EVOLUTION OF SECONDARY DEAN VORTICES IN SPIRAL MICROCHANNELS FOR CELL SEPARATIONS

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ABSTRACT

Spiral inertial microfluidic channels have been used for cell sorting with high efficiency and throughput, based on the assumption of two counter-rotating Dean vortices in the channel cross-section. However, this assumption does not explain the shift in focusing of the cells or particles upon increase in the flow rate. In this work, for the first time we show presence of a secondary set of Dean vortices in spiral microchannels. This observation redefines the way Dean flows are used for cell focusing at higher flow rates, potentially leading to higher efficiency separations at ultra-high throughputs.

KEYWORDS: Spiral inertial microfluidic channels, Dean flow, cell/particle sorting.

INTRODUCTION

Inertial microfluidics takes advantage of hydrodynamic forces (e.g., shear gradient and wall induced lift forces) acting on cells to focus them within the flow. These forces cause cells to migrate across streamlines and order in equilibrium positions based on their size, leading to label-free cell separation, purification and enrichment in a microfluidic device. In spiral microfluidic channels, these hydrodynamic forces are balanced by Dean drag arising from secondary flows and channel curvature [1-3]. The result of this new force balance is a single focusing position near the inner channel wall (Fig. 1), permitting continuous separation of cells or particles according to their size. This concept, for example, has been used by Kuntaegowdanahalli et al. [2] to separate neural cells in an Archimedean spiral and Guan et al. [3] to separate blood cells.

Figure 1: (a) Image of a typical spiral sorting device in PDMS [1]. (b) Schematic of the focusing principle based on two-dean vortices (F_L is the net lift force and F_D is the Dean force).

In a curved rectangular channel, the laminar Poiseuille flow is subjected to a centrifugal force (F_CF) and centripetal force (F_CP) (Fig.2a). These forces disrupt the parabolic profile of the laminar flow and cause the maximum point of velocity to shift from the center of the channel cross-section towards the concave wall of the channel. A sharp velocity gradient develops near the concave wall between the point of maximum velocity and the outer concave channel wall where the velocity is nearly zero. The decrease in the centrifugal force on the fluid near the concave wall leads to a stronger pressure gradient between the concave and convex channel walls (Fig.2b). The local velocity near the channel walls is not significant enough to provide complete balance of the pressure gradient, causing rise to hydrodynamic instability and development of secondary flow from the concave to the convex wall of the channel. This secondary flow manifests itself in the form of two counter-rotating vortices, known as Dean flow. Dean [4] defined a non-dimensional parameter, Dean number (De) to characterize the flow in curved channels:

\[ De = \frac{Re}{D_h} \left( \frac{1}{2R} \right) \]

where \( Re \) is the Reynolds number, \( D_h \) is the microchannel hydraulic diameter and \( R \) is the radius of the curvature of the convex surface of the curved channel. Hence, the strength of the secondary Dean flow is strongly dependent on the dimensions of the channel and the radius of curvature. As the curvature of the channel decreases with respect to the aspect ratio, there is increase in \( De \) which leads to increase in centrifugal force and there is development of additional regions of pressure gradient near the concave wall. To balance this increase in the pressure gradient, there is formation of additional counter-rotating vortices which recirculate the fluid near the outer channel wall or the concave wall. These vortices are called secondary Dean vortices or additional secondary flow vortices.
METHODS

We used a 3-inlet/1-outlet spiral device with channel dimensions 250 µm×100 µm to observe sequential development vortices as fluorescent dye progresses downstream towards the center of the spiral. Fluorescein (1µM) was pumped in one input (i.e. one-third of the channel width) to allow sufficient time for the dye to follow the streamlines before mixing downstream. The spatial development of the secondary flow was imaged with high-speed confocal microscope (Zeiss LSM710) and the images were taken at the interval of 60º along inner loop of the spiral microchannel at a flow rate up to 3mL/min.

RESULTS AND DISCUSSION

As the laminar flow of dye and DI water entered the curvature of the outer loop, the DI water started moving from concave wall to the convex wall due to the secondary instability causing the formation of primary dean vortices. As the flow moved further, due to increase in pressure gradient near the concave wall, the flow began to recirculate in the area closer to the concave wall (Fig. 3). As the flow continued into the second loop or the inner loop, the recirculation near the outer channel wall became more prominent and it developed into two small counter rotating vortices in addition to the primary Dean vortices (Fig.3f).  These vortices did not fully develop into larger secondary Dean vortices due to lower aspect ratio which requires a higher De to cause further increase in pressure gradient near the concave wall to lead to rapid recirculation. These secondary/additional Dean vortices were very prominent at De>19 and although significantly smaller in size than the primary vortices, they exhibited similar characteristics of counter-rotation and equivalent amplitude.

The effect of multiple vortices on cell focusing was demonstrated using 500× diluted blood sample introduced in the channel. Beyond the critical De, cells experience overwhelming Dean drag which pushes cells into the additional vortices near the inner channel wall leading to their entrapment in those vortices. Hence, RBCs (red blood cells) initially focused closer to the inner channel wall when the flow rate was <2mL/min (De < 14), but shifted toward the outer wall as flow rate increased to 3mL/min (De > 19) (Fig.4a,b).

These experiments lead us to conclude that cells are getting trapped in the secondary vortices, causing them to focus near the outer channel wall. Fig.4c suggests a possible path that cells may take to shift from primary to secondary Dean vortices.

To confirm our theory of entrapment, a sample of 10µm diameter fluorescent polystyrene particles was introduced at 3mL/min flow rate. The particles were trapped in the additional vortices and hence focused near the outer/concave wall.
channel wall. We measured fluorescence intensity of the entrained particles in an attempt to better understand the process. The results are presented in Fig. 5. The measurements were performed at five positions in the bottom half of the channel, spanning one of the lower secondary vortices (Fig. 5b). The intensity peaks at each position illustrate motion of the particles that get trapped in the vortex. Single peaks at positions Z1 (near centerline) and Z5 (near bottom) show a narrow region where a particle can get entrained. Both peaks are at approximately 75 µm from concave sidewall, which is expected for particles at the top and the bottom of the secondary Dean vortex as was shown in Fig. 4c. The two peaks at vertical positions Z3 and Z4 illustrate two particles entrained in the same horizontal plane, suggesting particles are located on two sides of the secondary Dean vortex. Thus, our confocal measurements support the concept of particle entrapment in secondary vortices.

CONCLUSIONS

This work improves our understanding of particle focusing in spiral microchannels. It finally explains apparent defocusing of cells and their shifts in focusing positions at higher flow rates, and paves the way to understanding the need for a precise range of $De$ for effective cell focusing and efficient cell separations. Ultimately we hope that improved understanding of Dean flows in spiral microchannels will lead to better designs of spiral inertial microfluidic devices with higher throughput and efficiency for numerous cell sorting applications.

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REFERENCES


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