so that they can attach to the electrodes. Unbounds beads are washed away, followed by injection of substrate to generate current signals.

Microfluidic valves are the basis of this microfluidic automation. As shown in Fig. 2(d), valves are created at the intersection between control channels (which are pressurized) and flow channels (where samples and chemical reagents flow). These two channels are separated by a thin PDMS membrane. When the control channels are pressurized, the membrane will be deformed and protrude into the flow channel, stopping the flow. A round flow channel is usually designed such that it can be fully closed [8]. However, in the push-down configuration, flow channels need to be shallow and a high pressure is required to close the valves [8, 13]. We designed a tall rectangular flow channel to accommodate sufficient sample. Even without full closure of microfluidic valves, partial closure is sufficient for this assay as demonstrated in our results.

III. Experiments and Results

A. Microfluidic valves

As valves operation is critical to assay automation, we first examined operation of microfluidic valves with a round-cross-section flow channel. Particularly, we investigated valve actuation to determine the optimal pressure parameters. Valves closure was estimated by dividing the width of closure area (l_v) by the width of flow channel (l_c). It was observed that valve closure increases as pressure rises. As in Fig. 3, when the pressure is 10 psi, the valve closure is 72.16% (l_v = 417.9 μm, l_c = 573.3 μm). The valve closure increases to 87.70% when the pressure is 30 psi. While the valve can close up to 91.06% under a pressure of 40 psi, we chose the valve operation pressure to be 30 psi, because it was found that pressure higher than that may cause the microfluidic device to leak. It is worth noting that due to the rectangular geometry of flow channels, our valves may not be fully closed as those valves with round flow channels. However, as shown in the result section, this type of valves can be still used to achieve automated protein testing.

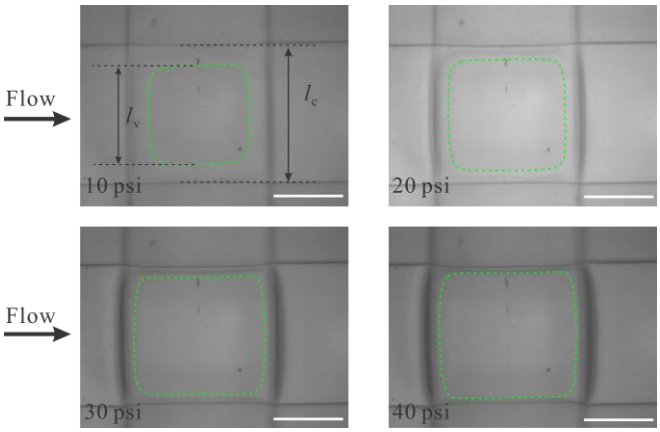

Figure 3. Images comparing valves operation at different pressures. The images show the top views of valves. The white scale bars are 300 μm. The green dash lines denote the part of valves that are closed. l_c is the width of flow channel and l_v is the width of the closed part of valve.

B. Peristaltic pump

Peristaltic pumps can be built by actuating multiple valves in designated sequences. Valve sequencing were optimized to enable mixture of beads with samples, and re-suspension of beads (flowing beads concentrated by an external magnet) [Fig. 2(c)]. Specific valve characteristics were investigated to refine the assay.

We modulated the three microfluidic valves in the antigen capture zone following a repeated sequence $P_iP_jP_k$ = 101→100→110→010→011→001 [Fig. 4(a)]. Each state in the sequence last for a duration of T_s. Fig. 4(b) presents a snapshot of bead motion under flow driven by the peristaltic pump, where T_s = 10 ms. The bead trajectory indicates the presence of flow. From the bead trajectories, we estimated that the maximum bead velocity is 773 μm/s and thus the flow rate is 1.11 μL/min. We further examined the influence of sequence duration (i.e., T_s) on flow rate [Fig. 4(c)]. It is observed that flow rate increases as the sequence duration becomes shorter. While the solenoid control circuitry can change state very fast, the response time of solenoid valves can limit the speed of peristaltic pumping. We decided to choose T_s = 10 ms, which is close to the response time of solenoids valves (3 Port Solenoid Valves S070 Series from SMC Corporation of America). These results also validated usage of valves with rectangular flow channels.

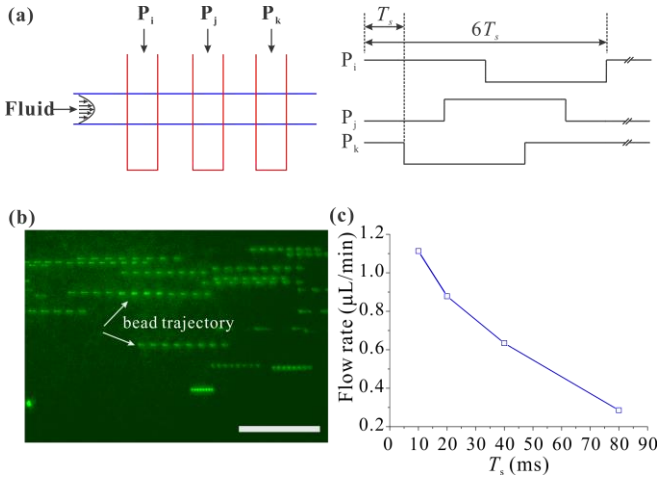

Figure 4. Microfluidic peristaltic pumping using three valves. (a) Illustratation of a microfluidic peristaltic pump using three valves (on the left) and its control sequences (on the right). (b) Movement of fluenrescent beads driven by the peristaltic pump. The scale bar represents 300 μm. The expsure time is 500 ms and T_s is 10 ms. (c) Relationship between flow rate and duration of controlling sequence.

C. Mixing within rotary mixer

In our design, we implemented rotary mixers to mix beads with sample, and re-suspend beads in the detection zone. Therefore, it is important to examine the performance of the rotary mixers. Experiments were conducted to investigate mixing of biomolecules and microbeads separately.

To study mixing of biomolecules, similarly as in an assay where we only fill half of the rotary mixer with sample, we load solution of bovine serum albumin (BSA) conjugated to Alexa Fluor 488 dye into one half of the rotary while the other half was filled with buffer. As BSA has similar diffusion coefficient with many protein biomarkers, the experiment is representative of mixing in a bioassay. Then we started the rotary pump (with T_s = 10 milliseconds) and observed the fluorescent intensity change over time [Fig. 5(a)]. It should be noted that because the solution has not been completely mixed in the beginning, the fluorescence intensity fluctuates with a period of 35 seconds which corresponds to fluid cycling time in the rotary. The intensity becomes flat after about 120 seconds, suggesting the BSA solution was well mixed.

We took a similar approach to examine mixing of micro-beads. Instead of measuring fluorescence intensity, we counted bead number over time and the results are shown in Fig. 5(b). After 60 seconds, the bead number does not change significantly. The mean value is 2809 and standard deviation is 188 (i.e., 6.7% of mean value), indicating the beads are almost uniform in the channel.

From these measurements, we conclude that it will take 1-2 minutes to mix the bead solution and the sample solution so that they are uniformly distributed in the rotary. This time is appropriate for the overall assay duration.

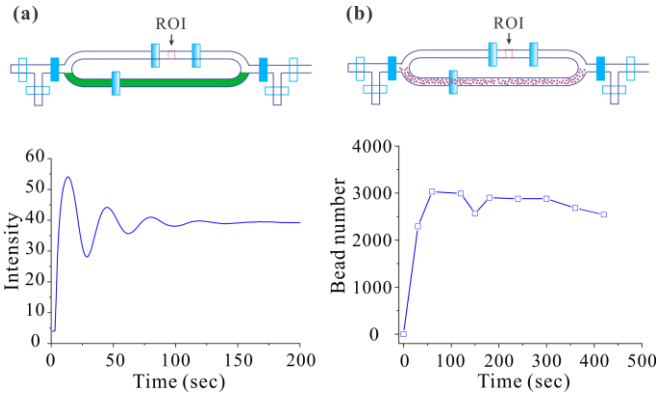

Figure 5. Mixing using rotary pump. (a-b) Results showing mixing of BSA (a) and magnetic beads (b) in the rotary mixer. The top schematic is the experimental setup, where we measure fluorescence intensity or bead number over time at region of interest (ROI) indicated by the red squares.

D. A 30-minute integrated and automated sensing of IL-6

Having validated the key components of our system, we finally demonstrated an integrated and automated measurement of human IL-6 in human plasma.

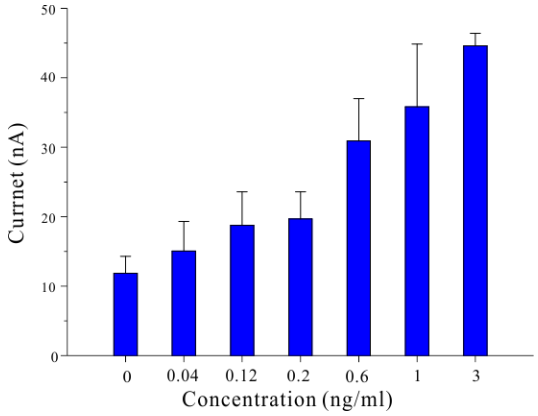

Figure 6. Example calibration curve obtained from the integrated and automated electrical sensing system. The samples were made by spiking human IL-6 into 4-fold diluted human plasma to different concentrations. The error bars represent measurement from two separate sensors. The total assay time was 30 minutes.

As illustrated in Fig. 2(c), the assay starts with manually and sequentially loading magnetic beads and samples into the capture rotary. After that, the assay runs automatically until it concludes. Briefly, the peristaltic pump drives the flow to circulate in the rotary mixer, mixing beads with samples. The bead-sample incubation time is 15 minutes. The mixture of bead and sample is sent to the detection zone, which takes 30 seconds. The beads are pulled down by a magnet controlled by a solenoid and the sample solution goes to waste. The peristaltic pump in the detection zone starts for 2 minutes and re-suspends the beads again into solution. After the pump stops, the beads are allowed to interact with electrodes for 8 minutes. A two-minute washing step is then carried out to remove unbound beads. Finally, a substrate is introduced into detection zone for amperometry measurement, which takes up to 3 minutes. The results are presented in Fig.6, which shows that the system can measure IL-6 down to 0.04 ng/ml within 30 minutes. Additionally, due to the usage of the microfluidic system, our assay needs less than 1 μL sample. Therefore, our all-electrical platform can be suitable for PoC applications.

IV. Conclusion

In this paper, we developed an integrated and automated electronic system for PoC protein testing. We demonstrate programmable fluidic control (e.g., microfluidic peristaltic pumping) by combining solenoid valves and microfluidic valves. Using human IL-6 as an example, the integrated platform is shown to be able to perform sensitive, rapid and automated protein assays.

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