

A DROPLET BASED MULTI-DRUG SCREENING SYSTEM CONTROLLED WITH ELECTROSTATIC MICROVALVES

Ender Yıldırım¹, Ebru Özgür², Haluk Külâh^{2,3}

¹Çankaya University, Mechanical Engineering Department, TURKEY

²METU MEMS Research and Application Center, TURKEY and

³METU, Electrical and Electronics Engineering Department, TURKEY

ABSTRACT

This paper presents a droplet-based drug effect analysis system utilizing electrostatically-actuated normally-closed microvalves to screen the effect of multiple drugs on a single type of cell. Proposed system minimizes the need for off-chip equipment by utilizing parylene based electrostatic microvalves. Prototypes of the system were fabricated and tested using colored DI water and 3 μm diameter micro beads, emulating drugs and cells respectively. During the tests, micro beads could be successfully entrapped in 137 pl droplets. Tests carried out with yeast cells also yielded successful encapsulation of the cells. It was shown that, switching between the drugs could be achieved by applying 200 V dc to operate the microvalves.

KEYWORDS: Multi-drug screening, parylene, microvalve, electrostatic

INTRODUCTION

Droplet-based systems are increasingly preferred in drug effect analysis, since picoliter size droplets solve the scaling problems observed in conventional methods (e.g. intermolecular effects and limited diffusion rate), thus improving the throughput of the analyses [1]. High throughput drug effect analysis is especially important in personalized therapies, such as cancer treatment. Traditionally, trial and error method is used to determine the most suitable drug for the patient. However, finding the most effective therapy generally takes time and may sometimes be fatal. Therefore, development of a fast, *simple-to-use drug effect analysis system with multi-drug screening capability* is important to determine the most effective therapy in a short period.

Several PDMS-based droplet-based microfluidic platforms have been proposed so far for drug screening [2, 3]. Brouzes et al. proposed a droplet based drug analysis system to screen cytotoxic effects of a drug library on U937 monocytes [2]. Similarly, Gong et al. proposed a droplet-based system to screen the effect of Cytochalasin-D on MDA-MB-231 breast cancer cell [3]. Although usefulness in monitoring the drug-effect is proven, fluid evaporation during long incubation periods and complicated off-chip fluidic control in case of multiple drugs restrains PDMS-based systems in practical applications.

In this paper, a **parylene-based droplet microfluidic system for multi-drug screening** is presented. Using parylene-C as the structural material solves the permeability problem [4], and reduces the need for off-chip control equipment by enabling on-chip integration of electrostatically-actuated microvalves. The device is capable of screening two drugs simultaneously with a precise on-chip fluidic control.

THEORY

Figure 1 shows the structure of the proposed system. The system utilizes normally closed electrostatic microvalves [5] to control the drug flow and a normally open electrostatic microvalve to control the oil flow (Figure 1.a). As actuation voltage is applied on one of the normally closed microvalves, the diaphragm collapses on the bottom of the actuation chamber allowing the corresponding drug flow underneath the valve seat (Figure 1.b). The drug flows through the Y-junction, where it meets with the cell-suspension. Flow of the resulting mixture is broken by the carrier fluid at the T-junction to generate droplets with the cells entrapped inside. In order to have an active control on the droplet size, a normally open electrostatic microvalve [6] is utilized. As actuation potential is applied on the microvalve, valve diaphragm collapses on the bottom of the channel resulting in a leakage flow at the periphery of the microvalve. As the oil flow is throttled by the microvalve, larger droplets are generated at the T-junction. Hence, it becomes possible to generate droplets of controlled volume and chemistry. Generated droplets flow through a serpentine channel for incubation, where the drug activity is monitored.

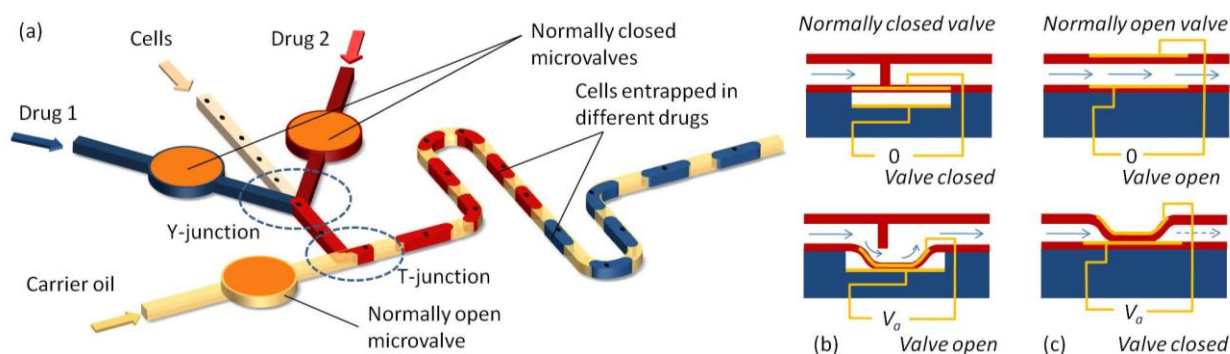


Figure 1: Operation principles of (a) the proposed system, (b) normally closed microvalve, and (c) normally open microvalve.

Flow characteristics of the microvalves, droplet generation at T-junction, and the microchannel dimensions affect the operation of the system. Flow characterization of the normally closed and normally open microvalves has recently been reported [5, 6]. Simulations are done to determine the droplet generation characteristics at T-junction (Figure 2). In order to determine dimensions of the microchannels, an equivalent electrical circuit model is used (Figure 2). During analysis, the system is simulated for two drugs only. However, increasing the number of drugs to be screened would only increase the number of normally closed valves on the system, which does not introduce a considerable operational complexity.

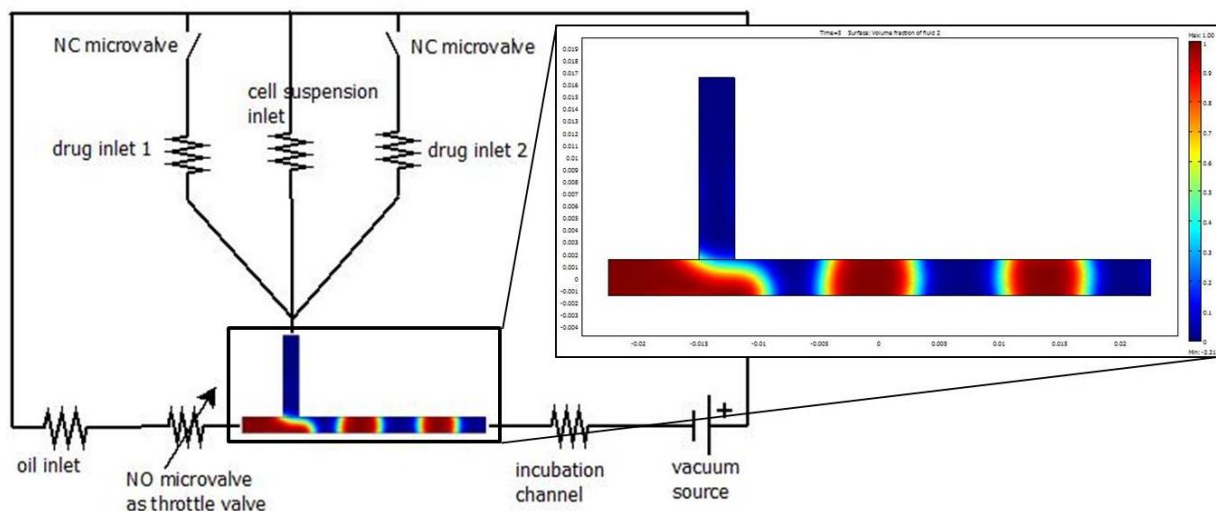


Figure 2: Electrical circuit equivalent of the system used to determine the channel dimensions and simulation of droplet generation at T-junction.

EXPERIMENTAL RESULTS

The prototype (Figure 3.a-b) was fabricated using five-mask process [5]. Fabricated devices were tested with colored DI water, micro-bead suspension, and silicone oil emulating the drugs, cell suspension, and the carrier fluid, respectively. For the micro-bead suspension, a bead solution carrying 3 μm diameter polystyrene beads with 1.68×10^7 particles/ml concentration was used. With this concentration, one single bead would be entrapped in a 120 pl droplet. To realize a bubble-free flow, 100 mbar vacuum was applied at the outlet. In order to supply the test liquids, reservoirs were mounted at the inlets and 20 μl liquid was dispensed in each reservoir. For electrical connection, an external 2-way switch was used to apply actuation voltage selectively to the microvalves (Figure 3.c).

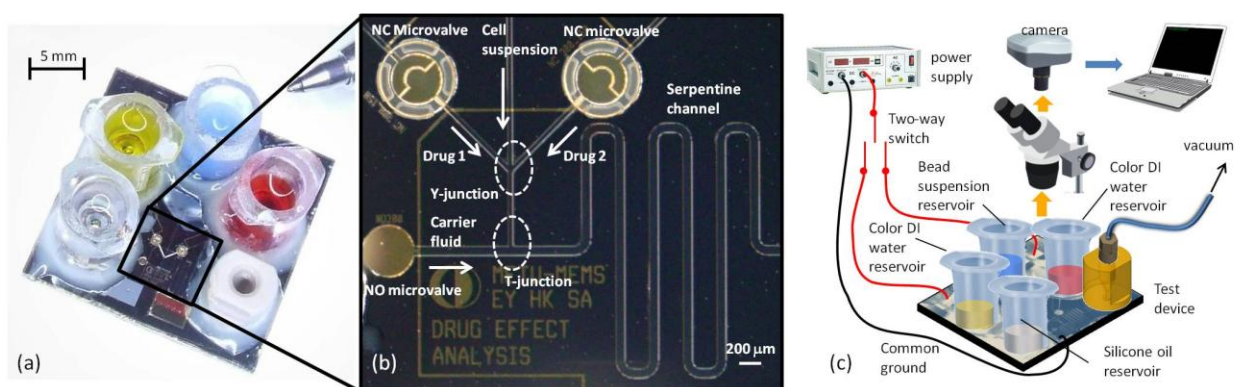


Figure 3: (a) Fabricated multi-drug screening systems and (b) its close-up view showing the components. (c) Set-up used for testing the system.

In order to demonstrate drug-switching, actuation potential of 200 V dc was applied to the microvalves selectively. During the test, droplets with different colors were generated at the T-junction upon actuation of the corresponding microvalves (Figure 4.a-b). It was also observed that, individual beads could be entrapped in these monodisperse droplets of 137 pl with 0.34 pl deviation (Figure 4.c). Cell encapsulation was also tested with live yeast cells. The tests show that yeast cells could also be entrapped in the generated droplets (Figure 4.d). However, it is observed that there is more than a single cell in some droplets due to cell aggregation. During the tests, normally open microvalve is not used for controlling the droplet size. However, tests with the normally open valve show that the droplet size can be controlled with 0.4 pl/V sensitivity.

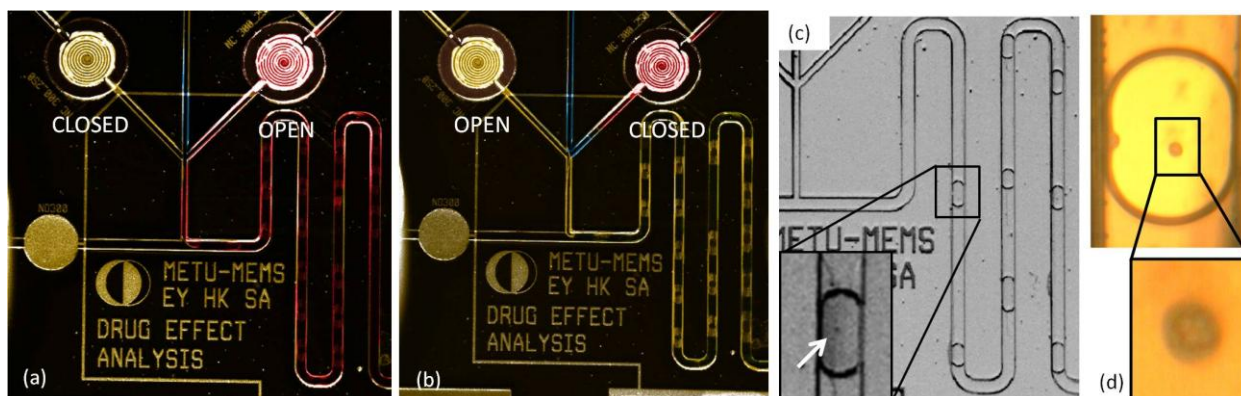


Figure 4: (a-b) Open and closed states of the microvalves. Figures are colorized for clarity. a) Droplets in serpentine channel and microbead entrapped in a droplet. (b) Yeast cells entrapped in the droplet. Due to cell aggregation, more than a single cell is observed in some of the droplets.

CONCLUSION AND DISCUSSION

In this study, a droplet based multi-drug screening system is proposed. The system is controlled by normally closed electrostatic microvalves to manipulate the drugs. The system is implemented to screen two drugs on the same chip. Prototypes are tested with color DI water as drug and microbeads as cells. It is shown that, droplets of different chemistry can be generated and particles can be entrapped in these droplets using the proposed system. The system is also demonstrated to entrap yeast cells in the droplets.

Fabricated prototypes allow screening of only two drugs on the same chip. However, number of the drugs to be screened can be increased by only increasing the number of the microvalves, which does not add a considerable complexity in terms of off-chip equipment and connections.

During the tests, normally closed microvalves were operated as on/off switches. However, by tuning the actuation potential only, flow rates of the drugs can be modified. Hence it can be possible to generate a mix of drugs, which is especially critical in combinational drug therapies.

Testing of the prototypes prove the feasibility of the system in terms of cell encapsulation and drug control using electrostatic microvalves. Viability of the encapsulated cells inside the droplets can be followed using fluorescent stains selective for dead or live cells. Change in shape, size and fluorescent intensity of the cells will indicate the drugs' effects on cells.

ACKNOWLEDGEMENTS

This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK) under Grant Number 111E110.

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CONTACT

Ender Yıldırım +90 312 233 1304 or endery@cankaya.edu.tr