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Particle Alignment in a Spinning Helical Microchannel

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Abstract: *Curved microfluidic channels are widely used in biomedical areas due to its compact structure and the physically interesting flow phenomena. Separation of particles based on different sizes, detection of pathogenic bacteria and isolation of leukocytes from whole blood with the aid of spiral and helical microchannel are typical applications for this. Several microfluidic point-of-care (POC) devices are there for monitoring HIV/AIDS by counting CD4 cells but researches are still pursuing to develop a more efficient low cost device that can be used in developing and underdeveloped countries. The present study was focused on developing a CD4+ T-cell counting device for HIV/AIDS monitoring with the aid of a helical microchannel. Numerical studies were carried out in a stationary and spinning helical microchannel to compare the effect of pressure drop and flow distribution for a high initial pressure and varying spinning speed and thereby stable conditions for the experiment was derived out. For the experiment 10 μm sized particles were used for visualization with a fluorescence microscope system. A sample with the viscosity as that of blood and other samples with different viscosities were also prepared to determine the effect of density and viscosity in aligning the particles. The samples were then injected into the channel and the particles were then traced in stationary and spinning channels. The channels were spun using a DC motor controlled by an Arduino board with a Bluetooth shield. It was found that for low viscosity samples the particles were not aligned when the channel was kept stationary and an alignment was achieved when the channel was spun due to the combination of centrifugal and gravitational forces. For a sample with viscosity as that of blood, alignment of particles was obtained even without spinning. Since an alignment of particles was achieved for a sample with viscosity as that of blood, the same approach can be applied for aligning and counting CD4+ T cells in blood samples from patients.*

Keywords: *Helical microchannel, spinning, alignment, particle, CD4 cells*

1. INTRODUCTION

Curved microfluidic channels are widely used in biomedical areas due to its compact structure and the physically interesting flow phenomena [1]. Separation of particles based on different sizes, detection of pathogenic bacteria and isolation of leukocytes from whole blood with the aid of spiral and helical channels are typical applications for this [2-5].

Studies predicted the emergence of microfluidic devices as the next generation point of care device which can be integrated with reliable and accurate devices for various biomedical applications [6]. A review study on emerging technologies for point-of-care (POC) T-lymphocyte counting showcase the application and technological developments in determining CD4 cells. It incorporates the details of POC and commercial CD4 counting technologies such as flow cytometry, manual CD4 counting methods, automated imaging cytometry, microflow cytometry, and instrumented and non-instrumented CD4 test devices [7]. A rapid, inexpensive and portable type fully automated microfluidic system was developed to count CD4 cells by processing an enzyme-linked immunosorbent assay (ELISA). The count was achieved by removing the "moving the substrate" in the conventional microfluidic methods [8]. Detection of antirecombinant bovine somatotropin (rbST) antibodies was developed based on cellphone imaging platform for microshphere fluorescence immunoaasay in milk samples [9]. Single nanoparticles and viruses of size 100 nm was demonstrated with a field portable florescence microscopy. The study showed remarkable promise for various point-of-care applications in biomedical filed [10]. A new technology was proposed to release the label-free captured CD4 cells in microchannel without using any fluid shear or enzymes. The study showed that the released CD4+ and CD34+ cells were vigorous for in vitro cell culture [11]. A prototype model was developed for an affordable CD4 lymphocyte counting. The device showed promising results for complex HIV diagnosis [12]. A cheap and time efficient simple microfluidic device was developed for CD4 counting which uses cell affinity chromatography to isolate CD4+T lymphocytes. The CD4 cells counted in the device agreed strongly with the flow cytometry among positive HIV subjects for a wide range of CD4 counts [13].

Recently we designed a particle counting and particle positioning system with an in-house developed florescent smartscope device and DC motor system which aims to develop a compact and inexpensive point-of-care device based on smartphone for developing countries. This motor

controlled particle positioning device can reduce manual processing and it can be integrated with our smartscope for point-of-care testing [14]. In the present work, particle alignment was demonstrated in a stationary and spinning helical micro channels. The optimized conditions for the experiment were derived with CFD simulations and particles were tracked with fluorescence imaging technique. The study includes the effect of density, fluid viscosity and channel spinning in aligning particles for counting.

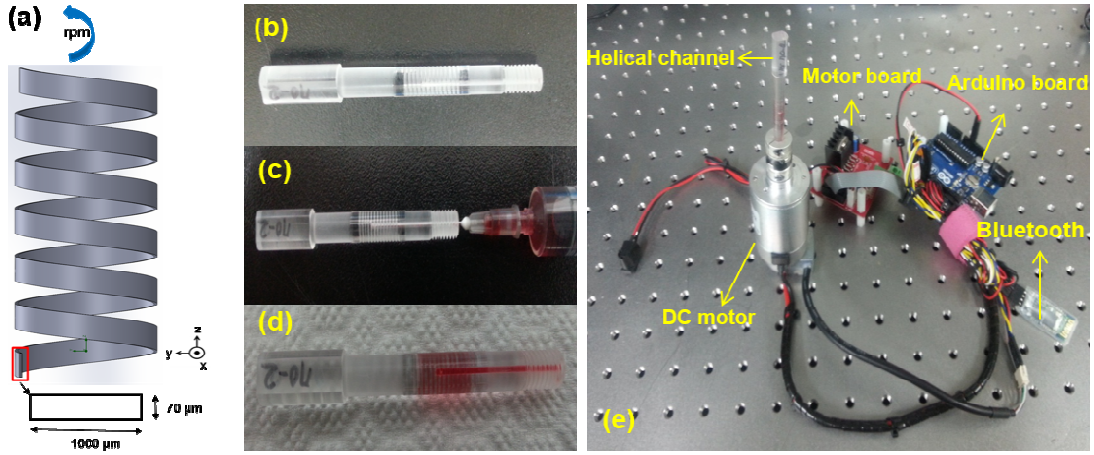

Fig. 1. (a) 3D physical model of a vertically oriented helical microchannel for simulation (b-e) Composition of the helical microchannel setup for the experiment, where (b) helical micro channel structure (c) fluid sample injection (d) filled sample in the channel (e) motor system with helical microchannel

2. MATERIALS AND METHODS

2.1. NUMERICAL SIMULATION

Numerical simulations were performed with the aid of the commercial computational fluid dynamics code SolidWorks Flow Simulation. Figure 1(a) shows the 3D physical model for the simulation with a channel aspect ratio of 14.28 (w/h (width/height) = 1000 µm/70 µm), radius of curvature of 3 mm, pitch = 1 mm and number of turns = 6. Two cases were considered for analysis, and in both cases simulations were performed for samples with two different viscosities, one was normal distilled water and the other, distilled water with 40% glycerol. For the first case, an inlet boundary condition of 50 psi was set and an atmospheric pressure condition was given at outlet for stationary and spinning channels. In the second case, fluid was assumed to be filled with a constant pressure inside for both fluids during spinning. The channels were spun from 500 to 3000 rpm to determine the effect of pressure drop and viscosity. The fluid was set to be steady, incompressible, laminar with no slip and heat conduction. Grid independent studies were conducted from coarser to finer meshes and an optimum mesh size of 5.3 million cells were used for analysis. The governing equation of the solver is given as follows,

$$\frac{\partial p}{\partial t}+\frac{\partial}{\partial x_i}(\rho u_i)=0 \tag{1}$$

$$\frac{\partial \rho u_i}{\partial t}+\frac{\partial}{\partial x_i}(\rho u_i u_j)+\frac{\partial p}{\partial x_i}=\frac{\partial}{\partial x_j}(\tau_{ij}+\tau^R{}_{ij})+S_i i=1,2,3 \tag{2}$$

$$\frac{\partial \rho H}{\partial t}+\frac{\partial \rho u_i H}{\partial x_i}=\frac{\partial}{\partial x_i}\left(u_j\left(\tau_{ij}+\tau^R{}_{ij}\right)+q_i\right)+\frac{\partial p}{\partial t}-\tau^R{}_{ij}\frac{\partial u_i}{\partial x_j}+\rho\varepsilon+S_i u_i+Q_H \tag{3}$$

where, $H=h+\frac{u^2}{2}$, u is the fluid velocity, S_i is mass distributed per unit mass, h is the thermal enthalpy, Q_H is the heat source per unit volume, τ_{ik} is the viscous shear stress tensor and q_i is the diffusive heat flux. The subscripts are used to denote summation over the three coordinate directions.

2.2. EXPERIMENTAL PROCEDURE

Figure 1(b-e) shows the combination of the helical microchannel setup for the experiment, which details all the components such as helical micro channel structure, fluid sample injection, filled fluid sample in the channel and DC motor system with helical microchannel. To determine the particle alignment in the helical microchannel, three kinds

of bead particle samples were prepared. The combination of the samples were as follows, for the first sample, 20% fluorescent 10 µm bead with distilled water and 1% read dye, in the second sample, 20% fluorescent 10 µm beads with 20% glycerol and 1% red dye and for the third sample, 20% fluorescent 10 µm beads with 40% glycerol and 1% red dye. The addition of glycerol was to find the effect of viscosity and the 40% sample's viscosity was same to as that of blood, hence the particle behavior similar to as in blood can be predicted. The prepared samples are then injected into the helical channel one after another using a 5 mL syringe for visualization. The particles in each sample were visualized horizontally using a 10x objective lens (0.3 NA) and a CCD camera (Sensicam, Cooke) in a fluorescent microscope (IX51, Olympus). For spinning the channel vertically, the helical channel was fixed to a DC motor with an encoder for rotation velocity improvement. The DC motor was controlled by an Arduino board with a Bluetooth shield, so that rotation velocity and time can be controlled using a smartphone by connecting to the Bluetooth. Camera focus was adjusted on the top to observe the particle position and particle alignment state. For analyzing the positions of the particles, the fluorescent bead area was checked by using image analysis program (ImageJ, http://imagej.nih.gov/ij/). For the images before spinning and after spinning, the edge of the fluorescent beads (N = 30) was selected and thereby the area of each bead was obtained. We checked the blurring degree by analyzing the area of bead and the depth wise position of the bead was found.

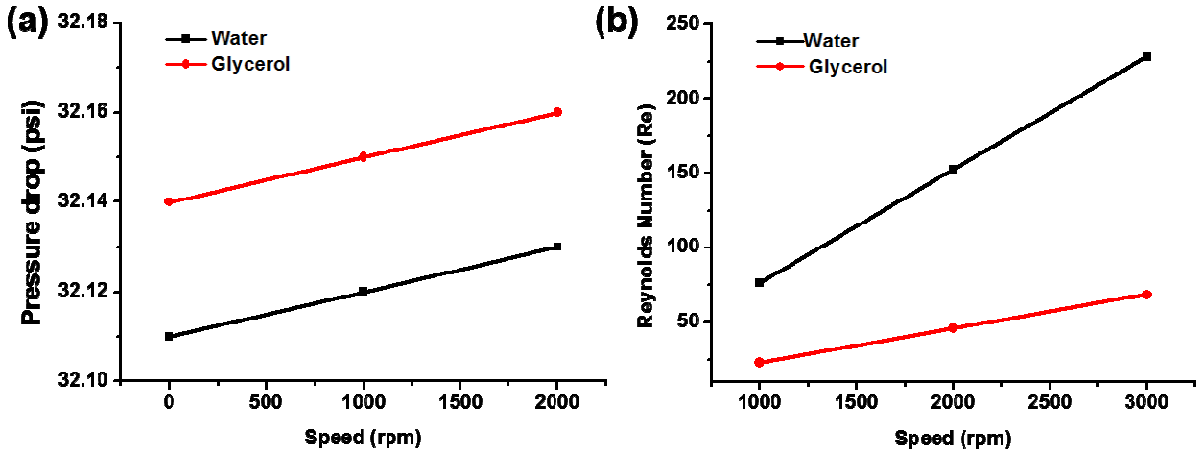

Fig. 2. (a) Variation of pressure drop for water and water mixed with 40% glycerol in stationary and spinning helical microchannels. (b) Reynolds number variation in the spinning microchannel with respect to different spinning speed (rpm) for water and water mixed with 40% glycerol

3. RESULTS AND DISCUSSION

Figure 2(a) shows the pressure drop in the helical microchannel as a function of spinning speed for water and water with 40% glycerol under different rpm. From the graph it was found that a high pressure drop was there in the channel and also as the channel spins the pressure drop tends to increase slightly as the speed of spinning increases. An increased pressure drop will affect the particles movement in the channel [15], so it was not advisable to conduct experiments at a high input pressure boundary condition for the present setup. Figure 2(b) shows the variation of Reynolds number in the microchannel with a constant pressure boundary condition inside for the working fluids, water and water mixed with 40% glycerol under increasing spinning speed. It can be seen from the graph that, the Reynolds number takes a value higher for water compared to that of water with 40% glycerol. Since there was no continuous flow inside the channel, there will be no velocity gradient along the axial direction, hence both the fluid takes almost same velocity as that of channel's spinning speed. The difference in the Reynolds number was mainly due to the difference in fluid viscosity so there will be an impact of fluid's viscosity with respect to an increasing speed inside the channel. Studies showed that, the effect of viscosity is significant in the microchannel for particle movement [16], so for the present case the effect was discussed along with the experimental results. Based on the preliminary findings, experiments were carried out to determine the particle alignment in the helical microchannel.

Figure 3 shows the alignment of particles in the microchannel with the first sample (0% glycerol), second sample (20% glycerol) and third sample (40% glycerol) before and after spinning. From Figure 3(a, b) it was found that after filling the channel and kept stationary, no particle alignment was obtained in the microchannel cross-section. When the channel was spun at 2000 rpm for 1 min, the particles were moved to the top due to the centrifugal force and as a result all particles were found to be seen on the same focus. Figure 3(d, e) depicts that, as in the previous case all particles were scattered inside the channel without spinning as displayed in the graph, but when the channel

was spun at 3000 rpm for 4 min particles were aligned at the same height due to the centrifugal force, which will lift the particles to the desired height towards the top of the microchannel cross section. It can be seen from Figure 3(g, h) for the third sample, particle alignment was achieved when the channel was kept stationary. More quantitatively, the particle positioning can be seen from the graph incorporated in Figure 3(i). Since the density of bead is smaller than that of 40% glycerol sample, the effect of gravitational forces will be minimal so the particle positioning was obtained even without spinning. In the other cases the density of bead was higher than that of fluid and also the fluid was less viscous so the particles will tend to move down from its initial position. When the channel was spun at 3000 rpm for 1 min, the particles approached little more to the top due to the effect of centrifugal force by keeping the same focus, but the displaced position was found less than that of previous samples. As we can see from Figure 2(b), the Reynolds number of 40% Glycerol sample was lower than that of 20% and 0% glycerol sample so the effect of fluid velocity in lifting the particles will be minimal in contrast to others. Also, here in this case, the fluid was highly viscous compared to other cases so the suspension due to particle size is negligible compared to a less viscous fluid [16].

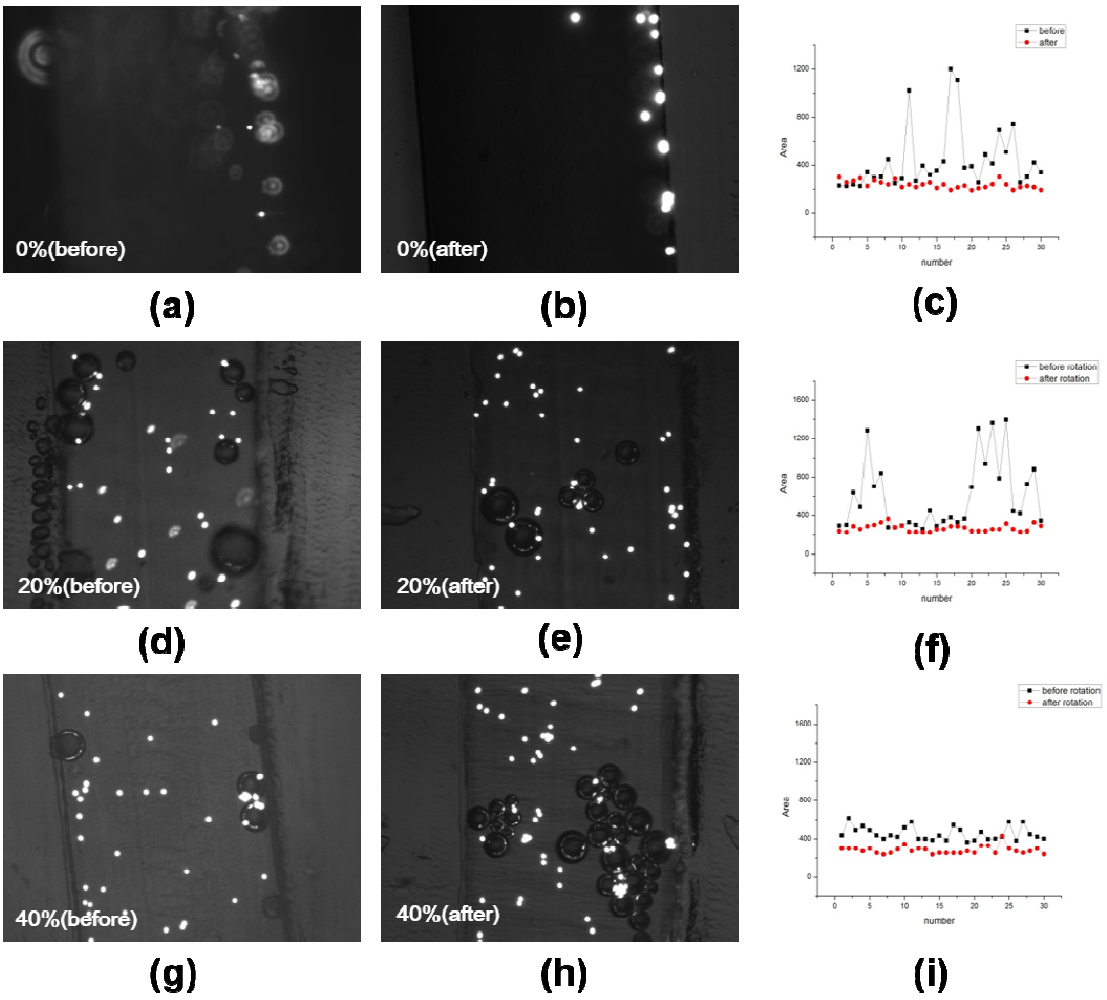

Fig. 3. Alignment of particles in the microchannel cross section for (a, b, c) first sample (0% glycerol), (d, e, f) second sample (20% glycerol) and (g, h, i) third sample (30% glycerol) before and after spinning

4. CONCLUSIONS

Particle alignment was investigated in a stationary and spinning helical microchannel systems using different fluid samples. CFD simulation results showed the effect of pressure drop and viscosity in stationary and spinning channels and thereby the optimum conditions for the experiment were derived out. It was found that when the channel was made stationary, no particle alignment was achieved for a sample with viscosity (0% and 20% glycerol) lower than that of blood, but when it was spun from low to high speed, an alignment was obtained at the top of the channel. When a sample with viscosity as that of blood was

used, particle alignment without spinning was obtained due to the difference of particle-fluid density and also because of the high viscous nature of fluid. The study demonstrates the effect of density, viscosity and centrifugal force in aligning particles. Since an alignment of particles was achieved for a sample with viscosity as that of blood, the same approach can be applied for aligning and counting CD4+ T cells in blood samples from patients for HIV detection.

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