Chapter 4

Micropatterning in BioMEMS for Separation of Cells/ Bioparticles

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Additional information is available at the end of the chapter

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Abstract

Biofluids remain a difficult issue in some drug delivery processes for separation of bioparticles through microchannels. This chapter reviews the techniques which have been substantiated and proven helpful for the separation of particles depending on mass and size with some constraints of high throughput. In this study, a key focus will be on separation criterion by patterning of a microchannel and utilize sieve type channels based on spherical bioparticles. The first part focuses on the designing of the pattern for issues of the network like clogging and theoretical experiments by both hydrodynamic and other passive methods for sorting/separation. The second part focuses on the simulations for separation for small and larger bio particles depending on mass and size, samples of blood and other Klebsiella infected fluidic samples for the experiment. The theme provided for mass and size-based separation is simple and can accomplish operations in microfluidics for several biological experiments, diagnosis approaches and zoological researches.

Keywords: microfluidics particle sorting, patterning, Klebsiella and other bioparticle, COMSOL Multiphysics 5.2a

1. Introduction

A portion of MEMS, that is, micro electro mechanical system technology has contributed in various applications of sensors and actuators, BioMEMS applications [1] in which it has played a crucial role for Micro/Nano fluidic devices and a key role for validating a factor in integration of multiple functions for different microdevice and miniaturization. These technologies of Microsystem are used widely in biomedical, disposability, low power consumption, low cost as well as it incorporates multiple phenomena physically and due to its design complications it is difficult to deploy these devices than other sensors [1].

BioMEMS has different types of devices which determines that device for this term have some manipulations in chemicals for about smallest part in form of microlitre for bacteria or proteins separation purpose of different cells (spherical and non-spherical) drug delivery and detection of contaminant with other manipulations necessary. However, some of the micro electro mechanical system (device instrument), which is attached to normal surgical instruments, is also called BioMEMS type but it is not included in such normal devices due to restriction honored and considered as technical type instrument. There are some other devices which involve itself under BioMEMS to accentuate the idea and perform all tasked from input of samples to the detection of the cells or proteins named *micro-total-analysis system*. Operations at chip scale can replace some familiar works of laboratory process known as *lab-on-a-chip* as well as for the approach for conducting measurements parallel an *array processor* are been included. There are some other term which does not belongs to the BioMEMS but it emerges with it are defined with self as *microfluidics* [1].

A splendid addition has been exhibited which allows for the sorting depending on selection and different interests in analytes [2–4]. Thus this chapter deals with the different techniques used for separation and for micropatterning of array channels used in different applications of biomedicals and other BioMEMS terms. First part of this chapter deals with the active and passive approaches for bioparticles and cellular separation depending on the fluid velocities and its concentration depending on applications and designing. The second half of the chapter focuses on the simulations of the sorting and the detection techniques [5].

2. Patterning, separation and detection in microfluidics

In Microfluidics system technology there is an separation methods which can manipulate individual cells having the potential and empowers the experiment sets larger with lower reagent costs and allow for faster reaction work compared to conventional methods [6] for separation and modeling area using micropatterning for the samples like blood and other species like mammalian cells (*K. pneumoniae*) focused on the size and mass of the bioparticle (Spherical and Non-spherical). Nonetheless in recent years Klebsiella species has now become one of the important antibody in infections like nosocomial even there are some important members of Klebsiella breed of Entero-bacteriaceae and some have been exhibited in human laboratory specimens like *K. rhinoscleromatis* and *K. oxytoca* [7]. However separation and detection for the manipulations from the fluidic sample of bioparticles using microfluidics are sensible and challenging issues in laboratories depending on the flow rates, throughput and clogging on microscale [5].

Separation of size-based particle and aborting is occupied in many filtration systems of commonly used tap water filters to a system with complex size separation chromatography systems. As separation of size-based particle has various intent which consists of distillation of fluids or air and analytes concentration (macromolecules or proteins, DNA and others) in separation of components and biological cells. Depending on the size and mass based separation the modeling like patterning or sieving methods are commonly used where it will allow

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those bioparticles (spherical and non-spherical) to flow through gaps according to hydrodynamic flow rate and based on fluid velocity and viscosity measurement the other particles can be sorted individually for precise outcome [8].

2.1. Bioparticles of spherical and non-spherical shape

Ideally for geometry, it has a definite shape associated with the index of shape for adequate characterization. Depending on the characteristics of the bioparticles and its recognition visually is straightforward as defining in words or numerical value of a shape can be in a limited portion. Despite the efforts made in previous works in evolving a sphericity or index have created many hurdles for distributing values on the 3-dimensional complex irregular shape [9–11]. For the shape factor there is no perturbation with it for changes in the drag force of particle for spherical and non-spherical as there has been a development in the dynamic shape factor relatively. This ratio of drag force is calculated for the forces experienced by the spherical or non-spherical particle over the ratio of other particles with the volume equivalently flowing with the medium and same flow velocity. Other than spherical particle there is a an phenomenon change in the non-spherical particle as understanding the indexing of non-spherical particle is important with the chemical, physical, biological functions and other phenomenon by which the changes in the effect of bioparticles cannot be denied. However the anatomy of some proteins molecule to the utmost dimensions of galaxies and stars neglecting the effect of shape can be made. Interaction with the analysis of particles deeply requires some considerations of shape for behavior in the system of the particle in applications practically to conclude [8] (**Figure 1**).

2.2. Techniques for sorting

In recent years for studies of application on cell separation there were many techniques extended which emerged in the field of microfluidics for biomolecular analysis which are initially driven as per requirement. While manipulation and Cell separation is a crucial sample step for processing in many medical assays and low Reynolds numbers as well as predictable flows, small fluid volumes, small dimensions, materials and the established microfabrication

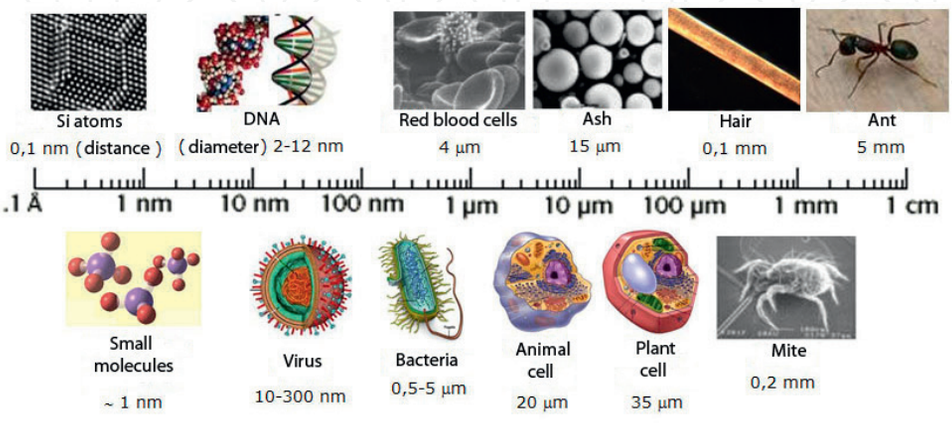

Figure 1. Types of bacteria present with its size of shape [12].

techniques which are typical microfluidic device factors allows user to work with cells on microscale. Existing microfluidic separation methods are categorized into two methods as active and passive methods where active methods incorporate an external force and passive methods rely on carefully designed channel geometries and internal forces to sort different particles. Classification is as shown in **Figure 2**.

In this chapter, we introduce various principles and related methods including some common separation methods for active type include immunomagnetic separation (IMS), acoustophoresis, electrophoresis, dielectrophoresis, optical force and flow cytometry or FACS [14]. There are different technologies or methods in microfluidics for precise, passive and continuous sorting systems invented including Hydrodynamic inertial force, Deterministic Lateral Displacement (DLD), Pinched-Flow Fractionation (PFF) and Hydrodynamic Filtration (HDF) [15, 16].

Microfluidic technologies that can manipulate individual cells with the potential enable larger experiment sets, lower reagent costs and allow for faster reaction work compared to conventional methods [6]. PFF is a separation technique where a field is applied to a fluid suspension pumped through channel which is narrow and long, flow in perpendicular direction depending on their differing "Flexibility" under the exerted force by the field and to cause separation of the particles present in the fluid. In recent years a number of microfluidic devices have been advanced for the continuous separation of bioparticles by employing unique techniques. One of the prominent separation method is DLD in which particles drives through the post to post arrays positions. The interaction of the different particles with different size and post to post array directions leads to different bacteria to drift in different directions with respect to the arrays thus causing the continuous fractionation and two dimensional of the sample mixture.

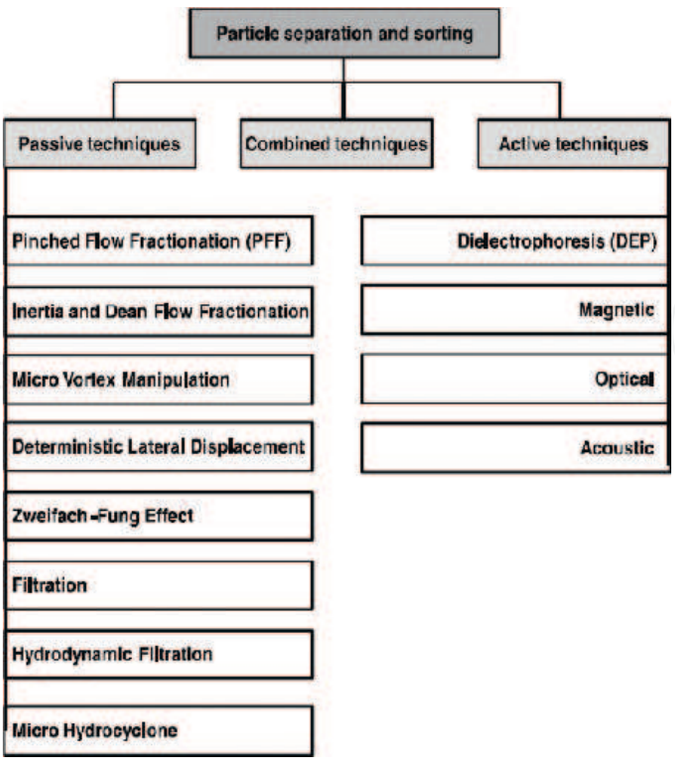

Figure 2. Classification of microfluidic sorting and separation techniques [13].

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Hydrodynamic force is one of the inherent physical principle and basic in system of microfluidics where inertial force and dean rotation force has been utilized, though hydrodynamic force based separation system can be created by fluid dynamic theory based microchannel network. An Acoustic method utilizes the ultrasonic standing waves and allowing a manipulation of cells then other separation methods like dielectrophoretic, magnetic and others. The basic principle of acoustic sorting is to use pressure gradients generated by ultrasonic standing waves since most microfluidic system uses liquid medium as the working fluid. DEP is the electro kinetic motion which occurs when polarisable particle is placed in non-uniform electric fields and particle motion induced by DEP force is influenced by the ambient electric field and the properties of electric particles or solutions. Magnetic sorting system can be of two different categories for separation (I) Attaching magnetic particles to the cells to react with the magnetic field and (II) Utilizing the native magnetic properties of the cells in laminar flow for some basic motion of particles expressed as Newton's second law [17] schematics shown in **Figure 3**.

Microfiltration is a method of basic concept for separating and sorting microparticles which utilizes the size of micropores and the gap between microposts as a lattice or sieve. Accordingly, microfiltration is highly dependent on the sizes of the microparticles. The advantages of this methods discussed are easy for understanding the separation principles for engineer or scientists to implement for an application to existing methods [17]. Thus by this study we have introduced a developed technique HDF which focuses on the shear flow of input associated with buffer streams which constricts the stream flow within the middle of the microchannel

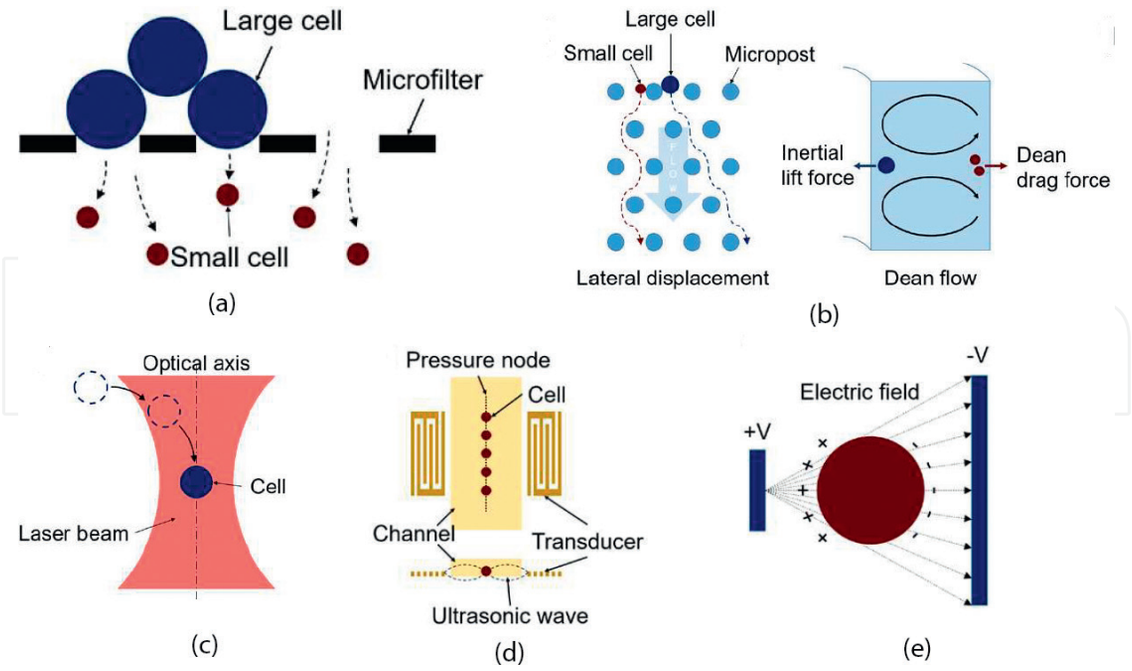

Figure 3. Cell Separation principle schematics (a) Geometrical constraints for cell size in microfiltration technique (b) hydrodynamic forces applied to the cells in the network of microchannel (c) Between the magnetic force field and the magnetic particles attached to the cells they are separated by the attraction force (d) The ultrasonic waves generated by the transducers from which acoustic cell separation utilizes acoustic primary radiation force (e) The electro kinetic motion of polarisable cells in non-uniform electric fields is utilized by Dielectrophoretic force (DEP) [17].

accommodated with particles in the plane of sidewalls with narrow width [18] where both concentration and classification of particles can be examined at the same interval of time by introducing a solution consisting of particle beads of various mass and size.

We preceded here with a method that has been adopted to invent a separation mechanism, Continuous processing, high precision for particle separation and high throughput [17]. When a particle flows in a microchannel the center position of the particle cannot remain present on a certain distance from sidewalls which is equal to the particle radius. The method of filtration utilizes this fact and is performed using a Sieve type-shaped microchannel network having multiple side branch channels/sieve-shaped networks. According to flow rates in microchannel by fluid flow (μl) it withdraws a small amount of liquid continuously on intervals from the main stream through the side microchannel network and particles are concentrated and aligned in the network according to size and mass. However the concentrated and sorted particles can be collected according to size and mass through all other output channels in the stream of the microchannel network. Therefore continuous introduction of a particle suspension into the microchannel enable particle sorting, concentration and classification at the same time with precision [19]. Whenever there is a difference in particle size and mass then separation becomes difficult for the result and mesh clogging is inevitable [20].

Hydrodynamic Filtration is one of the most frequently used technique to classify particles suspended in fluid flow due to sedimentation. Existing filtration methods performed either in batch or continuous manner and large-scale treatment can be easily achieved. Biological entities such as rod-shaped bacteria and disc-shaped red blood cells (RBCs), disproportional length and width which complicate the separation process designed for spherical particles and the narrowest width has to be considered for the separation criteria within the design parameters of the microfluidic devices. Some of the examples for cell separation include (**Figures 4** and **5**):

- Blood cells (WBC) isolation from tissue
- Circulating Tumor Cells (CTC) from blood
- Separation of some bacteria from food which are pathogenic to health and other systems
- Resistant cells isolation from peripheral blood

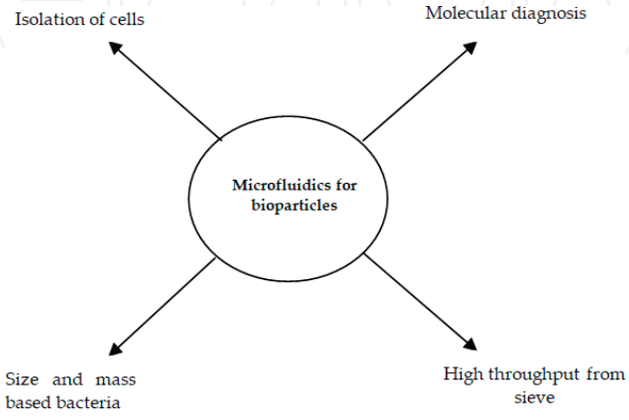

Figure 4. Role of microfluidics for the separation.

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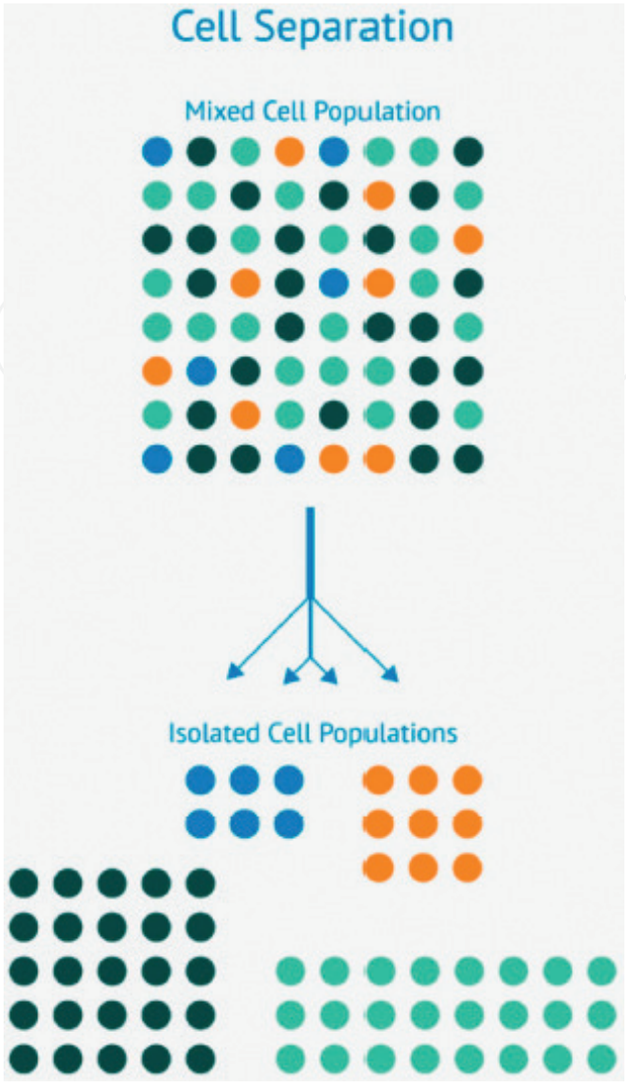

Figure 5. Sorting and separation for different cells from mixed population to isolated populations [20].

However there are some essential approaches through which separation takes in microfluids,

- From a biological sample if a single type cell is removed it can be called as **Depletion region.** Example mononuclear cells from which RBCs can be removed.
- Similarly when there is need of removing cells other then the single cell like RBC a **Negative selection** can be done where it will leave single cell type and other packet of cell needed to be removed can be separated. Example like bone marrow or whole blood sample, removal of cells varying with size and others.
- Whenever there is any downstream analysis then a mechanism of removal cell type is targeted whichever cell depending on size and mass can be separated this type of typical selection can be processed by **positive selection**. This type can be possible in monoclonal antibodies and performed by aiming a surface marker of cells (bioparticles) [20].

3. Analysis and modeling for microchannel

3.1. Sieve or lattice type microchannel network design

The sieve-shaped micro channel network is assured of two types of microchannels (I) The main channel and (II) Separation channel, In microchannel main channels are deeper than the separation channels as the main channels are at different angle positions of channel to lower right/left and perpendicularly-crossing are separation channels. Particle/cell is introduced continuously from the inlet at intervals, whereas a buffer solution without particles/cell is introduced from two side inlets shown in **Figures 6–8**. Smaller particles can reach the ceiling (upper region) of the main channels but the larger particles cannot because of the effect of hydrodynamic filtration. Consequently the repetition of the larger particles entering the separation channels will be higher than that of the smaller particles and the positions of the larger particles will shift in the direction according to flow rate more greatly than those of the smaller particles depending on the mass and size of the particle achieving continuous separation [21].

As discussed about the techniques employing the effect of hydrodynamics in microfluidic applications has been prominent in the decades to be fruitful in terms of efficiency, throughput and continues to develop in the future through some more improvements in separation processing rate and resolution. However there are many other new areas of hydrodynamic microfluidic phenomena for an application which demand further investigations and promisingly both explain and informs researchers theoretically about basics on physics and

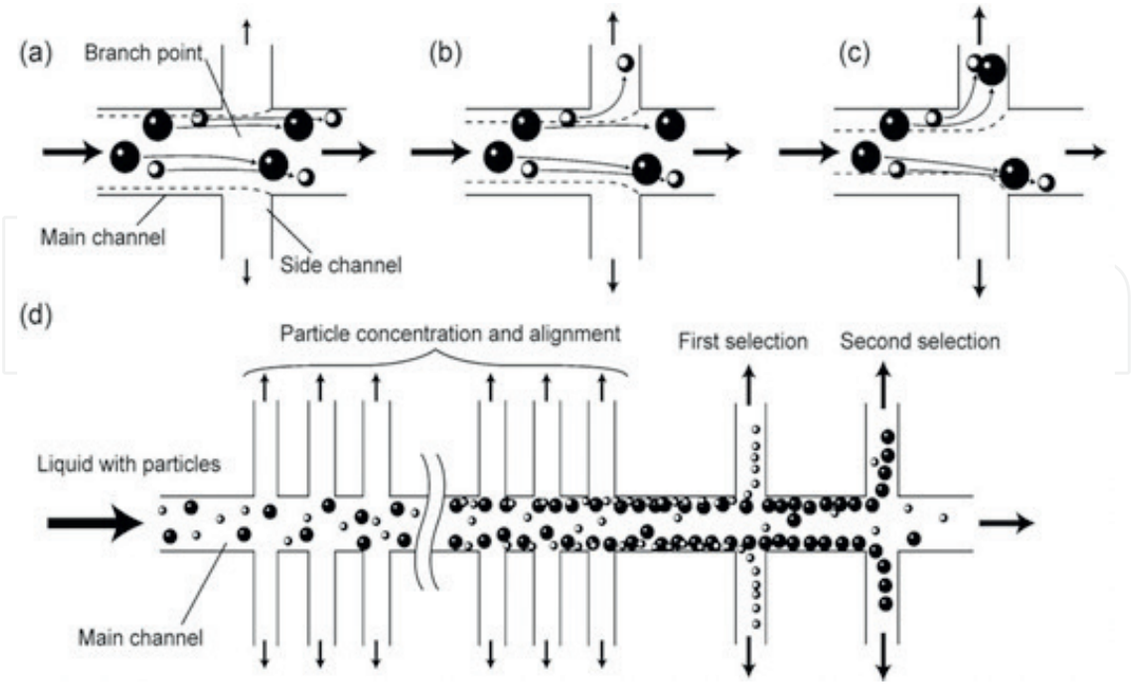

Figure 6. Hydrodynamic filtration principle showing behavior of particle at branch point according to different flow rates which are high, medium and low at multiple channels [19].

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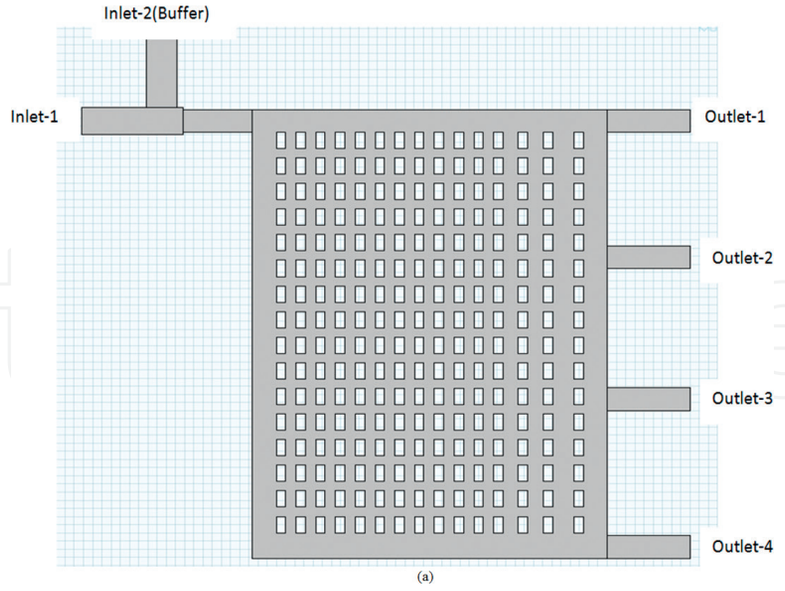

Figure 7. Schematic of Sieve type – shaped Microchannel network.

to exploit them experimentally in applications of biological terms. In spite of a few active separation techniques have been developed to conform the demand growing in these new area [22]. Presently a continuous particle/cell separation system utilizes a Sieve type-shaped micro channel network has been shown ahead. The difference in the densities, velocity, pressure and viscosity in sample it generates the asymmetric and symmetric flow distribution at each intersection with intervals resulting in the separation of large size particles through the streamline [23]. A modified mechanism of particle sorting using sieve type microchannel patterning is presented where it potentially enables the throughput separation highly and can prevent clogging problem of micro channel at some extent. The presented system would become a simple but valuable unit operation in the microfluidic apparatus for medical and biological experiments [21]. The presented network system would be highly useful because of sorting microparticles and cells with a high precision and would become an important useful tool for general chemical/ biological experiments in laboratories.

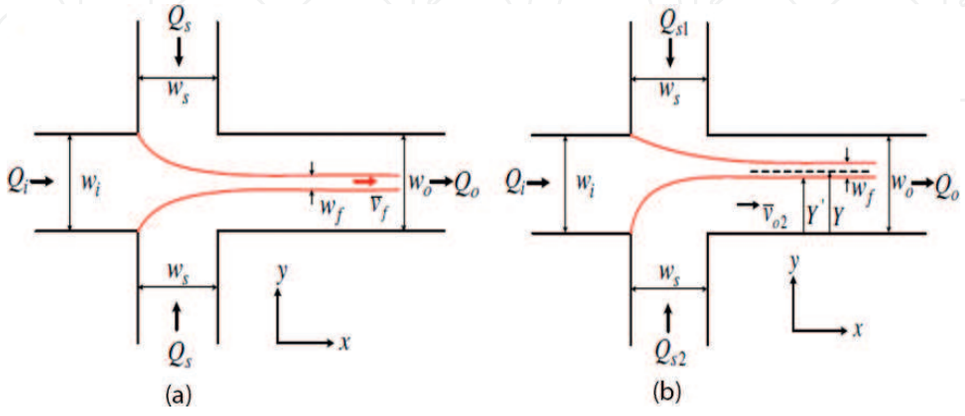

Figure 8. Schematic of: (a) Symmetric hydrodynamic flow focusing and (b) Asymmetric hydrodynamic flow focusing [27].

Here two inlets are employed from which one is used to introduce fluid without containing particles so that the particles flow along the sidewall. Multiple side channels are used in area so that particles larger than a certain size cannot pass through. As a result, such particles are concentrated and focused onto the sidewall. Microchannel network can be as shown in figure using COMSOL Multiphysics 5.2a software. In the downstream area the side channels are made gradually wider or shorter so that the particles are removed from the main stream in ascending order of size. Thus, the particles are sorted by size and concentrated with cytometry of flow with a basic laminar flow that focuses particles in same dimension, while at High flow rate inertial forces on particles cause are used to manipulate particles and inertial forces dominates when the particles Reynolds number is >1.

It is well known that a microchannel acts as a resistive circuit when an incompressible Newtonian fluid is continuously introduced into the channel. The micro device was therefore designed according to the concept that the volumetric flow rate Q, applied pressure P, and hydrodynamic resistance R are analogs of I, V and R in Ohm's law, respectively. In this study the following equation was used to estimate the hydrodynamic resistance R of each segment of the microchannel [24].

$$R \propto \frac{L}{l_1^3 l_2}\left[1-\frac{192\, l_1}{\pi^5 l_2}\sum_{n=1,3,5}^{\infty}\frac{\tanh\left(n\pi l_2/2 l_1\right)}{n^5}\right]^{-1} \tag{1}$$

where l_1 and l_2 are either the width or depth of the microchannel however, l_1 is the larger of these two values.

3.2. Theoretical and numerical analysis

For theoretical discussion prediction made with the width of two-dimensional hydrodynamically streams in rectangular shape micro channels is designed. Here critical diameter of bacteria particles will sustain a stable detonation for minimum diameter while the spacing that is center to center between the post is known as λ and d is known as the relative shift between adjacent posts, thus to measure the parameter λ ¸t can be measure with relative shift and tangent of the angle with respect to the vertical objects through the array as shown in (**Figure 8**) [25].

$$\varepsilon = \frac{d}{\lambda} \tag{2}$$

"Unconfined" and "confined" critical diameter was determined directly by inspecting the experimental distance/time data and examining the lattice-shaped microchannel at different angle [26].

Generally, ε (smaller) gives an result for smaller critical size in an array. However the clogging problem and large particle densities in an array do not occur easily. Therefore for a simple object [25],

$$D = \frac{K_B T}{6\prod \eta a} \tag{3}$$

where K_BT is thermal energy at temperature, D is diffusion coefficient of particle of radius 'a' inflow that we wish to separate from flow streamlines.

In this sieve type-shaped design a single region of posts will have a single threshold and particle flow will be in two directions. According to the principle of mass conservation, the supply of fluid flow passing through the dimension of the stream should be equal to the fluid passing through the inlet channel, i.e. [27]

$$w_f = \frac{Q_i}{\overline{v}_f h} \tag{4}$$

Moreover, the total fluid flowing through the outlet channel must equal the total amount of fluid supplied from the inlet and side channels, i.e. [27]

$$\overline{v}_o = \frac{Q_i + Q_{s_1} + Q_{s_2}}{w_o \times h} \tag{5}$$

Therefore, the relation between the width of the hydrodynamic focused stream (w_f) and the volumetric flow rates of the inlet channel (Q_i) and the side channels (Q_{s1} and Q_{s2}) can be as [27].

$$\frac{w_f}{w_o} = \frac{Q_i}{\gamma\left(Q_i + Q_{s_1} + Q_{s_2}\right)} \tag{6}$$

where,

$$\gamma = \frac{\overline{v}_f}{\overline{v}_o}$$

where the velocity ratio γ to be found, w_o is width of the outlet channel and v_f and v_o are the average flow velocities in the focusing stream and the outlet, respectively shown in **Figure 6** [27].

Thus the performance of the device using sieve type-shaped microchannel can be improved with the faster flow rate and clogging problem can be reduced. A critical hydrodynamic diameter can be designed easily with G between posts of the microchannel shown in **Figure 9**. Some assumptions on this study were made are:

1. Flow within the micro channels is steady and laminar. But, because of the smaller characteristic dimensions are involved the flow in microchannel is laminar.
2. The fluids are Newtonian.
3. The fluid has constant density in the inlet channel, side channels and outlet channel.
4. Inlet, outlet and all the channels are of the same measurement.

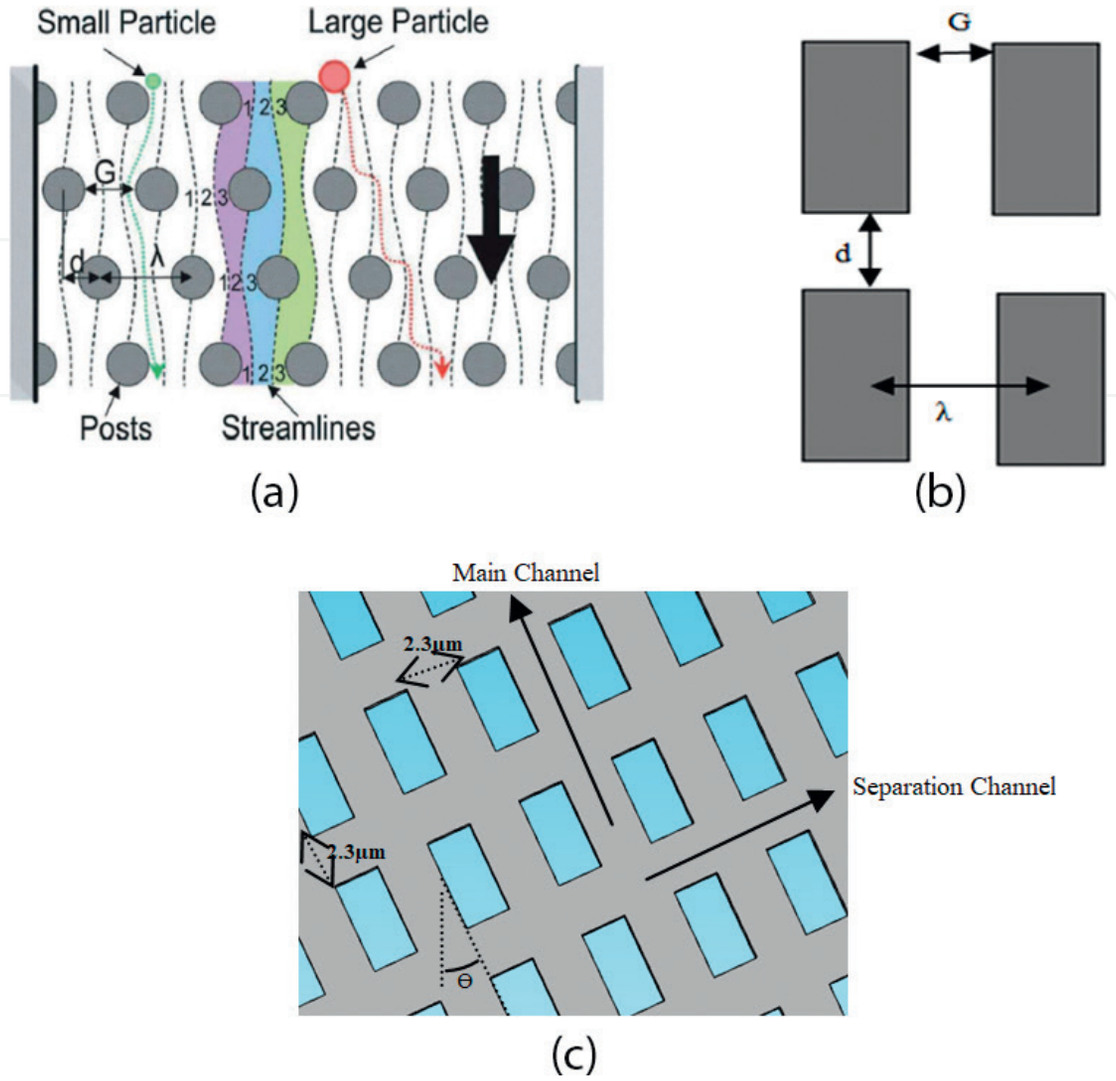

Figure 9. (a) Separation by a DLD in an array of micro posts with streamlines shown with G is spacing between the gaps in structure [28] (b) and (c) Structure and rectangular shape have and x and y distance of 4.3 and 2 μm showing main channel and separation channel.

For the particle movement depending on the volumetric flow rate was measured in COMSOL multiphysics 5.2a software by the Navier-stokes equation in compressible fluid flow [29].

$$\left\{\left(\rho\left(\frac{\partial u}{\partial t}\right)+u\cdot\nabla u\right)\right\} = \{-\nabla p\}+\left\{\nabla\cdot\left(\mu(\nabla u+(\nabla u)^{T})\right)-\frac{2}{3}\mu(\nabla\cdot u)I\right\}+F \tag{7}$$

There is a unique term that corresponds to the inertial forces, viscous forces, pressure forces and external forces which are applied to the fluid flow as Eq. (7) plays a vital role for the flow and to predict the movement for the particle according to the volumetric flow through the channel. For the different velocity magnitude at various streamlines for the particle in microchannel-1 is calculated on the basis of Eq. (7) precisely in COMSOL multiphysics 5.2a. By solving equation for specific conditions include inlets, outlets and walls predictions for the velocity and pressure in geometry can be observed and using sieves or grooves in a microchannel can be simulated.

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However instead of rectangle type shape in microchannel there are many related surfaces which can be used for sieving type or micropatterning of microchannel by which a precise result can be generated and can be modified using microchannel network for experimental work after fabrication of the microchannel for different surfaces are as shown in (**Figure 10**). For fabrication in micropatterning it has some processes used in BioMEMS and carried for micropatterning a channel as,

- **Deposition process** which is subdivided into,
  (1) Physical
  (2) Chemical
- **Patterning process** in MEMS is the transfer of pattern into a material lithography is widly used process which are framed as,

(1) Lithography where some types of categories are available with its process as Photolithography, Electron beam lithography, Ion beam lithography, Ion track technology and X-ray lithography

- **Etching process** are subdivided into,
  (1) Wet etching
  (2) Dry etching

Depending on different modeling, analysis of microchannel and sieve type patterns the fabrication can be proceeded with reliable material which can withstand some parameters for a patterned chip and can be sued in different areas for sample analysis like separation of particle and detection of bacteria from the given sample like CTCs, DNA, Klebsiella bacteria like *E. coli*, *K. pneumoiea* and other types.

The main task which can be carried for separation are the size and mass of the bacteria ranging between 0.3 and 10 μm and mass weight can vary from 1 to 10^{-12} Kg depending on the sample in biological terms precisely. Similarly for Klebsiella species one of the bacteria cells are *K. pneumoniae* whose particle size varies around 0.5–2.5 μm. However the mass of the

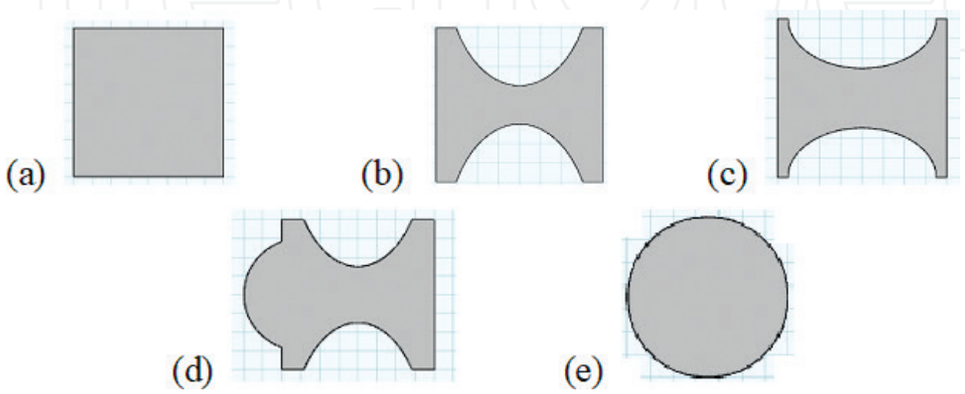

Figure 10. Different shape for microchannel (grooves).

bacteria particle of *K. pneumoniae* changes with the size of the particle and remains in a range of 10^{-12} to 10^{8}Kg. Depending on the volumetric flow rates in microfluidic system for a hydrodynamic filtration method the flow rates for the separation of *K. pneumoniae* bacteria from the sample can be precisely separated. Similarly the flow rates depending on the fluid viscosity and density can test at different flow rates in microliters (µl) numerous times for the inlets. According to model designed in (**Figure 7**) can be tested with two or multiple types of particles differing in size and shapes for the separation however it is possible for experimentation and simulation in software like COMSOL Multiphysics 5.2a, Intellisuite and other related tools. Whereas the clogging problem in the channel can be reduced using this structure which is a major issue for separation in micropatterned channels. Thus can achieve higher throughput sorting when a separation occurs at every intersections and robust against the problem of clogging in microchannel.

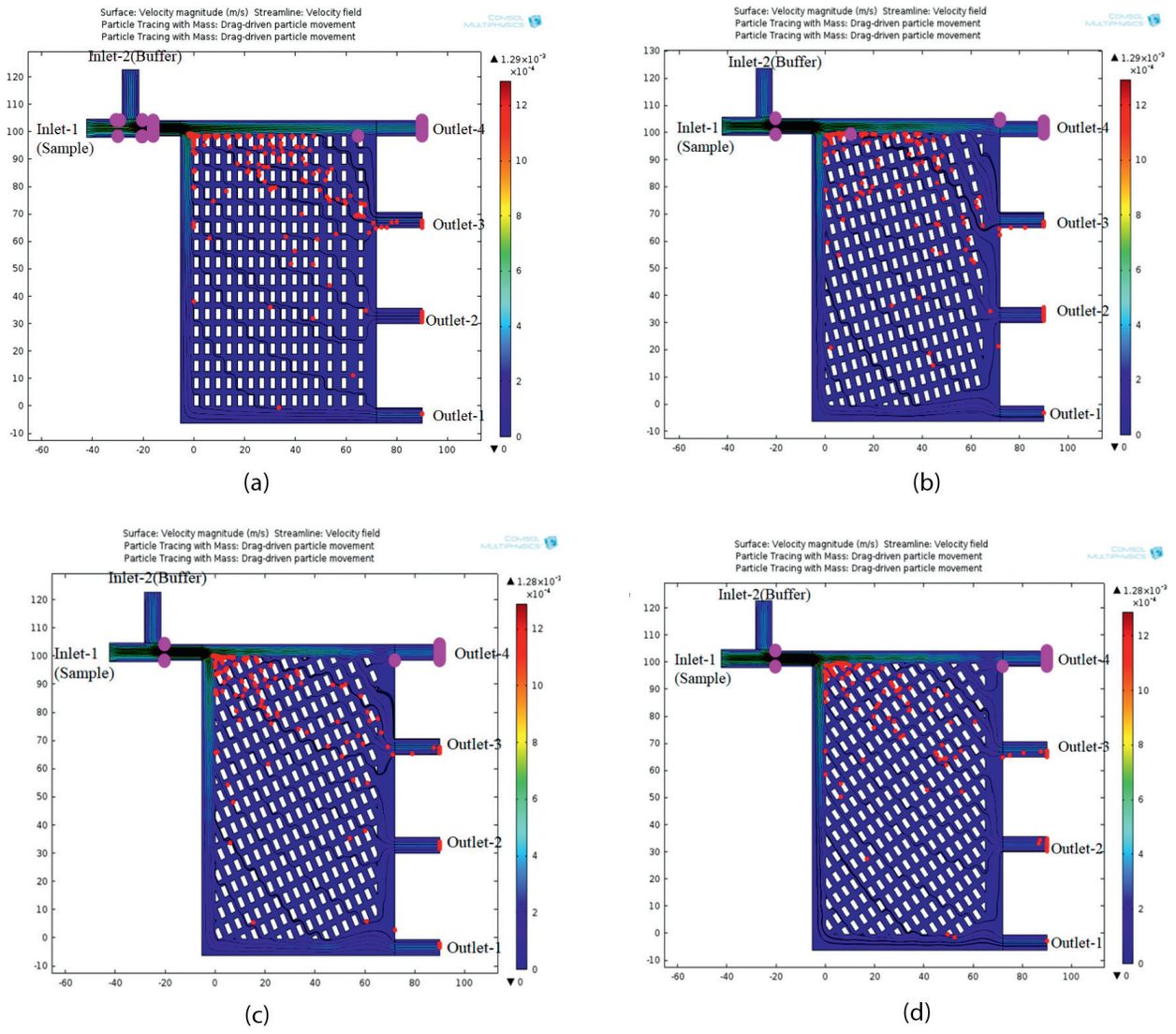

Figure 11. Shown in (a), (b), (c) and (d) Sieve type-shaped Microchannel at different angles with water solution at 50 µl/s of fluid flow rate in water. Similarly tested at different flow rate for different angles with measureable flowrates and simulated with blood sample properties also with the software COMSOL Multiphysics 5.2a.

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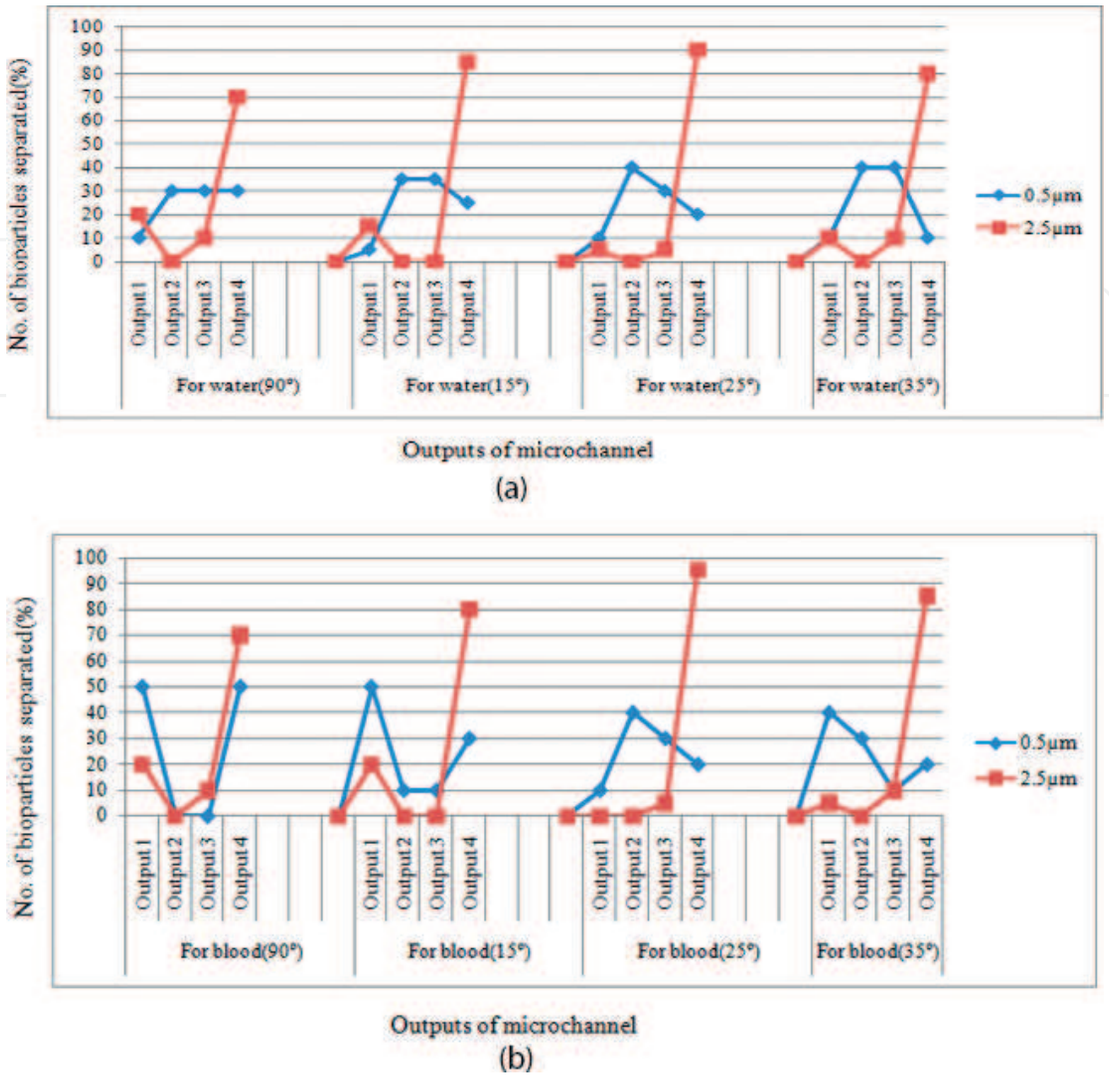

Figure 12. (a) Outputs for microchannel vs. Number of bioparticles seprated(%)(m/s) plot when water is passed through grooves at 50 µl/s for different angles, (b) Similarly, Outputs for microchannel vs. Number of bioparticles separated(%) (m/s) plot when blood is passed through grooves at 3000 µl/s for different angles.

Sieve type microchannel network is designed with main channel and a separation channel where the cells with sample are continuously introduced through an inlet-1 and the other inlet-2(buffer) through which the sample is injected without cells to precede the sample in the flow to decrease the fluctuations in flow when moving through the chip. Overall outputs (Four outputs) through which bacteria particles can be counted at it rates and pressure by which separation can be easily identified. As per the experimental simulations shown in **Figures 11** and **12** (Graphs) it give a probable results by which the larger particles of different mass are been detected through output-4 and the smaller particles of same mass and size are relatively sorted through the other outputs depending of the fluid flow rates which are mostly detected from output-3 and output-2. However Smaller particles will reach the ceiling (upper region) of the primary channel But the larger particles cannot because of the hydrodynamic effect. Owing to the change in frequency of particles they gets shifted toward right direction according to the fluid rate and buffer effect by which paticles are individually identified

through outputs. Similarly different shapes of particles like non-spherical or rod-type shape can be utilized and detected through microchannel network for sorting. By this system of sieve network it achieves a high throughput sorting while separation occurred at every intersection and is robust against the issue of clogging in microchannel network [6].

Micro device having sieve-shaped micropatterned channel has been designed and simulated for a distinct slanted angles at 90, 15, 25 and 35° through which separation of mass and size-based particles from a sample was carried for *K. pneumoiae* beads (by using its properties). The model for the Sieve type microchannel has been done based on the equation for critical diameter, Navier-stokes equations analysis and accordingly depending on the mass and size of the particle it was separated through the main and separation channels of micropatterned chip of sieve type model. The fluid flow simulation was tested with blood sample and distilled water properties in simulation at different angles of channels by which a bigger particle as well as smaller particles were separately identified via outputs for the precise separation (**Figures 11** and **12**). The main channel and the separation channel were crossing at right angle and also the particles with the size of 3 and 8 μm (aspect ratio = 0.3) were separated through outputs at various flow rates in μl/s for blood and water mixed with properties of *K. pneumoniae* beads by micro channel network as the size of the *K. pneumoniae* varies from 0.5 to 2.5 μm shown in **Figure 11**.

We examined the model of separation at different input flow rates concerning about 13, 20, 25, 50, 66, 80 μl/s with water and 133, 666, 800, 1000, 3000, 5000 μl/s with blood by which the separation behavior of the particles using COMSOL Multiphysics 5.2a were simulated and calculation is done. Similarly, at various angles of the channels the samples through input were simulated for the quick and precise performance for the sorting of bacteria at different fluid flow rates mentioned shown in **Figure 12**.

Finally a discussion can be made for the *K. pneumoniae* particles that it can be examined with different size of the spherical or non-spherical shape cells for separation which has been a big issue in hospitals and routine lifestyle. Since infections *K. pneumoniae* are air borne type which is controlled by mucosal vaccination through pulmonary route and nasal can be an assured approach in order to resemble natural infection. Other than traditional vaccines new vaccines like subunit vaccines are safer but they have less immune response [30]. Therefore to enlarge their immunogenicity, dynamic and delivery systems and safe adjuvant are necessary to be developed. Thus by using micropatterning system the sorting of particle from samples can be easily invented [30]. Furthermore this microchannel network can be carried for different purposes for biological or industrial work and even for the separation of rod-type particles from the *K. pneumoniae* by improving separation efficiency and change or modification in design for the better cause.

4. Conclusion

There was an good development in past, where various microfluidic techniques were developed which involves application in enrichment, particle sorting and detection. However

expected high throughput and clogging problem are the facts which has to be properly experimented for higher purpose of biological testings, their conditioning and patterning related fabrication techniques for developement with some characteristics and precise separation of cells should be one of the top priorities. Thus sieve-typed microchannel network can be a precise separation chip network which is efficient for hydrodynamic cell in microfluidic device of size and mass based separation. Effective for mucosal vaccine delivery systems, while flow can be related for immune protection against *K. pneumoniae* infection and suitable for group vaccination programs resulting in considerable health as well as economic benefits. Therefore to contribute to the different successful collaborations at this stage where the stability and the robustness of the microfluidic viability should be taken into care to steadily contribute to successful collaborations between different biomedical, clinicians, biologists, and other the microfluidic association.

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