Recirculating samples inside chambers using vacuum and centrifugal forces

Anyang Wang, Domin Koh and Kwang W. Oh*

SMALL (Sensors and MicroActuators Learning Lab), Department of Electrical Engineering,
The State University of New York at Buffalo, Buffalo, NY 14260, USA *kwangoh@buffalo.edu

ABSTRACT

In this paper, a combination of vacuum and centrifugal forces was studied for recirculate samples inside the chambers. Detection low concentration samples, rare molecules such as cancer cells or certain proteins among large volume of samples is still challenging in microfluidic based ELISA (enzyme-linked immnosorbent assay). In order to achieve high success rate of detection, recirculating of samples in assay chambers is one of the useful approaches. In this study, vacuum-driven microfluidic was used with the combination of the centrifugal force to achieve recirculating samples inside the chamber.

Keywords: microfluidics, centrifuge, vacuum-driven

1 INTRODUCTION

Microfluidic devices as lab-on a chip have been widely studied for point-of care testing. One of the major applications is ELISA (enzyme-linked immnosorbent assay). In the assay testing, immmobilization of the antigen of the intrest is a critical step, which can be achieved by capturing the antibody that has been attached to the plate or directly absorption to the plate. However, it is challenging to handle precious low concentration molecules or cells in large volume. Some examples including detecting rare protein [1] or cancer cells detection in the whole blood [2]. Thus, improving the limits of detection in immunoassay has been widely studied. [1 - 5]

One of the major approaches is to diminish the size of detection chambers. Phillips et al. [3] has developed a design equation for optimal channel height that yields the fastest response time for a given average flow velocity. However, reducing the channel dimension to the similar dimension of the molecules or cells sizes can cause the clogging. Another approaches is to use the low flow rates (~ nl/s) to let samples have enough time to diffuse to the surface receptors. One of the candidates is vacuum-driven microfluidics. As vacuum-driven microfluidic can provide ~ nl/s [6, 7], it can be an efficient way to help samples trapped to receptors. Furthermore, several authors have reported fluid recirculation in closed loop microfluidic devices to improve the DNA hybridization process. Yuen et al [4] reported 2–5 fold increase in reaction efficiency in hybridization of DNA. Recently, Garcia-Cordero et al [5], studied the interplay of capillary and centrifugal forces to demonstrate liquid recirculation in microfluidic devices. However, capillary force is highly dependent on the surface condition of the channel. Moreover, surface coating may be needed to make channel surface being hydrophilic to utilize capillary force.

In this study, vacuum-driven microfluidic was used with the centrifugal force to achieve recirculating samples inside chamber. Vacuum-driven microfluidics can drive the samples despite the surface condition [6, 7]

2 CONCEPT

Vacuum-assisted pumping is one of the passive pumping methods utilizing either the gas permeability or gas solubility of the materials such as Polydimethylsiloxane (PDMS). For instance, by pre-degassing the PDMS made microfludiic devices, samples can be driven as PDMS absorb the gas inside the fluidic channel to get back to its stable gas concentration. Some benefits using the vacuum-assisted pumping is its ability to avoid air bubbles. Since PDMS is absorbing the gas if any bubbles forming inside the channel. Moreover, the flow rate is constant despite the surface condition [6, 7]. Unlike other major passive pumpings such as capillary pumping uses capillary effect, the vacuum-assisted pumping is mainly relying on the gas permeability or solubility of PDMS. In other words, capillary pumping is governed by the interplay between surface tension of a liquid and the geometry and the surface of the device [8]. Thus, surface condition is critical in capillary pumping. On the other hand, the flow rate using vacuum-assisted pumping is mainly determined by the sample viscosity and the geometrical design of the device, which can be described as [6]

$$Q(t) \approx k\frac{FS}{C_{ATM}} = kD\frac{C_{PDMS} - C_{Chamber}}{C_{ATM}}\frac{S}{t_{wall}}$$

, where k is empirical factor related to viscous effect of the pumped liquid flow. F is steady state air flux diffusing into the micro-chamber. C_{PDMS}, $C_{\mathrm{Chamber}}$and C_{ATM} are the air concentrations in PDMS, micro-chamber and atmosphere respectively. S and t_{wall} are geometrical design of the channel, which is the total surface area that allows air to diffuse out from the microchannel, defined as the diffusion area and the PDMS wall thickness respectively. Surface modification is not necessary in vacuum-assisted pumping. The measured flow rate for the pre-degassing microfluidic in this study is shown in Fig. 1. Microfluidic chips were degassed inside the vacuum chamber at - 27 in.Hg overnight. After the device was taken out from the vacuum chamber, the flow rate was measured over the time, where exposed time = 0 min stands for the moment that the device was taken out from a vacuum chamber. Three devices with

a same design was tested for measuring flow rates. As reported in [9], the flow rate is time-variant since the stored air concentration inside PDMS decreases as the device started to be exposed to air. In this experiment, the device was used at least after the degassed device was exposed to air for 10 min to avoid abrupt flow rate change during the operation.

Another force used for the liquid reciculation is centrifugal force. Since the centrifugal force provides uni-directional force to the sample, it is easy to control samples movement direction inside the channel. The centrifugal-driven pressure as shown in Fig. 2 was calculated using [5]

$$P_c = \rho\omega^2 r\Delta r$$

, where ρ is the density of the liquid, ω is the angular velocity and r is the distance between the center of liquid mass from the center of rotation. The water was used for this experiment ($\rho = 10^3$ kgm^{-3}).

By switching the dominant force between the vacuum forces with centrifugal force, recirculating sample inside the channel can be achieved.

3 DEVICE FABRICATION

The device was fabricated using soft lithography to form microfluidic channels by PDMS. First off, a mold was fabricated using a 3-inch silicon wafer (University wafers, South Boston, MA, USA). Wafer was submerged into buffered hydrofluoric acid (BHF) at room temperature for 5 min to remove the native silicon dioxide layer. Next, wafer was cleaned using acetone and methanol, respectively and rinsed with deionized water following nitrogen gas blowing. Then, wafer is place on a hot plate at 120 ^{o}C for 5 minutes for complete dehydration. In order to make channel mold, negative photoresist SU-8 2050 (SU-8 2050, Micro-Chem Corp, Newton, MA, USA) was spin coated on top of the wafer by using the spin coater (Spin Coater - Brewer Science CEE-200) following the soft bake. Then the mask was aligned using the infrared (IR) mask aligner and Ultra-violet (UV) exposure system following the hard bake. Then the wafer was rinsed into SU-8 developer for 5 minutes to remove the unexposed photoresist following isopropyl alcohol (IPA) rinsing. Finally, the wafer is blown try by compressed nitrogen air to remove IPA. In order to protect the mold and facilitate releasing PDMS from the mold, release agent hexamethyldisilazane (hydrophobic substance) or HDMS was coated on the surface by exposing mold surface to vapor HDMS. After making mold structure, PDMS base and curing agent were mixed at the ratio of 10:1 following degassed at the vacuum chamber to remove air bubbles. Next, mixture was poured on the mold and baked at 80 ^{o}C for 3 hours for curing PDMS. After cool down the PDMS, PDMS replica was peeled off from the mold and every inlets and vents were punched using biopsy. Then, oxygen plasma treatment was performed for bonding PDMS and glass substrate. In order to stabilize PDMS surface condition, bonded device was placed on the hot plate and baked at 100 ^{o}C for 24 hours before testing.

4 RESULTS AND DISCUSSION

The test device to study recirculating sample inside chamber was shown in Fig. 3. The device has two vents, which vacuum driven flow and centrifugal driven flow can be switched by sealing/ unsealing these vents. The most simple way is to use tapes to seal the vents as shown in Fig. 3. After sample was loaded into a degassed device, vacuum-driven force and centrifugal force was applied reciprocally to pull and push sample inside the chamber. First, the sample was loaded into the inlet. Next, two vents were sealed using the tape to allow the sample flow inside the channel using the vacuum force. Next, the vents were unsealed by removing the tapes and the device was placed on a spin coater to apply 1200 revolutions per minute (RPM). Samples were pushed back to the left side of the chamber, which is the far side from the center of rotation. As a result, samples were pushed and pulled inside the chamber (one loop). By continuing the same process, the device can be used for further recirculation. The sample travel distance increases as with the time vents were kept sealed. For instance, the vents were kept sealed for longer time in Loop 6 compared with Loop 2. It would be helpful to coat the device surface with wax to prevent any gas diffusion through device surface so that the pre-degassed device can hold the pumping ability for longer time [9].

5 CONCLUSION

In conclusion, we proposed vacuum-driven microfluidic with combination of centrifugal force to achieve recirculating samples inside chamber. One of the advantages to use vacuum-driven microfluidics are its ability to drive the samples despite the surface condition as well as avoiding air bubbles generation. The study is useful in ELISA (enzyme-linked immnosorbent assay) to achieve high success rate of detection low concentration samples such as DNA and protein from large volume of samples.

REFERENCES

[1] Zheng GF, Patolsky F, Cui Y, Wang WU, Lieber CM (2005) Multiplexed electrical detection of cancer markers with nano wire sensor arrays. Nat Biotechnol 23(10):1294–1301

[2] Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L,Smith MR, Kwak EL, Digumarthy S, Muzikansky A, Ryan P, Balis UJ, Tompkins RG, Haber DA, Toner M (2007) Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature 450(7173):U10–U1235

[3] Phillips, Cynthia, et al. "Model-based optimal design of polymer-coated chemical sensors." Analytical chemistry 75.5 (2003): 1106-1115.

[4] Yuen, Po Ki, et al. "Microfluidic devices for fluidic circulation and mixing improve hybridization signal

intensity on DNA arrays." Lab on a Chip 3.1 (2003): 46-50.
[5] Garcia-Cordero, Jose L., et al. Microfluidics and nanofluidics 9.4-5 (2010): 695-703.
[6] L. Xu, et al. Microfluidics and nanofluidics 17.4 (2014): 745-750.
[7] A. Wang et al, NanoTech 2018, Vol. 3 Biotech, Biomaterials and Biomedical: TechConnect Briefs 2018, pp. 158-161, 2018
[8] Olanrewaju, Ayokunle, et al. "Capillary microfluidics in microchannels: from microfluidic networks to capillaric circuits." Lab on a Chip 18.16 (2018): 2323-2347.
[9] Liu, Bendong, et al. "Hermetic encapsulation of negative-pressure-driven PDMS microfluidic devices using paraffin wax and glass." Microsystem Technologies 24.4 (2018): 2035-2043.

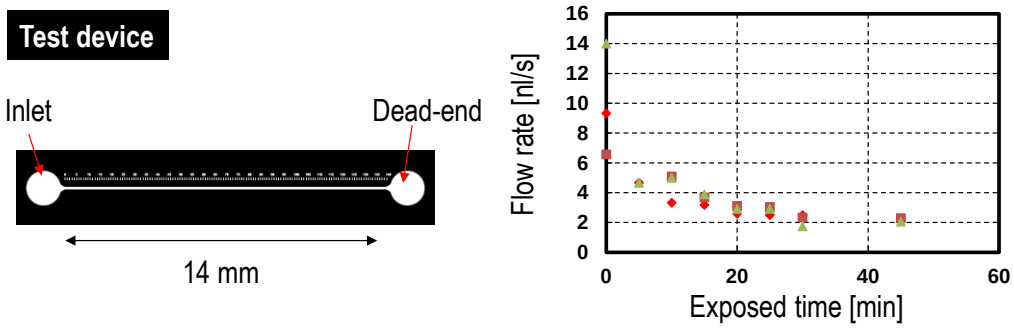

Fig. 1. The test device design for vacuum-driven force and measured flow rate. Three devices with a same design was tested for measuring flow rate. Degassed microfluidics has time-variant flow rate after the device was taken out from a vacuum chamber. The graph shows the measured flow rate for the test devices, where exposed time = 0 min stands for the moment that the device was taken out from a vacuum chamber.

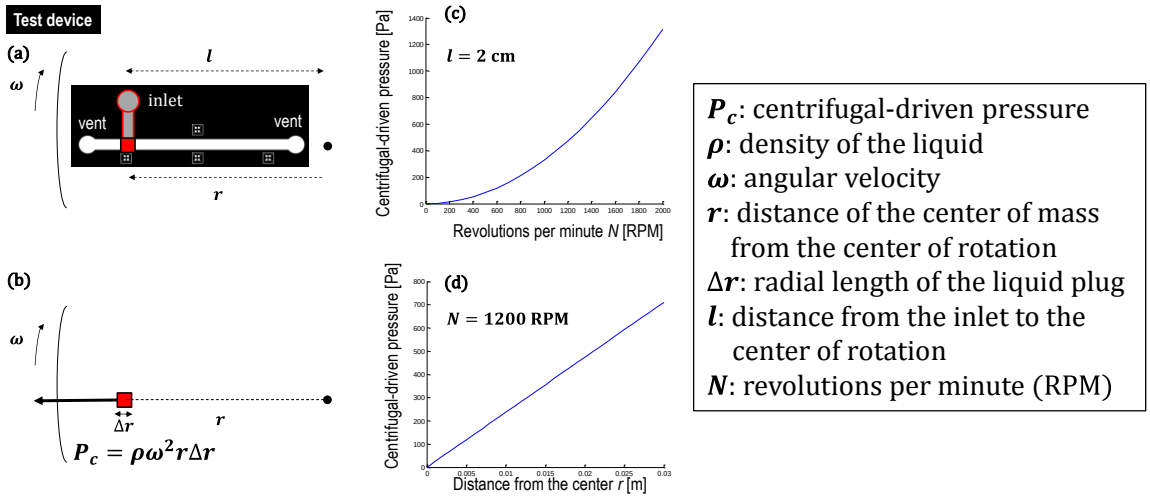

Fig. 2. (a) The test device structure and (b) its model, (c), (d) simulated centrifugal-driven pressure using MATLAB. The water was used for the test ($\rho = 10^3 kgm^{-3}$).

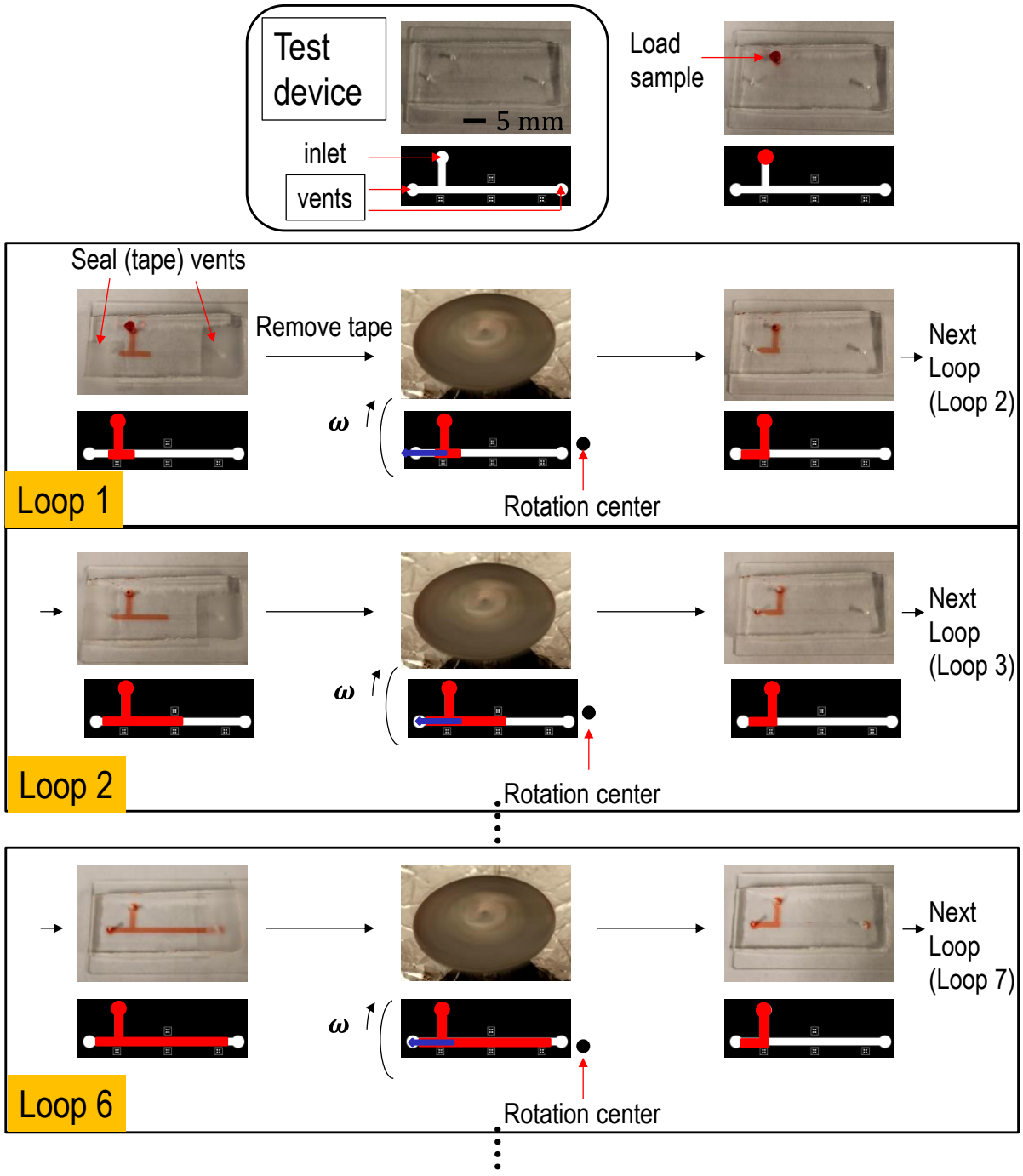

Fig. 3. The sequence images of the sample flow inside the chamber using vacuum-driven force and centrifugal-driven force. The device has vents, which vacuum driven flow and centrifugal driven flow can be switched by sealing/ unsealing vents. After sample was loaded into a degassed device, vacuum-driven force and centrifugal force was applied reciprocally to pull and push sample inside chamber. By vacuum-driven force the sample would fill the chamber, by centrifugal-driven force the sample would push back to the left side of the chamber, which is the far side from the center of rotation. Thus sample would be push and pull inside the chamber once (one set of loop). By continue the process, the sample can be recirculate inside the chamber.