A COMPACT MICROFLUIDIC CONTINUOUS FLOW SEPARATOR FOR PARTICLE AND CELL SORTING

Peter Lillehoj, Nan Li, Hideaki Tsutsui, and Chih-Ming Ho
Mechanical and Aerospace Engineering Department, University of California, Los Angeles, USA

ABSTRCT

This paper presents the development of a microfluidic device capable of continuous particle and cell separation. Pillars are placed within the microchannel to alter the fluid flow pathway, allowing particles of a certain size to be diverted toward a specified route. Polystyrene beads, 45 μm and 90 μm in diameter, were used to characterize the device; 100% of the larger particles and 94% of the smaller particles went to their respective bins at a rate of 5 beads/sec. Experiments were also performed with mouse embryoid bodies (EBs), which could also be separated into two groups according to their size.

1. INTRODUCTION

The separation/sorting of micro particles is an important microfluidic process. To date, a wide variety of techniques have been employed for the separation of particles and cells, utilizing acoustic forces, magnetic forces, optical forces, dielectrophoretic (DEP) forces, etc. [1]. These separation techniques require the employment of external force fields and the modification of the particles/cellular entities prior to separation, which are prone to particle damage and complicate the sample collection procedures. To alleviate these problems, researchers have recently been developing separation techniques based solely on the particle's size and the fluidic forces, thus eliminating the need for external fields or bead attachment. During separation, specimens remain intact, which eliminates damage resulting from bead attachment or applied fields and simplifies sample preparation procedures for lab-on-a-chip biological/chemical analysis. Ultimately, this method is a simple approach and offers greater compatibility with the specimens and buffers that can be used. Furthermore, by utilizing this technique onto a compact microfluidic platform, additional advantages of lower costs associated with production and smaller reagent consumption is achievable. Recent research on size-based separation has led to several techniques, including pinched flow fractionation (PFF) [2, 3] and deterministic lateral displacement [4]. Both these techniques allow for continuous particle separation, which is crucial for achieving large scale processing. Recently, researchers have fabricated microdevices which make use of deterministic lateral displacement, to separate human blood cells [5, 6].

Although the sorting techniques mentioned above are capable of high efficiency separation, their applications are geared toward smaller sized particles and cells (< 20 μm). An advantage of the presented device is its ability to simultaneously separate particles with very large size differences (from 0 – 100 μm), allowing for the separation of a wide variety of biological particles, which differ in orders of magnitude in size. Specifically, the device presented in this paper has been utilized to separate mouse embryoid bodies (EBs), which range from tens to hundreds of microns in size. EBs are spherical aggregates of spontaneously differentiating embryonic stem (ES) cells and the formation of EBs is an important step in many of differentiation protocols of ES cells. Existing methods of EB formation suffers from either heterogeneous size distribution or lack of scalability [7]. Therefore, continuous sorting of EBs by size can be a valuable tool for obtaining a large number of homogeneous EBs, potentially facilitating differentiation of embryonic stem cells. Unfortunately, a major drawback to studying EBs is the current manner by which they are separated (pipetting); it is time consuming, inefficient and results in poor size uniformity. Additionally, separation of EB cells through external force fields, especially electromagnetic fields, may raise potential issues in damaging the fragile cellular entities, thereby affecting the subsequent cell differentiation. This method of separation is solely based upon the modification of a fluid flow pathway within a microchannel through the strategic positioning and geometry of micro-sized pillars.

In this paper, computational modeling of the flow profile will be presented to guide the rationale in designing the device. This will be followed by detailed fabrication procedures and specific information regarding the experimentation. Next, device calibration and corresponding separation efficiency using polystyrene beads will be discussed. In conclusion, preliminary data on EB cell separation will be presented.

2. COMPUTATIONAL MODELLING

Computational simulations of the flow streamlines were performed using a finite element solver (COMSOL multiphysics) (see Figure 1). A 2D incompressible Navier-Stokes equation was solved with no-slip boundary conditions at the channel walls and pillars. Constant volume flow rates were assumed at both inlets and the outlet wells were fixed at atmospheric pressure. The simulations were utilized to design the geometry and spacing of the pillars; by changing these parameters, the resulting modifications in the streamlines could be observed. Visualization of the streamlines enabled us to predict the flow pathway of different sized particles, as well as optimize the configuration of the channel. Simulations were also performed for varying flow rates of the sample and the sheath solutions.

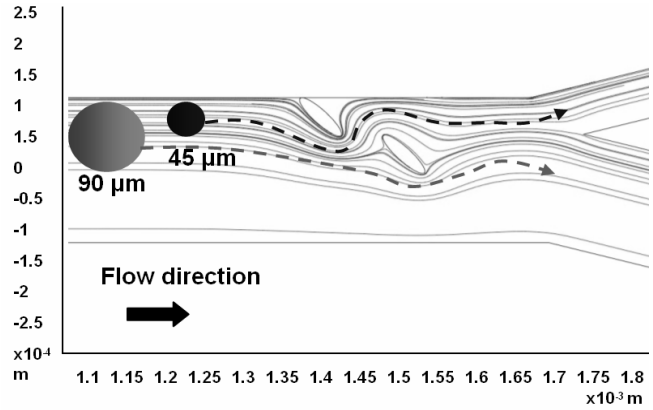

Figure 1. Computational simulation results showing the pathlines within a microchannel for separation into two groups; 0 - 50 µm and 50 - 100 µm. Particles are superimposed to indicate which pathlines they are going to follow.

3. FABRICATION PROCESS

A simplified schematic of the fabrication process is presented in Figure 2. The PDMS mold was fabricated on a silicon wafer. The wafer was first cleaned in a Piranha bath to ensure proper adhesion of the resist layer. Next, photolithography was performed to pattern SU-8 photoresist (MicroChem, Newton, MA) as an etch mask. The wafer was then etched using Deep Reactive Ion Etching (Unaxis Versaline), resulting in smooth, near-vertical sidewalls. Once the mold was fabricated, PDMS (Sylgard 184, Dow Corning) was mixed and degassed to remove bubbles. The mixture was poured onto the Si mold and cured for 2 hours at 80°C. Once the PDMS hardened, individual chips were cut and the inlet and outlet holes were punched. Prior to bonding, PDMS chips and glass slides (Fischer Scientific, Tustin, CA) were cleaned in an oxygen plasma.

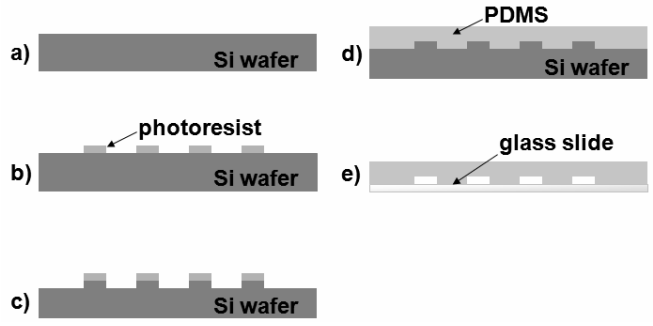

Figure 2. Schematic of the fabrication process for the particle separation devices.

A photograph of the fabricated device is presented in Figure 3A. The overall size of the PDMS chip is 15 mm x 15 mm. The width of the main channel where the separation occurs is 225 µm; the widths of the upper and lower branches are 70 µm and 170 µm respectively. The diameter of the outlet wells are 4 mm. The relevant pillar geometry and dimensions are presented in Figure 3B. The pillars are distinctly shaped as compressed ovals, having lengths and widths of 80 µm and 20 µm respectively. The pillars are positioned at a 45° angle with respect to the upper edge of the channel. The horizontal spacing between the pillars is 60 µm. Essentially, the separation region, where the particles are sorted, spans only ~200 µm. Although the separation region has already been optimized for length, further work could be done to decrease the overall size of the device, thus allowing for increased batch-fabrication and further reducing the footprint of the system.

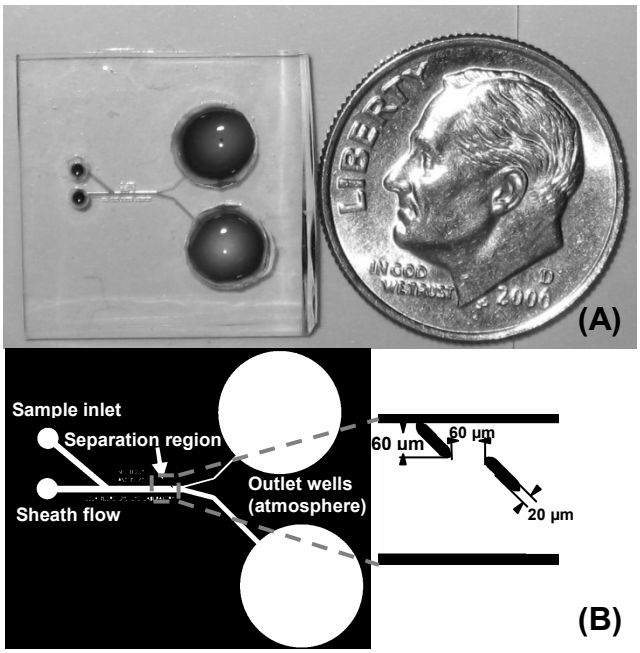

Figure 3. (A) Photograph of the device for separating 45 µm and 90 µm particles. The overall size of the device is roughly 15 mm x 15 mm. (B) Schematic diagram showing the different regions of the microchannel.

EXPERIMENTAL PROCEDURES

Polystyrene beads and Reagents

Polybead® microbeads were purchased from Polyscience Inc. (Warrington, PA). Phosphate buffered saline (PBS), bovine serum albumin (BSA), glycerol and Tween 20 were purchased from Sigma (St. Louis, MO).

EB preparation

Mouse embryonic stem cells were cultured on irradiated mouse embryo fibroblast (MEF) cells in Knock-Out Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, CA) supplemented with 15% fetal calf serum (HyClone, Logan, UT), 1000 U/mL leukemia inhibitory factor (Millipore, Temecula, CA), 100 µM 2-mercaptoethanol (Sigma-Aldrich), 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, 0.1 mM non-essential amino acid (all from Invitrogen). Prior to EB formation, ES cells were transferred to gelatin coated tissue culture dish and cultured for two passages to eliminate any residual MEF cells. For the generation of EBs, ES cells were trypsinized and plated (10^5 cells/well) in Ultra Low Attachment 6-well plate (Corning, Corning,

NY). Culture of EBs was carried out in Knock-Out Dulbecco's Modified Eagle Medium supplemented with 15% fetal calf serum (Omega Scientific, Tarzana, CA), 2 mM L-glutamine, 100 μM 2-mercaptoethanol, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.1 mM non-essential amino acid. EBs were fed with fresh medium every other day and cultured for 3 days before separation experiments. All cultures were maintained in a 37°C humidified incubator supplemented with 5% CO_2.

Experiment Setup

The experiments were performed under a Leica inverted microscope equipped with a MotionPro MP1000 high speed camera (Redlake MASD LLC, Tucson, AZ). Videos were captured using Redlake MiDAS software at a speed of 30 frames/second. The particle and buffer solutions were transferred into 1 mL glass syringes (Hamliton Co., Reno, NV) and driven by two syringe pumps (Harvard Apparatus, Holliston, MA) at constant flow rates. Tygon tubing (Fischer Scientific, Tustin, CA) of 0.5 mm inner diameter was used to transfer the solutions to both inlets of the device. Prior to running the experiments, a PBS/BSA solution was pumped into the device and set to incubate for 30 - 45 minutes. This procedure aided in priming the channels and allowed BSA to be absorbed onto the surfaces to prevent particle/cell adhesion. The sheath buffer was a solution of PBS and 1% Tween 20 to reduce bubble formation during experimentation. As a final preparation procedure, 5 - 10% glycerol was added to the sample solution to slow particle sedimentation.

4. RESULTS

Separation of Polystyrene beads

Experiments were performed to separate polystyrene beads into two groups; 0 – 50 μm and 50 – 100 μm. Particles smaller than 50 μm followed the corresponding streamlines and remained near the upper wall of the main channel. Due to the spacing between the pillars, the smaller particles were able to move through the gap and follow the path leading to the upper branch (Fig. 4A). The particles larger than 50 μm were also focused on the upper wall of the channel as they encountered the pillars; however they are too large to fit through the gap. Larger particles flow past the gap, under the second pillar, and exit out of the lower branch (Fig. 4B). By observing a simulation of streamlines in the channel (Fig. 1), we can see that the experimental results follow very closely with the theoretical predictions. An advantage of this design is the perfect purity of the 0 - 50 μm collection well; larger particles cannot enter the upper branch due the gap size between the pillars and the narrower width of the branch, thus enhancing the separation specificity. In addition, the smaller particles will always enter their respective bin if they are focused prior to encountering the pillars. From these observations, we determined that the separation efficiency is affected by how well particles are focused and the ability of the particles, particularly the smaller particles, to remain in their streamline throughout the channel. This can be accomplished by maintaining laminar flow, which is easily achievable at this length scale and experimental flow rates.

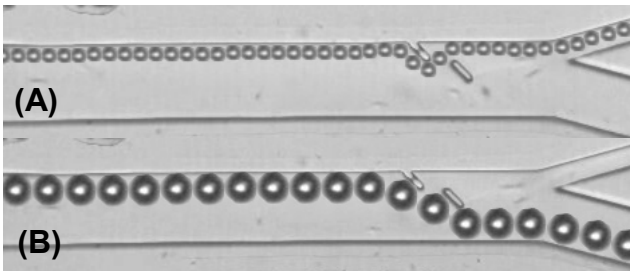

Figure 4. Trace of polystyrene beads. (A) 45 μm in size. (B) 90 μm in size. Sample and sheath flow rates were 1 μL/min and 4 μL/min respectively. Images were generated by superimposing video frames.

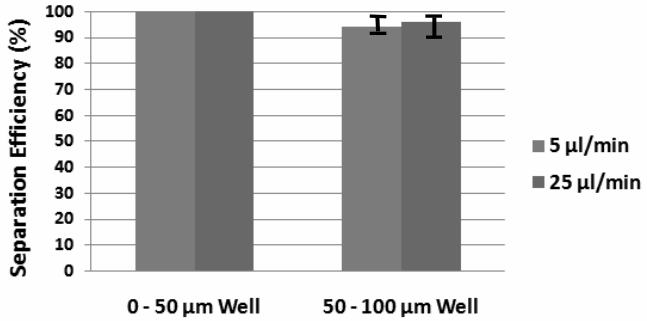

Figure 5. Separation efficiencies at two different flow rates. The separation efficiency refers to the purity of the beads in the collection wells. These results are based on continuous bead separations, excluding clogging events.

A crucial element in ensuring proper operation of this device is maintaining good particle distribution within the microchannel. A problem that occurred occasionally during experimentation would be clogging within the channel, due to particle clumping. Particles clustered together as they entered the separation region would clog the pillars and block proceeding particles. Additionally, clumped particles were poorly focused, causing them to veer outside their intended streamline and flow toward the incorrect well. A solution to alleviate these problems is to ensure that the particles are dispersed within the microchannel; this is performed during sample preparation by diluting the beads to a concentration of approximately 10^3 beads/mL. Unfortunately, diluting the sample solution too much will also reduce the overall throughput of the device. To compensate for this dilemma, experiments were performed at higher flow velocities. The results showed that increasing the flow rates by 5 times did not have negative impacts on the separation efficiency (Figure 5); 100% of the larger particles and ~94% of the smaller particles went to their respective bins at both flow velocities.

Separation of EBs

EBs less than 100 μm were first separated from the main population by pipetteing. This process was required

to isolate the EBs of interest from larger ones that would clog the device. Unfortunately, this technique is not very precise and several larger EBs were collected as well, occasionally clogging the device. The purpose of these experiments, however, was not to separate all EBs from the entire population, but to test the feasibility of this application. Future devices that can separate EBs into multiple size-dependent groups will be designed to accommodate for all the EBs. The results match very closely with those from bead experiments; separation of smaller and larger EBs is presented in Figure 6. The effects of flow rates on EB separation were also studied; increasing the flow rates did not have a negative impact. However, it was observed that having too fast of a flow rate damaged the EBs. EBs traveling at high velocities would break apart upon impact of the pillars. To ensure integrity of the EB cells, it was found that the operational flow rate should be less than 20 μL/min.

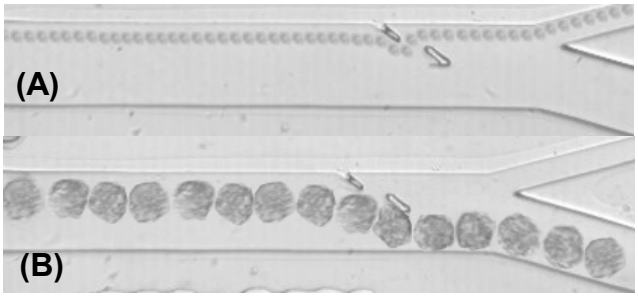

Figure 6. Trace of mouse EBs. (A) 30 μm in size. (B) 100 μm in size. Sample and sheath flow rates were 1 μL/min and 4 μL/min respectively. Images were generated by superimposing video frames.

The issue of clogging that was previously addressed was also evident during the separation of EBs. The frequency of EB clustering was more prevalent than during bead experiments. Additionally, smaller EBs isolated for this experiment were not fully mature, resulting in non-spherical shaped cell formations. The uniformity of the EBs plays a crucial role in the proper operation of this device. EBs that were inhomogeneous in shape are likely to either shift toward different streamlines further down the channel or clog the device. While it is not required for EBs to be perfectly spherical in shape, they need to poses a certain degree of homogeneity so that they can follow the separation principals of this device. This is possible through the study of EB growth and formation, which is outside the scope of this work.

5. CONCLUSION & SUMMARY

A compact device, which is capable of separating polystyrene beads and EBs has been demonstrated. The unique design employed in this device allows for separation into size-dependent groups with a greater degree of uniformity. Compared with conventional microfluidic devices employing similar separation techniques, this method offers two orders of magnitude reduction in the separation region, while still achieving the same functional purposes for particle separation. This is possible through the innovation of this method, which is the simultaneous sorting of all the particles within a narrow separation region. Experimental results qualitatively matched closely with the simulations, thus enabling for rational designing of the device. Potentially, this technique could be employed for the separation of cells, such as erythocytes and leukocytes in blood. The ability to separate whole blood into its sub-populations on a handheld device will aid the medical diagnostic in remote areas and this proposed microfluidic sorting device presents one of the key components to achieving such a device for further portable point-of-care medical diagnosis

Future work will be directed toward designing a device capable of multi-level separation. This will be achieved by applying the principals of the current device and adding additional pillars within the microchannel.

6. ACKNOWLEDGEMENTS

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