Design and simulation of a novel MEMS based microfluidic circulating tumor cell (CTC) detection system for a lab on a chip device

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Abstract. This paper proposes a novel microfluidic system for a lab on a chip device which includes three major systems: a microchannel, micromixer and a droplet generator. The novel system proposes electroosmotic fluid flow control combining droplet generation and immunocapturing based CTC separation. ANSYS Fluent is used to optimize the fluid flow parameters, droplet size and justify the mixing capability of the micromixer. COMSOL Multiphysics simulations justify the integration of the electroosmotic fluid flow control for precise generation of droplets and optimization of dimensional parameters of microchannels, followed by a fabrication method for the microfluidic system.

1. Introduction
According to the ‘National Vital Statistics Reports’ for 2016 and 2017, cancer has identified as the second highest cause of death in the USA [1],[2]. Cancer cells are described as cells that divide relentlessly which in turn forms solid tumors and flood the blood with abnormal cells. Therefore, early stage cancer detection is a vital necessity to reduce deaths caused by cancer. Generally, cancer diagnosis is done through the extraction of tissue from the affected area (solid biopsy). Tissue extraction through biopsy is invasive, time consuming and expensive. Furthermore, cancer state monitoring is important, hence to give a better insight into the evolution of tumor dynamics during treatment and disease progression. A liquid biopsy is the other option which is the analysis of biomarkers in a non-solid biological tissue. There are several biomarkers that have been identified. CTCs, circulating tumor DNA (ctDNA) and exomes are three of the most common markers [3]. CTCs are the most commonly captured biomarker to detect cancer as it provides a unique identification of the type of cancer and the progression stage as it contains distinct morphological and phenotypic features. The concentration of CTC in the patient’s blood can be measured in counts. The number of cells is countable compared to the exosomes. There are few techniques to capture CTCs from whole blood. A positive selection of anti-bodies using anti-EpCAM was selected as the technique to follow in this paper [4]. Based on the available traditional methods, Lab on a Chip (LoC) devices have been developed to perform the separation process on a single chip rather than using complex integrated sub systems. LoC devices do not require...
bulky setups and those systems can provide quick test results and able to manipulate a single cell for further diagnostics and reliable observations. Microfluidic CTC separation is done in two different methods: physical property based and immunocapturing based. Physical property-based cell separation techniques are inexpensive and does not require additional surface markers. But physical properties like size and density can create overlap regions with other blood cells at extreme limits and cause false results [4]. Physical based separation requires complex geometries which are difficult to fabricate. Immunocapturing is the other method that is used to separate CTCs in microfluidic systems. Immunocapturing facilitates downstream analysis of cancer cells such as proliferation, genotyping and drug sensitivity tests. Most immunoaffinity methods make use of the Epithelial Cell Adhesion Molecule (EpCAM) protein for immune interactions.

CTC capturing chips utilizing both separation methods are available in literature. ‘CTC-chip’ captures viable CTCs with high purity in a single step from non-pre-treated blood samples [5]. ‘HB-chip’ [6] isolated CTC clusters successfully using transverse flows within the micro channel to realize maximum collisions between cancer cells and the antibody-coated chip surface. ‘CTC-iChip’ is designed to obtain deterministic lateral displacement (DLD) followed by immunomagnetic separation of bead-labelled white blood cells (WBCs) and unlabelled CTCs [7]. Presented solution contributes towards the development of non-invasive methods for cancer risk prediction. Finding the number of CTCs available in a certain blood volume is difficult but the exact count is critical to get the maximum reliability of results. The aim of this research is to obtain efficient isolation of CTCs through droplet formation. This leads into identifying the threat of cancer and saves time by eliminating the requirement for performing various other tests which delays the consequential treatments. Proposed microfluidic system design can also be modified to capture different biological cells for several other diagnostic applications.

This paper discusses the microfluidic system design for immunocapturing based CTC detection.

Section 2 presents the detail design of the microchannel, micromixer and the droplet generator. Section 3 proposes a fabrication method for the microfluidic system. Section 4 presents the results which are optimized through simulations.

2. Proposed MEMS based microfluidic system for CTC detection

The microfluidic solution proposed in this paper contains three reservoirs for blood, CD326 anti-EpCAM microbeads and oil as illustrated in figure 1. HFE-7500 fluorocarbon oil is selected for the droplet generation since it is biocompatible [8]. All three fluids are driven as electroosmotic flows. Blood and the microbeads are mixed using a micromixer which contains magnetic particles that are attracted by the CTCs. The blood and magnetic bead mixture are formed into droplets such that a droplet may contain only one CTCs. Then a magnetic system is used to separate the droplets which contains CTCs. These cells can be further observed through fluorescent microscopic techniques [9].

![Figure 1. Designed microfluidic system.](image-url)
2.1. Microchannel design
Microchannels are fabricated with different cross sections [10]. The most common types are rectangular, square, circular, half circular, U-shaped and gaussian beam shaped microchannels. The microfluidic system shown in figure 1 is designed using a square shape micro channel with a T-junction to transport blood and anti-EpCAM microbeads to the micromixer. Two separate square cross section paths are designed for the oil flow to generate droplets.

Microfluidic platforms have capillary driven, electroosmotic, electrowetting and pressure driven fluid flow systems. Electroosmotic fluid flows are generated naturally when a potential is applied and can be controlled using voltage and flat symmetric flow. Electroosmotic flow is much more controllable compared to other common techniques [11]. Therefore, the designed micro droplet generator uses electroosmotic methods for fluid flow control. Electroosmotic flow simulations performed in COMSOL Multiphysics were based on the required velocities for the droplet generation and mixing of fluids.

Desired velocities within the microfluidic channel is obtained by optimizing the voltages at the reservoir and junctions of the microfluidic system. Figure 2 shows the obtained tip velocities for corresponding potential drops along the microchannel. These velocities satisfy the velocity requirements for mixing and droplet generation of the complete microfluidic system.

2.2. Micromixer design
Micromixers are classified as passive and active micromixers [12]. Depending on fabrication feasibility and cost, a passive mixer is used for the initial design. With the simulation results Considering the mixing efficiency, a square wave shaped convergence-divergence based micro mixture is designed [13]. Convergence-divergence points increase the efficiency of the mixing process. For an accurate magnetic based separation, a 20:1 mixing ratio between blood and CD326 anti-EpCAM microbeads is recommended [14]. Designed micromixer enhances molecular diffusion and chaotic performance for mixing purposes. Depending on the required velocities from the droplet generation, mixing process was simulated using ANSYS Fluent. According to figure 3 convergence divergence geometry, length and cross-sectional dimensions are optimized to have a mixing ratio closer to the recommended.

![Figure 2. Electroosmotic flow simulations.](image1)

![Figure 3. Finalized dimensions of the micromixer.](image2)
2.3. Droplet generation design
There are several active and passive techniques to generate fluid droplets [15]. In passive techniques such as squeezing, dripping, jetting and tip-streaming as shown in figure 4, the dispersed fluid is introduced into the continuous fluid to generate droplets. Active techniques require additional energy input through external sources, and it provides another level of controllability for droplet formation. Active techniques are categorized further as the additional force driven and intrinsic force driven.

![Passive and active techniques of droplet generation.](image)

Generated droplets in the system contains blood and magnetic particles. CTCs have a diameter around 20 \( \mu \text{m} \) [4]. Droplet diameter should be less than 40 \( \mu \text{m} \) to ensure that it does not contain two CTCs and should be greater than 20 \( \mu \text{m} \) to contain one CTC. Therefore, the droplet size is decided to be around 30 \( \mu \text{m} \).

Simulations are carried out using blood and HFE-7500 fluorocarbon to select the most suitable technique for the blood droplet generation. Three different geometrical designs are compared. Fluid velocity based active technique and the double T-section geometry are selected to generate desired size droplets. Geometry 2 generates droplets closer to the desired size whereas geometry 1 and geometry 3 results in undesirable droplet formations as shown in figure 5.

![Comparison of different geometries for droplet formation.](image)

3. Fabrication method
The entire microchip mould is designed on a silicon substrate according to the steps shown in figure 6. The first negative photoresist of SU-8 2050 [16] is spin-coated on silicon with a 50 \( \mu \text{m} \) thickness and patterned using photolithography. After photolithography, a second SU-8 negative photoresist layer is deposited for 2 mm in thickness and patterned to create the boundaries of chip batch production [17]. The PDMS layer is deposited with Si elastomer curing agent with a 10:1 ratio. PDMS is then peeled
off from the mould and finally bonds with a glass of 2 mm thickness through glass-oxygen plasma bonding [18].

Figure 6. Schematic of microfabrication on silicon substrate (a) device fabrication steps (b) microfluidic system batch fabrication steps.

4. Results and Discussion

Microchannel dimensional parameters were optimized to obtain the optimum velocities, mixing ratio and droplet diameter. Finalized values for the microchannels are included in table 1. Micromixer design is optimized by using a convergent divergent point which minimizes the cross-section area of the mixing micro channel by half. This improves the mixing of blood and CD326 anti-EpCAM microbeads to a degree where the mixing ratio is 15:1 at the mixer output. Total length of the micro mixer is 70mm.

Table 1. Finalized dimensional and velocity values of the microchannel.

| Fluid section | Width of cross section (µm) | Width of cross section end (µm) | Length of microchannel (mm) | Velocities of inlets to mixer and droplet generator (mm/s) | Voltages between the reservoir and inlet (V) |
|---------------|-----------------------------|---------------------------------|-----------------------------|----------------------------------------------------------|---------------------------------------------|
| CD 326        | 50                          | 2.5                             | 9.5                         | 9.44                                                     | 90 – 0                                      |
| Blood         | 200                         | 50.0                            | 9.5                         | 9.41                                                     | 300 – (-50)                                 |
| Oil           | 50                          | 50.0                            | 46.5                        | 20.10                                                    | 350 – 0                                    |

Droplet diameter is determined by observing the blood volume fraction of two deviated points with respect to the centre line. The diameter of the droplet is observed to obtain the optimum velocity combination of blood and oil.

Figure 7. Droplet diameter observation.

The volume fractions at point 1 and point 2 which are 15µm and 20µm apart from the centre line, are obtained to determine the droplet diameter. For the optimum velocity combination of blood and oil: $V_b$
and $V_o$, the volume fraction of point 1 is less than 0.00025 and point 2 is greater than 0.8 as shown in figure 7. Hence, the droplet diameter is deduced to be greater than 30 $\mu$m and less than 40 $\mu$m. Optimum flow velocities of blood ($V_b$) and oil ($V_o$) were obtained as 10 mms$^{-1}$ and 20 mms$^{-1}$ to generate droplets of diameter around 30 $\mu$m. Therefore, the blood mixture and oil velocities of 10 mms$^{-1}$ and 20 mms$^{-1}$ were used to perform all other connected simulations.

5. Conclusion
ANSYS Fluent simulations supported the optimization of fluid flow parameters to get desired size droplets and the analysis to justify the mixing capability of the designed micromixer. COMSOL Multiphysics simulations aided the design process to optimize critical dimensional parameters of the microfluidic channels and justified the integration of electroosmotic fluid control to the system to obtain fluid flow velocity requirements. It would be interesting if future research could be conducted on the design of a magnetic system which is used for separation of droplets with CTCs. The additional integration of a microscopic system to count the number of CTCs available in blood would complete the microfluidic system for cancer risk prediction.

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