Characterization of Droplet Trapping Structures

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Abstract: **Trapping of the cell-laden microgel droplet has been a topic of interest due to the possibilities of static investigation. However, on-chip bioassay, such as viability analysis, has not been widely reported. This paper specifies the various droplet trapping device designs, where the droplets are generated by the flow-focusing method, and provides test procedures to determine the performance of these devices and estimates their corresponding efficiency. Moreover, it describes the problems faced in fabricating and testing the designs with their potential causes and recommends suitable enhancements. Finally, it proposes alternative designs of trapping structures for future implementations.**

Index Terms **— Flow-focusing Droplet Generation, Microfluidics, Multifluidic Trapping Network**

I. Introduction

In microfluidics, droplets consist of two immiscible phases: biological materials or chemicals dispersed in a continuous phase medium where biological materials can range from a single cell to a multi-celled organism. Droplets can be used to investigate the behavior of materials encapsulated inside them, act as microreactors or synthesize proteins; several of these applications have been widely reviewed by Shaojiang Zeng et al. [1]. For instance, Weiwei Shi et al. trapped a species of worms, *Caenorhabditis elegans*, in droplets to study the effect of neurotoxin [2]. Consequently, droplet generation devices are designed in tandem with trapping structures to store droplets from several hours to days so that it could be retrieved later.

Various trapping devices had been proposed in the literature. The device proposed by Courtois F et al. consisted of a large reservoir to trap 10^6 droplets of proteins [3] and store for 6 hours to be retrieved later. However, the device could not store the droplets in the same order as its input sequence. Wei-Heong Tan et al. [4] proposed an alternative approach with a number of trapping elements arranged in an array to preserve the sequence of droplets stored. Furthermore, Huebner A et al. proposed a device with C-shaped baskets in a reservoir to trap droplets of cells [5].

Although several of the droplet trapping devices have been widely reviewed in the literature, no studies have been made to characterize their performance. This paper attempts to fill this missing gap by determining their efficiency through various test procedures as described in the later sections. The designs of the droplet generator and multiple trapping structures utilized for the estimation of efficiency are discussed. Moreover, the challenges posed during the fabrication and testing of these devices are identified and their potential reasons are recognized. Additionally, possible solutions to these problems are suggested. Finally, two alternative designs for trapping droplets are proposed for future implementations.

II. Experimental Method

The experimental setup used to carry out the testing is described in Fig. 1. Water-in-oil droplets were used for the experiment. Phosphate Based Saline (PBS) solution was used for the dispersed phase of the droplet and mineral oil with 5 % SPAN 80 surfactant was used for the continuous phase. A generic flow-focusing droplet generator was used for testing all the trapping devices. Water and oil were pumped into the droplet generator with a flow rate of 60 µL/hr and 180 µL/hr respectively using syringe pumps. The generated droplets were trapped by various trapping structures and the testing is carried out using images acquired from a camera fitted to the microscope.

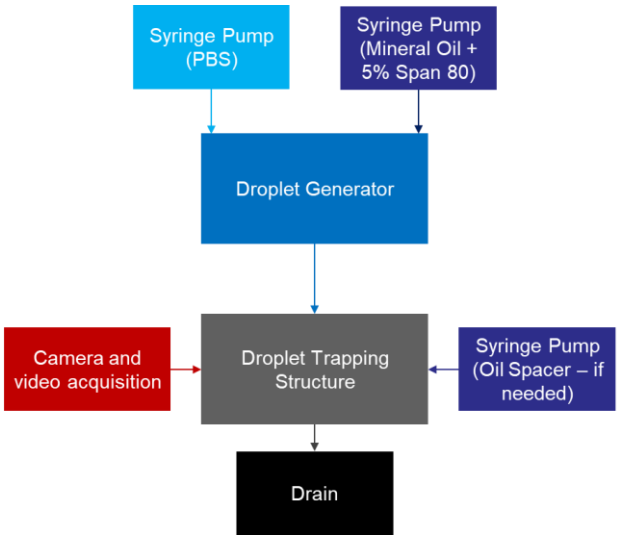

Fig. 1. Experimental setup for the generation of water-in-oil droplets and trapping the droplets

Several methods of droplet generation are available as discussed by Pingan Zhu et al. [6]. In this paper, the flow-focusing method [8] was used due to the ease of controllability of droplet size. In the flow-focusing method, the flow of a dispersed phase through a central channel is pinched off at regular intervals by two symmetric channels perpendicular to the main channel with a continuous phase flowing through them. By controlling the flow rate of the two phases, the size of the droplets was controlled. Fig. 2 is the

flow-focusing water-in-oil droplet generator. The generated droplet is of diameter 90 µm.

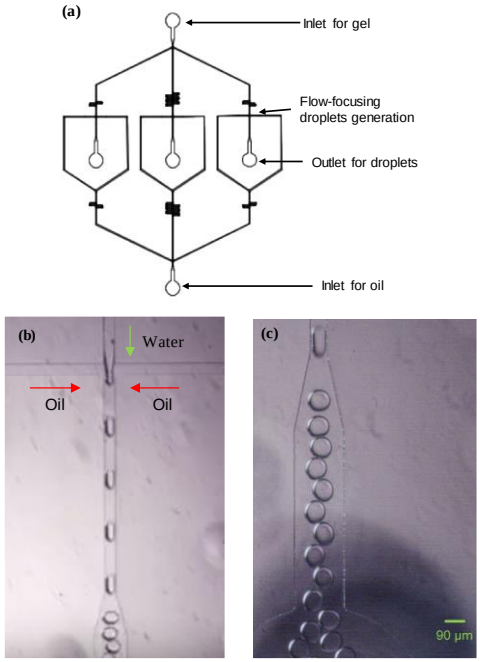

Fig. 2. (a) Flow-focusing droplet generator design with three outlets (b) Flow of water through central channel was pinched off at regular intervals by two symmetric channels perpendicular to the main channel with oil flowing through them (c) Generated droplet was of diameter 90 µm

The layers of all the devices were fabricated using soft-lithography techniques with Poly(dimethyl) siloxane (PDMS) as the material; Sylgard™ 184 was cured with a 10:1 base to curing agent ratio to prepare PDMS layers [19]. The glass substrates for the devices were treated with a 40:1 base to curing agent ratio of PDMS before stacking the PDMS layers.

The following droplet trapping structures were fabricated for testing their performance. The materials and techniques used were similar to the above section.

A. *Big Basket Trapper*

The big basket trapping structure (Fig. 3) consists of an inlet for droplets gradually expanding into a reservoir terminated by a waste outlet to drain the waste oil. The design is similar to the device fabricated by Courtois F et al. [3]. However, pillars, of size 400 µm with a 50 µm gap between each, were added around the periphery of the reservoir to drain waste.

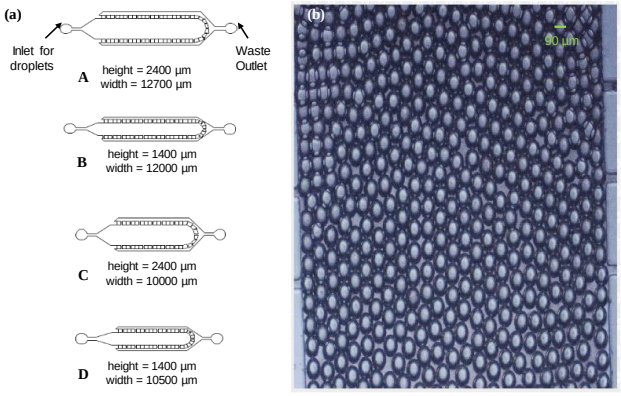

Fig. 3. (a) Big basket trapping structure of various dimensions (b) Droplets trapped by structure C of height = 2400 µm and width = 10000 µm

When the droplets enter the device through the inlet, they were collected in the reservoir while oil seeped through gaps in the pillars and finally, drained through the outlet.

B. *Multifluidic Trapping Network*

Multifluidic Trapping Network (MTN) as in Fig. 4 are widely used in droplet microfluidic applications. The original design as proposed by Wei-Heong Tan et al. [4] was modified to include a T-shaped spacer circuit instead of a T-shaped generator; the spacing between each droplet was controlled by the flow rate of the spacer phase. The device consists of serpentine channels intercepted by circular trapping elements of radius 100 µm. The trapping element is connected with the adjacent channel through a narrow restriction channel of width 30 µm.

The working principle of MTN is described in Fig. 4(c); initially, the droplet sees lesser resistance along the trapping element path than the main channel path due to the inherent construction of the device; as the droplet approaches the restriction channel, the resistance offered by main channel decreases and eventually is lower than the restriction channel resistance; hence, subsequent droplets flow through the main channel and get trapped in sequence in the next set of trapping elements.

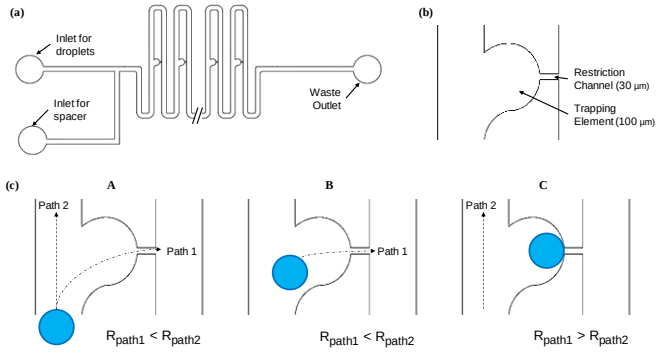

Fig. 4. (a) Multifluidic Trapping Network with 30 trapping elements (Only 4 is shown here) (b) A scaled view of the trapping element of radius 100 µm and restriction channel of width 30 µm (c) Working principle (A) Resistance offered to droplet along path 1 is lesser than path 2 (B) Droplet approaches restriction channel due to lower resistance (C) Resistance of path 2 is lower compared to restriction channel resistance. Subsequent droplets flow through path 2

C. *Rail Guided Micro-Well Trapping Structure*

Rail guided micro-well trapping structure consists of a large reservoir with trapping wells at various points [8]. The inlet side of the device has a number of rail structures to guide the droplets into the wells as in Fig. 5. The maximum width of the rails is 95 µm and the width of the well is 250 µm.

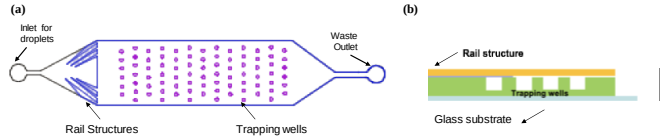

Fig. 5. (a) Rail guided micro-well trapping structure with rails of 95 µm and well size of 250 µm (b) Side view of the trapping structure

When the droplets flow into the device, they are guided by the rails to be trapped into the wells of comparable size. Thus, a larger number of droplets are stored in these wells.

D. C-Shaped Basket Trapper

This trapping structure as in Fig. 6 consists of several baskets which are inverted C-shape surrounded by pillars of size 400 µm with a 50 µm gap between each [5,10]. The C-shaped pillars act as guiding structures as well as storing elements.

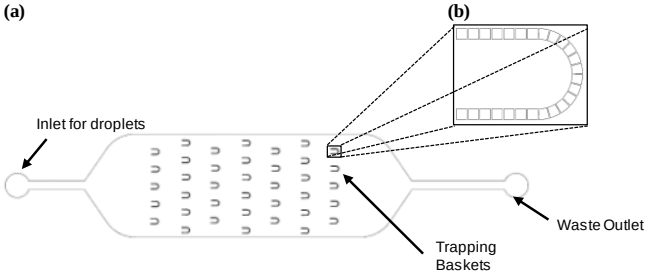

Fig. 6. (a) C-shaped basket trapper with baskets (b) A zoomed in view of the basket surrounded by pillars of size 400 µm with a 50 µm gap between each

III. Results

The droplets generated by the generator were introduced into each trapping device and tested for their performance. The test procedure and the results are discussed below.

A. Testing of Big Basket Trapper

The testing was performed for two trials of each structure A-D in Fig. 3(a). The number of droplets stored in the reservoir across various rows was counted by visual inspection. Since the droplets stored can be squeezed, the mean value of several rows (8 rows) at various distances from inlet was calculated to offset the error. Using the mean, the trapped distance for each structure was calculated as T_d = mean x D, where D is the droplet diameter (= 90 µm.) The actual width of the device was determined at the design step as A_d. Finally, the percentage of efficiency was calculated as $\frac{T_d}{A_d} \times 100$

Table 1 shows the efficiency of various structures and the values used to obtain the results. It can be observed that the minimum efficiency achieved in this device was 90.75% and maximum efficiency was 98.57%.

Structure No.	Actual device width (A_d) µm	Mean value of droplets trapped per row	Trapped Distance (T_d = mean x D) µm	Efficiency % ($\frac{T_d}{A_d}$ x 100)
A	2400	24.2	2178	90.75
B	1400	15.33	1380	98.57
C	2400	24.67	2220	92.50
D	1400	14.17	1275	91.07

Table 1. Efficiency of big basket trapping structures A-D of Fig. 3(a). D is the diameter of the droplet = 90 µm

B. Testing of Multifluidic Trapping Network

MTN designed has a total of 30 trapping elements (= n_{actual}). The number of droplets stored in all the trapping elements was counted as $n_{trapped}$ = 30. Then, the percentage of efficiency is given by $\frac{n_{trapped}}{n_{actual}} \times 100 = 100\%$ Thus, a 100% efficiency was achieved in both the trails of the device. Fig. 7. shows the trapping of droplets by the device.

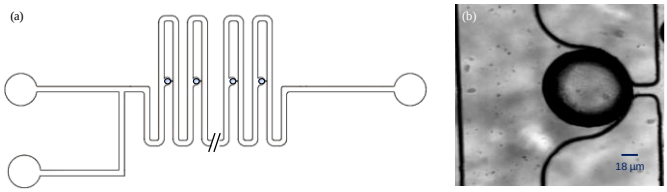

Fig. 7. (a) Representation of droplets stored in Multifluidic Trapping Network (b) Droplet trapped in one of the trapping elements

Rail guided micro-well trapping structure failed due to lower well height than the diameter of the droplet. As a result, the droplets escaped from the well structures. A potential solution to solve this issue is to increase the height of the well to at least 1.2 times the radius of the droplet. Furthermore, the C-shaped basket trapper also failed due to the lower rigidity strength of the pillars surrounding the basket. The droplets bent and squeezed through the pillars and hence, were not trapped in the baskets. This could be prevented by baking PDMS for a longer time or using a higher amount of curing agent than the elastomer base to increase the elastic modulus of pillars [11].

IV. Discussion and Future Work

From the testing, it can be noted that the Microfluidic Trapping Network showed superior performance compared to the big basket trapper. Although the number of droplets that could be stored in MTN for the same size of the big basket trapper is limited by the channel and trapping size, the sequence of droplet flow is preserved.

During fabrication and testing, several challenges aroused limiting the performance of the trapping structures. For instance, the extremities of big basket trapper near the outlet had air bubbles due to an increase in flow rate near the ends. The possible solution is to gradually decrease the width of the extremities with a reduced flow rate of the continuous phase. Moreover, wetting the device with the continuous phase prior to the experiment may be helpful. As can be observed in Fig. 8, some of the droplets squeezed through the gaps between the pillars or through the restriction channel in MTN because the continuous phase pressure was greater than the Laplace pressure exerted by the walls of the droplets [12]. This could also be prevented by reducing the flow rate of the droplets and decreasing the size of the gap. Furthermore, some of the droplets coalesced or broke up due to a lesser surfactant ratio which can be reduced by increasing the surfactant ratio to stabilize the droplets [13].

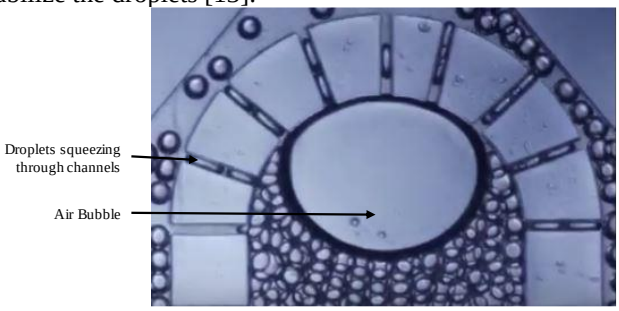

Fig. 8. Air bubbles were formed in the extremities of big basket trapper near the outlets. Also, droplets squeezed through the gaps in the pillars.

An alternative approach to store droplets is proposed as in Fig. 9. The design consists of a big reservoir as in big basket trapper interspersed with vertical wells. Unlike Fig. 5, these

wells have a hemispherical cross-section with a vertical cut at one end; the circular profile provides a means to guide the droplets into the well gradually without breaking their structure. Initially, the operation typically involves trapping the droplets in the structure keeping it horizontally parallel to the ground as in Fig. 9(b). The retrieval of droplets is achieved by flipping the device such that the trapped droplets fall from their respective wells as in Fig. 9(c).

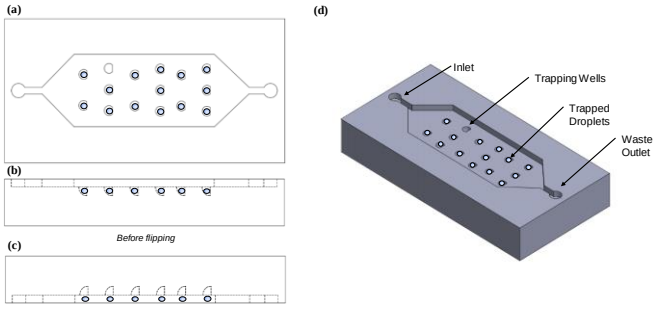

Fig. 9. Proposed design for trapping droplets with micro-well structures (a-b) Droplets trapped in the wells before flipping the device (a) Top view of device with droplets trapped in several wells (b) Side view (Note the circular profile near the inlet side of the well) (c) After flipping the device, the droplets are retrieved from the wells. (d) An isometric view of the device with radius of wells 1.2 times the radius of droplet.

Another design proposed for trapping droplets is shown in Fig. 10. The design is similar to Fig. 9 with the addition of guiding ridges at one end of each slot. The guiding ridges partially trap droplets of a particular size, which rolls into the hemispherical wells with the flow of continuous phase; the working principle is as explained in Fig. 10(c-d) [14]. This proposed design is expected to be capable of filling several trapping elements efficiently due to the presence of guiding ridges and to have less droplet break-up possibility due to the gradual increase in the radius of wells.

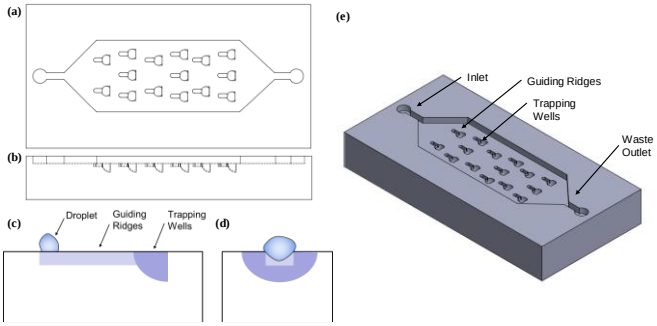

Fig. 10. Another proposed design for trapping droplets with wells and guiding ridges (a) Top view of design (b) Side view (c-d) Working principle of the design for one of the trapping elements (c) Side view of a well with a droplet trapped in the guiding ridge (d) Front view of one of the trapping elements. Note the droplet trapped in the ridge (e) An isometric view of the device. Radius of each slot is 1.2 times droplet radius (108 µm) Width of guiding ridges is 0.6 times radius (54 µm) and height is 0.5 times radius (45 µm)

V. Conclusion

The various droplet trapping designs were studied and their respective testing procedures were enumerated. Consequently, the test results were discussed wherein the Multifluidic Trapping Network showed a 100% efficiency. Some of the challenges and solutions to prevent them were also discussed. Finally, alternative designs to trap droplets were proposed for future works.

VI. Author Contributions

R.R. and A.V helped SungJin Kim in fabricating the devices. A.V. set up the experiment for droplet generation. A.V. and R.R. collected the data for big basket trapper. R.R. performed the computations of efficiencies for big basket trapper. A.V. verified the results. R.R. and A.V. tested the performance of MTN and calculated its efficiency. R.R. performed the experiment of the C-shaped basket structure with the help of SungJin Kim. R.R. conceptualized and designed the proposed alternative designs in SOLIDWORKS. A.V. provided the solution to the air bubbles issue. R.R. provided the solutions to the issue of droplets squeezing through pillars and to the failed trapping devices. All authors discussed the results and contributed to the final manuscript.

VII. Acknowledgment

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