Bioengineering Research

Label-free Impedance-based Detection of Encapsulated Single Cells in Droplets in Low Cost and Transparent Microfluidic Chip

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Abstract

This study reports a label-free impedance-based detection of cells inside picoliter droplets. The fabricated fully PMMA micro-chip consists of a double-T junction channel in order to generate same sized droplets for increasing volume fraction and pairs of gold-sputtered differential coplanar electrodes for impedimetric analysis. When same-sized droplets containing cells pass through differential electrodes due to an instant change in electrical impedance, output voltage encounters a significant peak. First we succeeded in generation of same sized droplets. According to our output signal data, an acceptable label free electrical detection of cells inside droplets is obtained. This detection was due to differentiates between empty and non-empty droplets. Moreover we stepped forward in obtaining additional data of cells encapsulated in droplets like number, morphology and size. Furthermore, this electrical approach is in line with development of integrated LOC devices that deals with detection of cells. In this paper, we detected Jurkat cells with an average diameter of 13 µm using less than 80µm droplets by the impedance detection technique in the microfluidic channels. When a droplet passes through the electrodes, the amplitude of output voltage increases. Results showed that droplets with encapsulated Jurkat cell have 1.24% higher output voltage amplitude (320 mV) comparing with empty droplets (260 mV). Our simple fabricated transparent chip has the capability of detection 3 to 4 droplets per second containing living cells through label free characterization according to the size of cells inside droplets.

Keywords: Droplets Encapsulation, Micro Impedance Cytometry, Cell Detection, Droplet Counting

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1. Introduction

Microfluidics generally incorporate technology that uses microstructures to manipulate and precisely Simple detection of micro-particles and encapsulated cells in droplet is an important research field in microfluidics systems. Counting micro particles and different cells in medical analysis has been considered as an important issue in recent century [1]. Miniaturization and development of point of care systems led to very cost effective, low sample volume usage and also prevents time consuming processes. Thus, microfluidic systems are a suitable choice for noted purposes [2]. Electrical impedimetric detection is one of the most widespread methods in microfluidic based bioanalysis systems. The method is label free, non-invasive [3], high throughput [4], cost effective and also huge data recording is unnecessary. Although fluorescent labelling provides fast and accurate detection of cells and micro-particles, but this technique is time consuming, laborious and expensive [5].

In many applications such as cell separation [6], micro sensors [7-10], lab-on-chips [11], and cell counting devices [10], polymers such as Polydimethylsiloxane (PDMS), poly(methyl methacrylate) (PMMA), and SU-8 are in favour. Among them, PMMA as a transparent, thermoplastic material [12] with proper mechanical and chemical characteristics (like hydrophilic behaviour) is suitable for micro-devices [13-15].

In addition droplet based microfluidics is going to be an appropriate platform for cell analysis and experimentations such as polymerase chain reactions [16], cell printing technologies [17], cell based assays [18] and proteome analysis [19]. Micron sized Droplets help cell analysis by reducing ambient parameters interfering in main reaction through isolating chemicals or bio materials physically. Smith et al [20] used the double-T junction geometry for droplet generation, same as what we used in this paper, but they used it for detection of proteins using nanoelectrospray ionization mass spectrometry. Their method had a droplet collection and storage part and oil was for spacing between droplets. They used a prepared droplet generator to form symmetrical droplets. They mentioned that existence of empty droplets is one of the drawbacks of this method and consequently tried to propose a method to address this issue [20]. However, best flow rates were not investigated in their microsystem. Most label free methods utilize cell alignment in microfluidic channels which is a very challenging process [21,22], but our method doesn't need cell alignment. Also the low cost fabricated chip in this paper has the potential to be used in low frequency characterization of living cells according to their size and morphology, simply. In what follows fabrication processes, stages to make a well-shaped droplet and final results have been discussed.

2. Materials and Methods

2.1. Chip fabrication

Two transparent 3 mm thick poly methyl methacrylate sheets were used for micro-electrodes deposition and micro-channel patterning. A laser machined PMMA sheet with thickness of 1mm was used as a mask for deposition of gold layer on transparent PMMA layer.

In the first step a commercial CO2 laser system (Universal PLS 6.75, maximum highest scan speed of 300 mm⁄s) was utilized to pattern micro channel layouts onto the PMMA sheets. Channel configuration let two fluids to be inserted at the same time in the channel and generate droplets. During the process of fabrication, the power was set on 30W and 200W with the scan speed of 120 mm/s and 300 mm/s for cutting and patterning respectively. Fabricated micro-channel is shown in Figure 1(a). Since our tubes had outer diameter of 2.36 millimeter, the fabricated chip inlets and outlet was then drilled by 2 mm diameter for entering the exchangeable tubes into the microchip without any leakage problem.

Electrode deposition on substrate was performed using a polymeric shadow mask which was provisionally bonded to the substrate. For this purpose PMMA were laser cut to make mask for gold sputtering. CO2 laser system was used to create the desired patterns. During the cutting process, the power setting of the laser was 30 W at the rate of

150 mm⁄s. After patterning, shadow mask was provisionally bonded to a 3-mm thick transparent PMMA sheet by casting mixture of isopropyl-alcohol (IPA) and double-distilled water (DI) with the ratio of 7:5. Then, these two layers were attached to one another by two paper clamps as a source of low pressure for bonding utilizing thermally-solvent technique and were placed in a fan-assisted oven (Figure 2a)

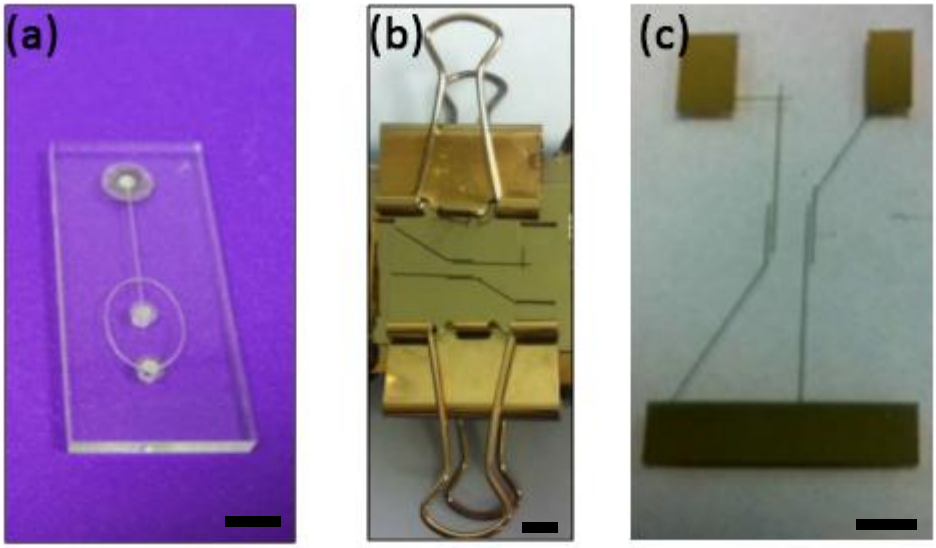

Figure 1. (a) channel after micro-machining (b) provisionally bonded PMMA layer to shadow mask after gold sputtering. (c) Coplanar electrode pattern on PMMA sheet. Scale bars are 10 mm

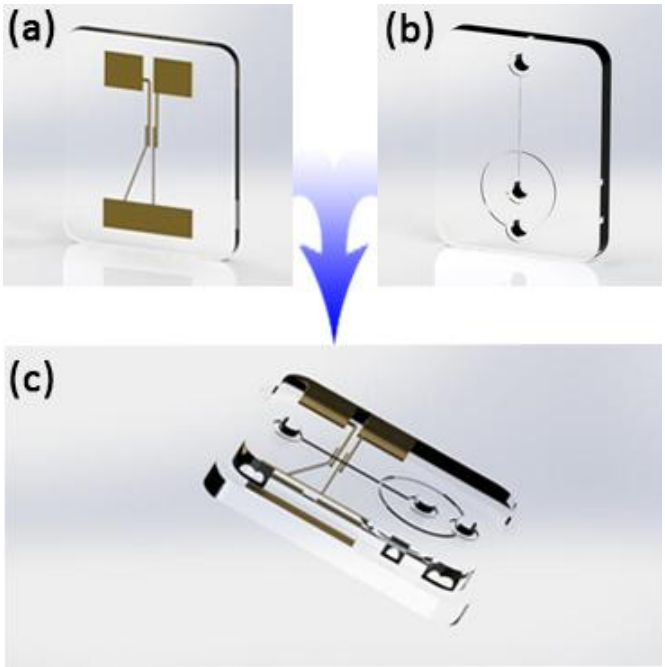

Figure 2. Schematic of fabrication process. (a) gold sputtered electrodes (b) fabricated micro-channel using laser ablation (c) final microfluidic chip after thermally-solvent bonding technique.

Utilizing a desktop sputtering system, the sample was then sputtered with the 80 nm gold layer. After peeling of the shadow mask (Figure 1b), pattern was appeared as it is seen in Figure 1c.

The next step was bonding two PMMA sheets containing gold electrodes and microfluidic channels, respectively. Both parts of the chip were soaked in IPA and water solution with the ratio of 7:3 respectively and then mechanically connected with each other with previous mentioned recipe [23]. The solution will also help to remove any residual of attaching the mask that previously connected.

At the end, after detaching paper clips, conductive silver glue was used to connect wires to the sputtered gold electrodes. The schematic final fabricated chip is shown in Figure 2c.

2.2. Droplet generation

Two 10 ml syringes were filled with coloured DI water as a discontinuous and coconut oil as a continuous phase and inserted to a quadra syringe pump, minimum 0.1 ml/min flow rate, with suction and injection capability. Syringes were connected to microchip using feeding tubes on both inlet and outlet for injection of fluids into micro-channel (Figure 3).

Several pump flow rates was tested to meet the appropriate droplets with best size and shape. In this regard, for oil flow rates of 9, 18, 27 and 36, water flow rate was changed up to 80(ul/min)and frequency of droplet generation in each one achieved. By changing both flow rates the droplets began to appear but in most cases, the droplets had improper shape and size. Observations showed frequency of droplet generation increases by both increasing discontinuous and continuous phases but continuous phase had greater impact on frequency. Figure 4. illustrates change in droplet generation frequency versus water flow rate in different oil flow rates. Inset shows optical images of droplets in different frequencies. High yield and reproducible droplet were resulted in lower frequencies and optimum frequency was lower than 4(Hz). Best droplet generation rates of oil and water has been selected as shown in Table 1.

Certain numbers of rates were contained similar-sized droplets which are demonstrated in table 1; moreover, the deviation of droplet sizes from the average for these 5 cases is seen in Figure 5 for same number of droplets. As it is derived from figure 5, 18 µl/min of continuous phase and 10 µl/min of discontinuous phase provide the least deviation of droplets size from the average and are appropriate for final study.

In vertical axis of Figure. 5, $\overline{D}$ represents average diameter of generated droplets and Di is diameter of ith droplet.

2.3. Encapsulated cells in droplets

Prepared solution containing Jurkat cells (c8166 cell line) was provided from the Pasteur Institute (Tehran, Iran) without any purification. The solution containing cells as discontinuous and coconut oil as continuous phase were used for final tests. Similar procedure followed as described in the last section for droplet generation. Optical images ensured that droplets encapsulated with maximum one Jurkat cell due to low concentration cells in solution utilizing optimum flow rates achieved in previous sections. Generated same-sized droplets are shown in Fig. 6 and also an encapsulated cell in a droplet is highlighted.

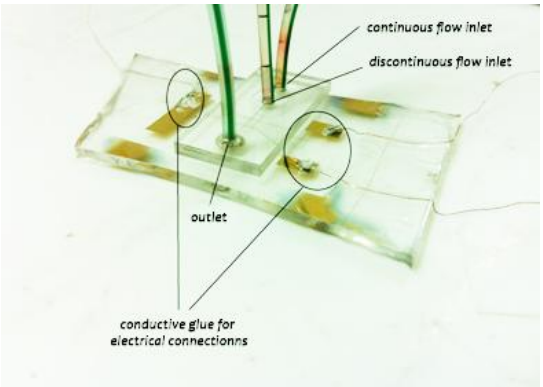

Figure 3. different parts of fabricated chip

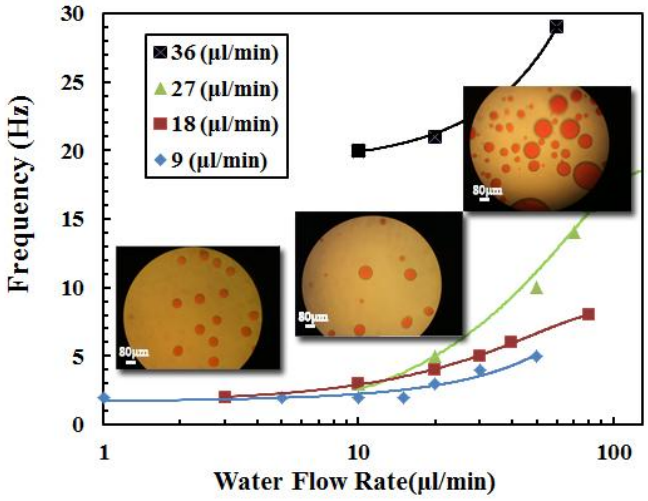

Figure 4. Relation between droplet generation frequency and function of continuous and discontinuous flow rates

Table 1. 5 considered cases of droplet generation flow rates

Label	Flow rate (µl/min)	
	Continuous phase	Discontinuous phase
W1,O1	9	5
W2,O2	9	10
W3,O3	18	10
W4,O4	9	20
W5,O5	27	10

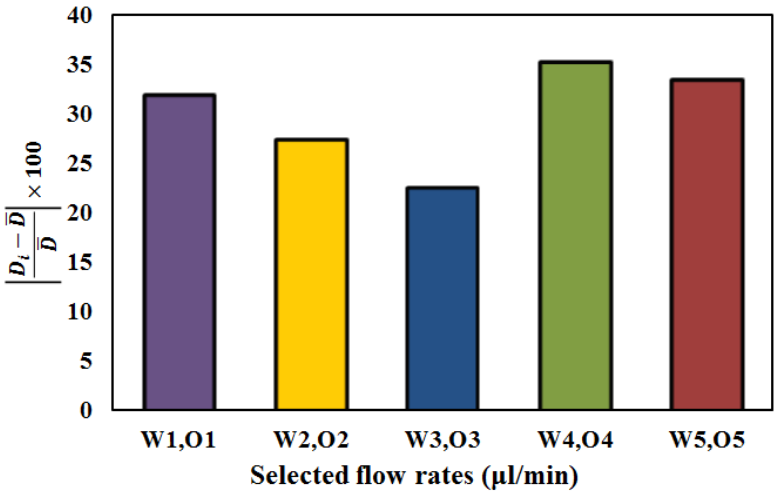

Figure 5. deviation of droplets sizes from the average of 5 cases of droplet generation

2.4. Impedometric detection of single cell

As described in previous section, single cell in a droplet was encapsulated. In Figure 8, differential output voltage of test in a time interval is demonstrated while flow of droplets in micro-channel is highlighted. As shown in this figure, a train of droplets flow through the differential electrodes. If we consider second pair of electrodes as a reference, the output signal shows a significant voltage peak when a droplet place between first pair of electrodes. By the time the droplet located between the second pair, another voltage peak with opposite sign is obtained. In Figure 7 approximate equivalence of output signal and location of droplets in micro-channel are shown.

Electrical approach was performed for detection of trapped cells. Impedance was measured using AC at frequency of 101 KHz and 5 Vpp. the output from the differential electrodes was then measured using digital oscilloscope assisted with internal high yield analog to digital circuit transferred to and further analysis was performed in PC. Figure 8 shows output signal in a time interval.

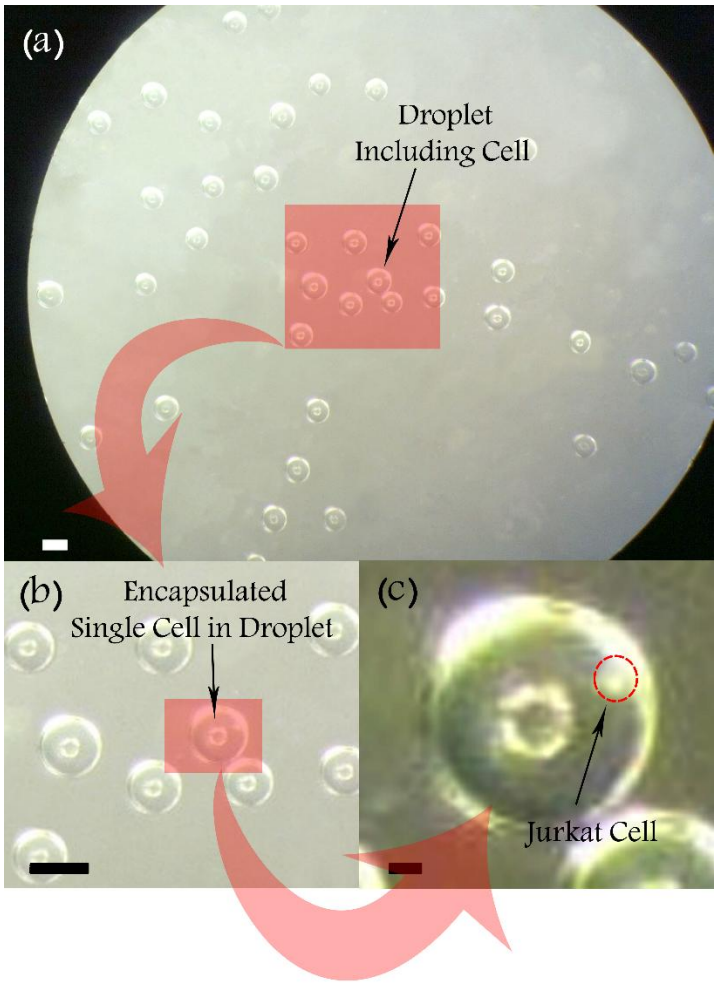

Figure 6. generated same sized droplets. (a) Optical image of same sized droplets while red rectangular region include an encapsulated cell; scale bar is 80 µm. (b) Higher magnification of the red region; scale bar is 80 µm. (c) Encapsulated Jurkat cell; scale bar is 11um.

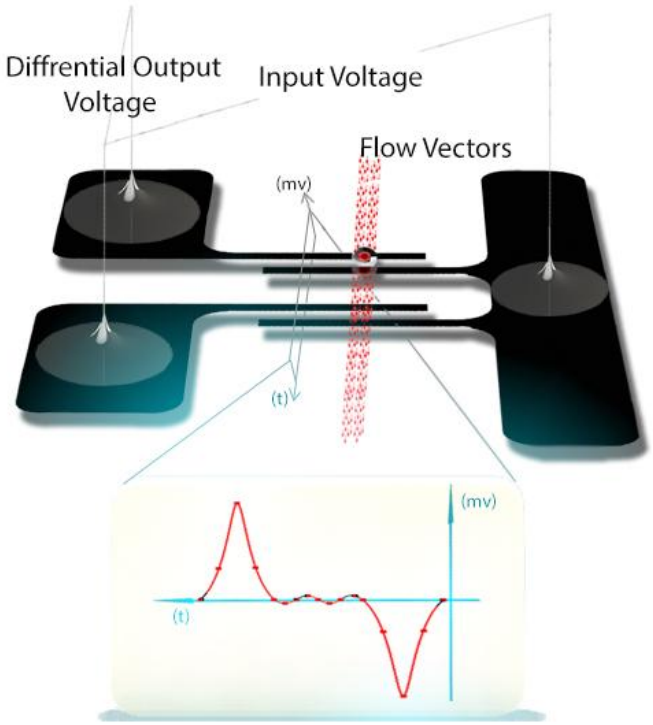

Figure 7. input and output voltage connections, location of droplets in micro-channel and chip output signal

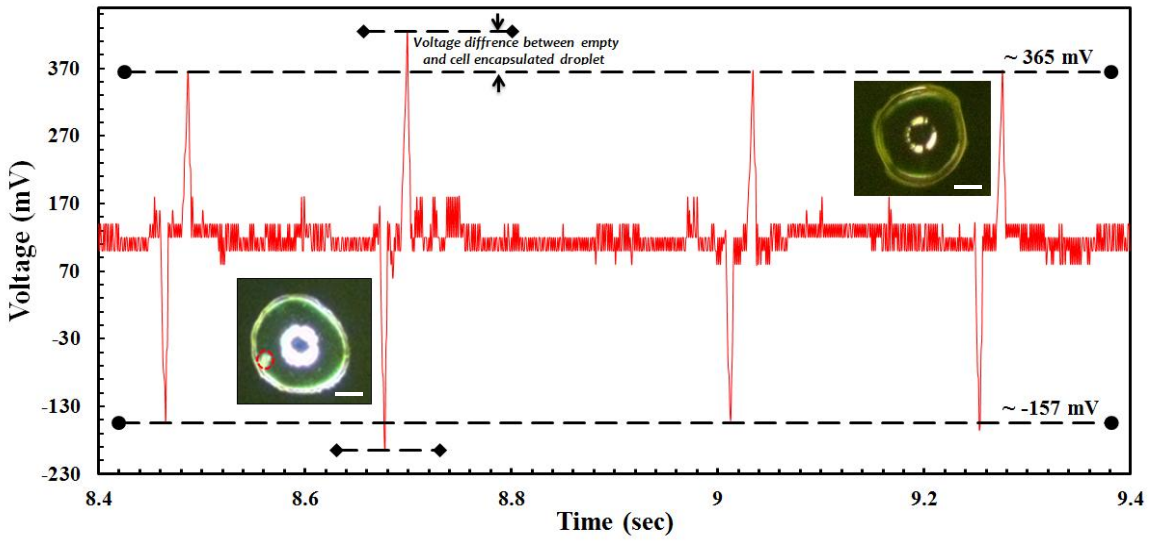

Figure 8. Differential output signal represent pulses related to empty and a cell encapsulate droplets. Inset also shows two optical images equal to their adjacent pulse and the red circle in left one highlights a cell; scale bars are 20 µm.

Although a few number of pulses in differential output voltage were included different amplitude, numerous recorded data indicated approximately similar amplitude when pulses occur. On the other hand, our observations showed that most of the droplets were empty. Considering these two, the similar size of droplets and pulse train contained similar amplitudes, the pulses with higher amplitude belongs to single cell encapsulated in a droplet. Due to the fact that dielectric properties of viable Jurkat cancer cells is different from surrounded medium, its conductivity and permittivity varies, so signal amplitude in cell contained droplets are higher than empty ones. This low cost and easy to use electrical detection of particles can be comparable with optical detection results.

3. Conclusion

Droplet based clinical analysis are one of the most interested subcategories in microfluidics bio analysis researches in recent years. By the way exact calculation of cells inside droplets has been rarely performed. In this work a microfluidic chip was fabricated utilizing PMMA chip for detection of droplets including cells. Particularly, passage of droplets in the device is specified by differential output signal measured using connection of the microchip to oscilloscope and more analysis in PC. The electrical technique takes the advantage of being a label free method instead of using optical laborious technologies. For future studies calibration of this device may help to analyze more cells and utilizing it for cell counting applications. The microfluidic devices fabricated using this technique have also many potential applications in detection of other micro-particles.

Conflicts of interest

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

Acknowledgments

No applicable.

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