LOW-POWER ELECTRICALLY CONTROLLED THERMOELASTIC MICROFLUIDIC VALVE ARRAY FOR MULTIPLEXED IMMUNOASSAY

Chun-Wei Wang, Shancy Augustine, Toshi Nishida, and Z. Hugh Fan*

Interdisciplinary Microsystems Group (IMG), Department of Mechanical and Aerospace Engineering & Department of Electrical and Computer Engineering, University of Florida, Gainesville, FL, USA

ABSTRACT

We report a low-power, thermally actuated, and elastomer-based microvalve, which is integrated in a thermoplastic microfluidic device for multiplexed immunoassays. The thermal-actuating material used in this valve also enables a latching capability. Thirteen microfluidic valves have been integrated in a microfluidic device, enabling a network of fluid channels for reagent manipulation and sequential delivery. With a unique combination of antibodies, the device is able to analyze six proteins simultaneously. The total energy consumption for a device during the immunoassay is only 4 mWh.

INTRODUCTION

Immunoassays are extensively used in biomedical research, clinical diagnostics, and food/water safety testing [1-3]. Although they can be performed in a microplate to increase sample throughput, immunoassay in each well is generally limited to one analyte per test. Such a format is inefficient and not cost-effective for the requirement of detecting multiple analytes in one sample. For instance, a set of biomarkers should be detected for a particular disease to enhance the accuracy and reduce false positives or negatives. Similarly, a spectrum of pathogens should be simultaneously monitored in foods and drinking water.

A microfluidic network (μ FN) has potential to achieve multiplexed immunoassay in a microfluidic device [4, 5]. Figure 1 shows the process of μ FN for protein patterning. First, multiple antibodies are introduced into each channel respectively (Figure 1a) Then sample solutions are introduced through other sets of channels perpendicular to the previous ones (Figure 1b). Each intersection area is a detection area for a specific protein. Although multiple target proteins can be detected simultaneously, many time-consuming steps of manual control of flows still required. A microvalve can be utilized to solve the problem.

Figure 1: Microfluidic networks for protein patterning. (A) Each antibody is immobilized on each channel (blue). (B) The channel set for samples (in pink) is oriented perpendicular to the previous set (in blue). Each intersection area (in black) is an assay site for a specific analyte.

Among all microvalve designs, an electrically controlled and thermally actuated microvalve is the most compact, hence portable [6-9]. However high power consumption is a drawback of this type of microvalve, especially when multiple valves are required in one device for multiplexing. Moreover, utilizing thermal actuation in a low-cost plastic device is a challenge because the maximum temperature that plastic can withstand is lower than silicon or glass.

In this study, we design a low-power, thermally actuated

microvalve array which is featured by the latching capability. The average energy consumption per valve during an immunoassay is only 0.31 mWh, which is about 300-500 times less than other thermoelastic microvalves [10, 11]. With such a low-power valve, we are able to integrate the valves into an all-plastic microfluidic device that enables portable operation. We have demonstrated a multiplexed immunoassay on 6 assay sites in the device.

MATERIALS AND METHODS

Reagents and Materials

COC resin and film (Zeonor 1020R and 1420R) were purchased from Zeon Chemicals (Louisville, KY, USA). Polyurethane elastomer (Bayhydrol 110) was purchased from Bayer Material Science (Pittsburgh, PA, USA). COC sheet (8007) from Topas Advanced Polymer Inc. (Florence, KY). Paraffin wax (MP 47°C-65°C) was purchased from Spectrum Chemicals (New Brunswick, NJ, USA). UV curable epoxy is purchased from McMaster-Carr (Atlanta, GA, USA). Avidin was purchased from Fisher Scientific and the proteins C-reactive protein (CRP), transferrin (TfR), resistin (Res), and erythropoietin (EpoR) with their respective biotinylated capture and detection antibodies were purchased from R&D systems (Minneapolis, MN, USA). The dyes Alexa Fluor 647 was purchased from Life Technologies (Carlsbad, CA, USA).

Device Design and Fabrication

A low-power, electrically controlled thermoelastic-based thermoplastic microvalve consists of 4 layers, which are channel, cavity, elastomer, and heater layers, as illustrated in Figure 2a. The channel layer is formed by compression molding COC resins to 1.5-mm thick. The microchannels in this layer are 100 μ m wide, 35 μ m deep. The elastomer layer is a 4- μ m-thick polyurethane

Figure 2: (a) Illustration of a microvalve consisting of 4 layers indicated. (b) Top-view picture of the electrically controlled thermoelastic valve array in a microfluidic device. Thirteen valves and 6 inlet/outlet were integrated in the device. The metallic wire is the electrical pattern of the heater layer. The channels were filled with a red dye solution to show the channel layout. elastomer, which is made by spin-coating a polyurethane solution

on a sacrificial layer, and then bonding it chemically with the channel layer as described in our previous work [12]. The cavity layer is a 254- μm -thick COC sheet containing 1-mm-radius cavities, which are made using a cutting plotter (Graphtec America, Irvine, CA, USA). This layer is chemically bonded to the elastomer layer [12] as well. The heater layer consists of a thin copper film patterned in a serpentine shape by photolithography on a COC film. One mg of paraffin wax is placed in the cavities, and melted by putting the device on a hot plate (75°C) until wax fill the cavities entirely. The cavity layer is then bonded to the heater layer with UV curable epoxy.

Figure 2b shows the top-view of the device with a microvalve array. The device consists of 13 valves and 6 inlets/outlets. To visualize channels, a red dye solution is introduced into the device. The dark metallic line is the pattern of the copper wire on the heater layer. The size of device is only 2.25 inch x 1.25 inch.

Principle of Valve Operation

Figure 3 illustrates the valve operation including closing, latching, and opening as we recently reported [13]. When power is on, the wax changes from solid to liquid, expands, and deforms the elastomer to close the channel (Figure 3b). As the power is off, the wax returns to solid while the elastomer is still under deformation. Consequently, the valve remains closed or latched (Figure 3c). The valve is reopened by applying a short power pulse with a pressurized fluid from a syringe pump (Figure 3d).

Figure 3: Principle of valve operation. (a) Initial state of a valve. (b) When the heater is on, wax melts, expands, and deforms the elastomer layer to close the channel. (c) As the heater is off, wax changes to the solid phase faster than elastomer recovery. Consequently, the valve remains closed without power. (d) A pulse of power and a pressurized flow in the channel are simultaneously supplied to open the valve.

Multiplexed Immunoassay

To achieve simultaneous immunoassays of 6 proteins (4 target proteins and 2 control sites) in our device, the scheme of valve operation and immunoassay process are described as follows. First, 1 mg/ml avidin solution is introduced into all channels and incubated for 30 min. Then by controlling an appropriate set of valves as shown in Figure 4a, two vertical channels are accessible. Two mixtures of 10 $\mu\text{g}/\text{ml}$ capture antibody solutions (antibody 1,2 and antibody 3,4) are then introduced into each channel and incubated for 30 min. Next, by controlling another set of

microvalves as shown in Figure 4b, three horizontal channels in the device are open. The sample solution which contains all target proteins is introduced in top and bottom channels, while the control solution which is bovine serum albumin (BSA) is introduced in the middle channel. After incubation for 30 min, three mixtures of 2.5 $\mu\text{g}/\text{ml}$ fluorescently labeled detection antibody solution (dAb 1,3, dAb 2,4 and dAb 1,2,3,4) are introduced to each channel as shown in Figure 4b. Note that washings with a PBS buffer are performed among different steps. As a result, each target proteins can only be detected in one assay site, which is the intersection area of channels. Moreover, the total energy consumption for a device during the immunoassay process is 4 mWh.

Figure 4: Immunoassay scheme for simultaneously detection of 4 proteins with 2 assay sites as negative controls. The device consists of 13 valves (solid blue circles and open circles with a cross), and 6 wells as fluid inlets/outlets. (a) As specific valves close (circles with cross), the capture antibodies flow in the vertical direction of pink arrows and are immobilized on the channels. (b) As the valve layout changes, the sample and detection antibodies flow in the horizontal directions as indicated by pink arrows. Due to the unique combination of antibodies indicated at the inlet, 4 proteins are distinctly detected at the 4 assay sites (red circles). The number at each red circle indicates the presence of a particular protein if a signal is detected there. The middle picture illustrates the sandwich immunoassay scheme at each assay site.

RESULTS AND DISCUSSION

Valve Performance Test

In order to test the valve performance, a single valve testing setup was designed as shown in Figure 5a. A resistor is connected in series with the channel containing a sodium chloride solution. The electrical current (I) indicates whether the valve is open ($I \neq 0$) or closed ($I = 0$) since a closed valve can prevent the current passing through the solution in the channel.

Figure 5b shows the result of the valve testing. The red curve indicates the electric power for the heater, and the blue curve indicates the measurement of an electrical current. First, the valve is closed with a power of 56 mW in 24 s. After power is off, the valve remains closed (latched) as the current is around zero. To reopen the valve, a pulse of power is supplied for 5 s with a 1 $\mu\text{L}/\text{min}$ flow. The valve is reopened as the current indicated $\sim 0.5 \mu\text{A}$.

Figure 5: (A) Experimental setup for valve performance test. Electrical current (I) determines whether the valve is closed ($I = 0$) or open ($I \neq 0$). (B) Cyclic operations of microvalve. The blue Y-axis (left) and blue curve represents the state of the valve; the red Y-axis (right) and red curve shows the power for the heater. The valve closes in 24 s with power at 56 mW and reopens with the same power in 5 s (pulse).

Assay Calibration Curve

In order to verify the immunoassay protocol in our device, a CRP regression analysis is performed. The CRP concentrations of 20, 40, 70, 140, and 250 ng/mL are used for this analysis. The detection antibody is labeled with Alexa Fluor 647. The immunoassay protocol is the same as described above, except that only one protein is detected. Figure 6 shows the calibration curve of CRP detection. The negative control in this analysis is BSA, and the data at zero CRP concentration is included in the calibration curve. The recorded data points are fitted well to a reverse exponential curve with an R^2 value of 0.99.

Figure 6: Graph of CRP calibration curve. Each data point represents the fluorescence intensity from CRP measurement (20 - 250 ng/ml). Data point at 0 ng/ml CRP is the negative control using BSA. The curve is fitted with reverse exponential curve ($y = 4970 - 4828e^{-0.0046x}$) with a R^2 value of 0.99.

Multiplexed Immunoassay

Our microfluidic device is capable of detecting 6 different proteins simultaneously. To demonstrate the multiplexed immunoassay, 4 assay sites are used to detect 4 target analytes (CRP, transferrin, resistin, and erythropoietin) and the remaining 2 assay sites are used for negative controls (BSA). Since the real biological sample always contains multiple analytes, we mimic this situation by mixing all target proteins and BSA together as the sample solution. Figure 7 shows the fluorescence intensity in 6 assay sites. The signal-to-noise ratio for CRP (100 ng/ml), TfR (300 ng/ml), Res (198 ng/ml), and EpoR (134 ng/ml) are 5.4, 2.2, 3.0, and 3.1 respectively. Furthermore, as the fluorescence intensity of CRP is fitted with the calibration curve from Figure 6, the corresponding CRP concentration is 85 ng/ml, which gives 15% forecast error.

Figure 7. Fluorescence intensity of each assay sites with their corresponding protein concentration.

CONCLUSION

A thermoelastic microvalve using wax as the actuation material has been fabricated and characterized by investigating its latching behavior of the microvalve. The thermoelastic microvalve is controlled electronically, making the device portable as compared to pneumatic valves which require bulky accessories such as a gas tank. The latching capability reduces the power consumption, enabling us to integrate these valves in a low-cost, all-plastic microfluidic device. Using a microfluidic network strategy, we have successfully demonstrated a multiplexed immunoassay in the device. Moreover, the total energy consumption during the 1 hour immunoassay process was 4 mWh, which can be provided by a 9 V lithium battery. The low power, low cost, compact, and electrically controlled microvalve has a potential to enable portable, battery operated, disposable microfluidic devices for point-of-care diagnosis.

ACKNOWLEDGMENT

This work is supported in part by National Institute of Health (R21GM103535-02), the Flight Attendant Medical Research Institute (FAMRI-082502), and Defense Advanced Research Projects Agency (DARPA) via Micro/Nano Fluidics Fundamental FocusCenter at the University of California at Irvine (HR001-06-1-0500), and the University of Florida via the Research Opportunity Fund. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

REFERENCES

- [1] Q. Xiao and J.M. Lin, "Advances and Applications of Chemiluminescence Immunoassay in Clinical Diagnosis and Foods Safety", *Chinese Journal of Analytical Chemistry*, 43(6), 929 (2015)
- [2] E. Morales-Narvaez, T. Naghdi, E. Zor, and A. Merkoci, "Photo luminescent Lateral-Flow Immunoassay Revealed by Graphene Oxide: Highly Sensitive Paper-Based Pathogen Detection", *Analytical chemistry*, 87(16), 8573 (2015)
- [3] R. Tozzoli, M. Bagnasco, D. Giavarina, and N. Bizzaro, "TSH receptor autoantibody immunoassay in patients with Graves' disease: Improvement of diagnostic accuracy over different generations of methods. Systematic review and meta-analysis", *Autoimmunity Reviews*, 12(2), 107 (2012)
- [4] Y.L. Gao, P.M. Shermanb, Y. Sun, and D. Li, "Multiplexed high-throughput electrokinetically-controlled immunoassay for the detection of specific bacterial antibodies in human serum", *Analytica Chimica Acta*, 606(1), 98 (2008)
- [5] A.H.C. Ng, U. Uddayasankar, and A.R. Wheeler, "Immunoassays in microfluidic systems", *Analytical and Bioanalytical Chemistry*, 397(3), 991 (2010)
- [6] A.K. Au, H.Y. Lai, B.R. Utela, and A. Folch, "Microvalves and Micropumps for BioMEMS", *Micromachines*, 2(2), 179 (2011)
- [7] H. Takao, K. Miyamura, H. Ebi, M. Ashiki, K. Sawada, and M. Ishida, "A MEMS microvalve with PDMS diaphragm and two-chamber configuration of thermo-pneumatic actuator for integrated blood test system on silicon", *Sensors and Actuators a-Physical*, 119(2), 468 (2005)
- [8] D. Baechi, R. Buser, and J. Dual, "A high density microchannel network with integrated valves and photodiodes", *Sensors and Actuators a-Physical*, 95(2-3), 77 (2002)
- [9] A. Ruzzu, K. Bade, J. Fahrenberg, and D. Maas, "Positioning system for catheter tips based on an active microvalve system", *Journal of Micromechanics and Microengineering*, 8(2), 161 (1998)
- [10] J.C. Yoo, Y.J. Choi, C.J. Kang, and Y.S. Kim, "A novel polydimethylsiloxane microfluidic system including thermopneumatic-actuated micropump and Paraffin-actuated microvalve", *Sensors and Actuators a-Physical*, 139(1-2), 216 (2007)
- [11] G.V. Kaigala, V.N. Hoang, and C.J. Backhouse, "Electrically controlled microvalves to integrate microchip polymerase chain reaction and capillary electrophoresis", *Lab on a Chip*, 8(7), 1071 (2008)
- [12] P. Gu, K. Liu, H. Chen, T. Nishida, and Z.H. Fan, "Chemical-Assisted Bonding of Thermoplastics/Elastomer for Fabricating Microfluidic Valves", *Analytical Chemistry*, 83(1), 446 (2011)
- [13] S. Augustine, P. Gu, X. Zheng, T. Nishida, Z. H. Fan, "Low-power electrically controlled thermoelastic microvalves integrated in thermoplastic microfluidic devices", *Microfluidics and Nanofluidics*, 19, 1385 (2015).

CONTACT

*Z. Hugh Fan, tel: +1-352-846-3021; hfan@ufl.edu