A MICROMACHINED CLOG-FREE EJECTOR FOR LONG-TERM RELIABLE HYDROGEL PRINTING

Riberet Almeida* and Jae Wan Kwon

Department of Electrical and Computer Engineering, University of Missouri, Columbia, MO, U.S.A.

ABSTRACT

This paper presents a novel technique for long-term reliable hydrogel printing by employing a thin liquid-film based microfluidic valve mechanism. Common and major clogging problems of microfluidic ejectors by various bio-fluids such as hydrogels and oligonucleotides are very well known. By employing electrowetting technique to drive a thin liquid film of oil, which is immiscible to the hydrogels to be printed, an effective microfluidic valve mechanism has been implemented. Based on the new microfluidic valve technique, we have successfully demonstrated very reliable printing of hydrogels (carrying media of tissue cells) without any clogging problem. While the nozzle was clogged completely in an hour under ambient condition without any protection from the microfluidic valve, it shows clean and fresh printing even after 72 hours of standby time due to the discontinued evaporation by closing of the thin liquid-film based microfluidic valve.

INTRODUCTION

In recent years, contactless and controlled microfluid delivery techniques by ejection have been widely used in many applications in chemistry and life sciences. Many of continuous or drop-on-demand ejections of the bio-fluids have been achieved by utilizing various piezoelectric-based microfluidic ejectors due to no-heat requirements [1][2]. Especially, printings of cells and biomaterials have been broadly used to generate three-dimensional tissue constructs. Former attempts in tissue printing offer the ability to deposit cells and other biomaterials in a rapid layer-by-layer fashion for creating tissue structures with the aid of gels [3]. Hydrogels are widely being used in tissue printing because they provide an aqueous and porous environment for cells' health and help retain cells' shape after printing. Protein and carbohydrate based polymers are used in the design and fabrication of organic scaffolds in skeletal tissue engineering [4][5].

Despite lots of the impressive results of organ printing, nozzle failure due to clogging is one of the biggest problems that need to be circumvented before such a process can gain widespread acceptance [6-8]. Most of the nozzle-based ejectors are prone to clogging due to fast evaporation and accumulation of the biofluid and biomaterials at the nozzle tip. In general, clogging problem is caused when the ink fluid (the solution to be ejected) is exposed to the air and dried very quickly. Similarly, the polymers in the aqueous solutions begin to crosslink and transform into hydrogel networks when the solution's evaporation occurs. To avoid the known problems above, we have invented a microfluidic ejector equipped with a microfluidic valve having a similar protective mechanism for evaporation control as seen in the human eye. An oily lipid layer protects tear evaporation avoiding dryness of the eye balls very effectively. As seen in nature, a liquid is a good substance to control another liquid. For example, when oil (non-volatile and immiscible liquid with water) covers a water surface, a thin layer of oil is formed on the top and isolates water completely from direct air exposure. Thus, we have employed the thin oil layer as a microfluidic valve to control the evaporation speed of

the ink fluid (hydrogel) as illustrated in Figure 1. The thin oil layer provides a complete separation and protection layer at the bio-fluid/air interface in the nozzle.

Figure 1. Cross-section view of liquid-film based microfluidic valve. It can open and close the ejector nozzle by using electrowetting technique.

THEORY

In this device, an immiscible liquid droplet which acts as a protective sheath is actuated over the ejector nozzle by using the electro-wetting mechanism. The actuation force is caused by the change in contact angle or wettability of the surface which in turn is caused by the change in the solid liquid surface tension γ_{SL} that results in the droplet motion. The change in γ_{SL} occurs on the hydrophobic Cytop layer. The relationship between the applied voltage across the electrode and the liquid and the change in surface tension is given by the Lippmann equation:-

$$\gamma = \gamma_0 - \frac{1}{2} CV^2 \quad (1)$$

where γ_0 is the surface tension when there is no voltage applied across the liquid-electrode interface and 'C' is the capacitance per unit area of the dielectric layer. In electrowetting-on-dielectric (EWOD) devices, γ needs to be interpreted as γ_{SL} , the surface tension in the solid-liquid interface.

The total electrostatic energy $E(x)$ of the system can be estimated as

$$E(x) = \frac{1}{2} C_{eqv}(x) V^2 \quad (2)$$

where C_{eqv} is the equivalent capacitance arising from the different energy storage elements in a typical electrowetting system employing a sandwiched droplet between electrodes. The actuation force acting on the sandwiched droplet during transition can be estimated by the positive derivative of this energy function as follows;

$$F_{act}(x) = \left(\frac{dE(x)}{dx} \right)_V \quad (3)$$

FABRICATION AND EXPERIMENT

The device consists of three main components (microfluidic valve, reservoir and ejector), which are prepared separately and then integrated into a single system. The microfluidic valve can be built on the top surface of the microfluidic ejector with an indium tin oxide (ITO) coated glass as a cover substrate. Thus, a thin and immiscible liquid droplet can move and slide over the nozzle of the ejector to provide the function of microfluidic valve as illustrated in Figure 1. An orifice in the cover substrate was vertically aligned very well with the ejector's nozzle so that the cover will not disturb the clean ejection path. The ITO served as a transparent ground electrode for the valve. The orifice in the cover substrate was fabricated by using laser micromachining.

Figure 2. Fabrication Steps outlined

As briefly shown in Figure 2, fabrication of the microfluidic ejector starts by RIE patterning the backside of a 3 inch silicon nitride coated wafer for silicon etching in a solution of potassium hydroxide (KOH). The KOH etching was done in a 45% solution at 80°C. It forms thin diaphragms on the other side of the wafer. The wafer is then gently sonicated in deionised (DI) water for a minute to break away the thin silicon nitride diaphragms to make open-tip nozzles for the ejectors. A 1mm-thick lead zirconate titanate (PZT) substrate has been used as a

driving piezoelectric crystal. Then, the microfluidic valve was built on the front side of the ejector. A 5µm thick parylene deposition was followed by a 0.5µm thick aluminum deposition and patterning for the electrodes of the microfluidic valve. The underlying parylene layer was used as an insulating layer to prevent electrical breakdown through the silicon nitride since the thin oil film needs relatively high voltage to drive. Cytop was then spin-coated on the cover substrate and cured on a hot plate at 150°C for 10min to render the surfaces hydrophobic. The cover substrate was placed over the ejector with a 150µm gap formed by SU8 spacer. For the last step, a 250µm thick SU-8 spacer layer was added between the bottom of the silicon substrate and PZT crystal to create a large reservoir and to accommodate an inlet tube (Zeus PTFE 190µm OD).

To begin the ejection experiment, the microfluidic ejectors were first primed with DI water and tested for finding successful ejection conditions by varying many different ejection parameters such as the driving pulse width and amplitude. Once we confirmed good ejections, the ejector was filled with a freshly prepared aqueous solution, which is one of naturally occurring hydrogels such as gelatin, agarose and collagen. All these solutions were tested with the ejectors employing the liquid-film based microfluidic valve. Fluorescent dyes such as rhodamine 6G (R6G), acridine and alcian blue were added to the hydrogel solutions to aid visualization of the ejected droplets onto coverslips in using fluorescence microscopy. Once the ejectors were filled with the hydrogel solutions, a 700mV_{pp} sinusoidal signal (2.1MHz) was fed into an RF switch (Mini-circuits, ZX80-DR230-S+) which was modulated with a 29.97Hz signal having a pulse width of 40µs and amplified. The microscope was positioned properly for capturing side view of the vertical ejection of hydrogels and the horizontal movement of the liquid-film valve (oil droplet) simultaneously. In the microfluidic valve, silicone oil was chosen as valve-core material since it is one of the most inert and immiscible liquids with many water-based bio-fluids. Silicone oil has a dielectric constant of 2.71 and a dielectric strength of 390KV/100mil. In addition, the silicone oil has superior properties over various liquid metals such as mercury and galinstan, which have serious toxicity and oxidation problems, respectively. However, the silicone oil requires relatively high electrowetting actuation voltage for droplet movement as shown in the result section.

RESULTS

We have operated the ejector containing DI water under various operating conditions (e.g. frequency and amplitude of the driving ac signal), and found three distinct modes of ejection; (1) no ejection (idling mode) (2) ejection of individual droplets (also known as drop on demand (DOD) mode) and (3) ejection of a continuous jet (jet mode). The microfluidic ejector runs at its resonance frequency of 1.9-2.2MHz to generate acoustic pressure gradients to eject small droplets or continuous streams. The formation of capillary waves at the liquid-air interface located at the nozzle tip plays a critical role in determining which modes dominate resulting in either DOD or continuous jetting modes [9].

As shown in Figure 3, the movement of the thin silicone oil droplet for the microfluidic valve operation was precisely controlled by using the electrowetting technique. The nozzle was completely closed when the thin liquid-film was actuated

with a voltage of 400VDC and moved over the nozzle area. In addition, the microfluidic valve was kept open while the microfluidic ejector was running as shown in Figure 4.

Figure 3. Images show that a thin liquid (silicone oil) film valve opens and closes the ejector nozzle by using eletrowetting technique.

Figure 4. Photo taken by using strobing white LED shows an ejected jet (about 50µm in diameter) passing through the top cover orifice

Since the silicone oil droplet and the hydrogel solution are immiscible with each other, no material exchange has been observed across the liquid-liquid interface. It was observed that for any given ejector, the nozzle ejecting the hydrogel solution completely clogged within a few hours when the valve was left open under ambient conditions and failed in any further ejection. On the other hands, a good quality of ejection was continuously achievable even after a standby period of 72 hours, when the nozzle was closed and protected by the liquid-film based microfluidic valve. The evaporation speed of ink fluids (water and hydrogels) and nozzle condition was summarized in Table 1. We believe that this technique can be extended to a wide range of micro-engineered hydrogels that are currently used in tissue printing.

As can be seen in Figure 5, fluorescence microscopy of the glass coverslips onto which the hydrogels were ejected revealed droplets of different sizes including larger agglomerates

of greater than 500µm. Size of single ejected droplets ranged between 10-30µm.

All ejection tests were repeated after every few hours to check the durability of the hydrogel ejectors by closing the microfluidic valve between tests thereby preventing evaporation.

Figure 5. Ejected hydrogel(a-d:gelatin, e,f:collagen) with Rhodamine 6G & Acridin orange on glass slides using Fluorescence microscope.

Larger sized features as seen in every sub image of Figure 5 were due to the overlap of single ejected hydrogel droplets. Once ejected from the nozzle and upon reaching the target coverslip, the ejected hydrogel droplets began to crosslink at a faster rate due to the loss of liquid content through evaporation which is similar to the drying mechanism as seen in commercial inkjet printers. Also since no crosslinking agents that could otherwise lead to instantaneous hydrogel crosslinking were used on the substrate, overlapped ejected droplets simply coalesce into larger diameter features.

Further imaging and surface characterizations of the substrates onto which the hydrogel droplets are ejected would be required to determine the vertical growth of overlapped droplets. Since during these tests neither the substrates nor the piezo ejectors were connected to X-Y manipulation stages, ordered placement of ejected hydrogel droplets in defined paths as seen in the printing of 3D hydrogel scaffolds could not be achieved. Future testing using this technology would involve the use of manipulative stages for printing pre-defined hydrogel patterns as used in tissue printing setups elsewhere. At this point, further improvements in the microfluidic valve in terms of fabrication and material selection for immiscible liquids is being looked into which could possibly lead to a higher ejection quality.

Table 1. Evaporation speed data when the liquid-film based microfluidic valve is opened or closed.

Liquid-film valve (silicone oil)	Evaporation time of ink fluids stored in the ejector	
	D.I. water	Hydrogels
Close	N/A (> 30 days)	N/A (>72 hrs)
Open	25 min	Dried and Clogged in 1hr

CONCLUSIONS

We have successfully demonstrated very reliable printing of hydrogels for a long time without any clogging problem in the microfluidic ejector. Electrowetting technique was used for the actuation mechanism to open and close the fluidic valve. The liquid-film based microfluidic valve built on the ejector using an environmentally friendly immiscible fluid was proven to be a very effective way to prevent evaporation of various volatile fluids from the air-liquid interface at the nozzle of the microfluidic ejectors. This mechanism could have high impacts on many applications requiring evaporation controlled ejection processes such as various inkjet-based printers including bio-ink printers for tissue printing.

ACKNOWLEDGEMENTS

Authors would like to thank Dr. Luis Polo-Parada in the Department of Biological Engineering for his help in fluorescent imaging. This material is based upon work supported by the National Science Foundation under Grant No. ECS0901618.

REFERENCES

[1] H.B Hsieh et al; "Ultra-High-Throughput Microarray Generation and Liquid Dispensing Using Multiple Disposable Piezoelectric Ejectors" J Biomol Screen 2004; 9; 85

[2] J.W. Kwon, H. Yu, Q. Zou and E.S. Kim "Directional droplet ejection by nozzleless acoustic ejectors built on ZnO and PZT" J. Micromech. Microeng. 16 (2006) 2697–2704

[3] S. Moon, S.K. Hasan, Y.S. Song, F. Xu, H.O. Keles, F. Manzur, S. Mikkilineni, J.W. Hong, J. Nagatomi, E. Haeggstrom, A. Khademhosseini, U. Demirci "Layer by Layer Three-dimensional Tissue Epitaxy by Cell-Laden Hydrogel Droplets" Tissue Engineering Part C: Methods

[4] N. Fedorovich, J. Alblas, J.R. de Wijn, W.E. Hennink, A.J. Verbout, and W.J. Dhert, "Hydrogels as Extracellular Matrices for Skeletal Tissue Engineering: State-of-the-Art and Novel application in Organ Printing" Tissue Engineering Volume 13, Number 8, 2007

[5] D. Ben-David, T. Kizhner, E. Livne, S. Srouji "A tissue-like construct of human bone marrow MSCs composite scaffold support in vivo ectopic bone formation" J Tissue Eng Regen Med 2010; 4: 30–37

[6] A.Khademhosseini, R.Langer; "Microengineered hydrogels for tissue engineering." Biomaterials 28: 5087–5092.

[7] A. Khademhosseini, Y. Du, B. Rajalingam, P.Joseph "Microscale Technologies for Tissue Engineering;"

[8] V. Mironov, T. Boland, T. Trusk, G. Forgacs and R.R. Markwald, "Organ printing: computer-aided jet-based 3D tissue engineering." Trends Biotechnol.21, 157–161 (2003)

[9] Eric R. Lee Microdrop generation

CONTACT

Riberet Almeida; Ph: +1 573-673-7735;
rab5b@mail.missouri.edu