

PHENOTYPE-DEPENDENT AND INDEPENDENT INERTIAL FOCUSING

Soojung Claire Hur, Mahdokht Masaeli, and Dino Di Carlo

University of California, Los Angeles, USA

ABSTRACT

The ability to precisely position flowing particles in microchannels would aid development of clinically useful cell identification and purification technologies. Here, we investigated the effect of fluid inertia on lateral focusing positions of flowing deformable cells over a wide range of flow rates. It was found that, at lower flow rates, cells can be differentially positioned in the lateral direction based on deformability variations, while, at higher flow rates fluid inertia dominates and all cell types, regardless of their mechanical properties, were more uniformly positioned. The presented study provides improved design criteria for high-throughput cell manipulation system development.

KEYWORDS: Inertial focusing, phenotype discrimination, flow cytometry, cell separation

INTRODUCTION

Positioning of cells in continuous flow is important for research and clinical diagnostics as a critical component of flow cytometers (FC). Recently, we exploited inertial effects in microfluidic systems for sheath-free, parallel focusing to improve image-based cell identification [1]. Briefly, particles/cells flowing through a high aspect ratio microchannel are focused to unique lateral and vertical positions as a result of counteracting inertial lift forces, specifically wall-effect, F_{WL} , and shear-gradient lift, F_{SL} . However, the deformable nature of cells introduces additional nonlinearity that leads to an additional force away from the channel wall, F_{DL} , counteracting F_{SL} (Figure 1). We recently observed that deformability and size differences among cell types resulted in different focusing positions in the lateral direction [2].

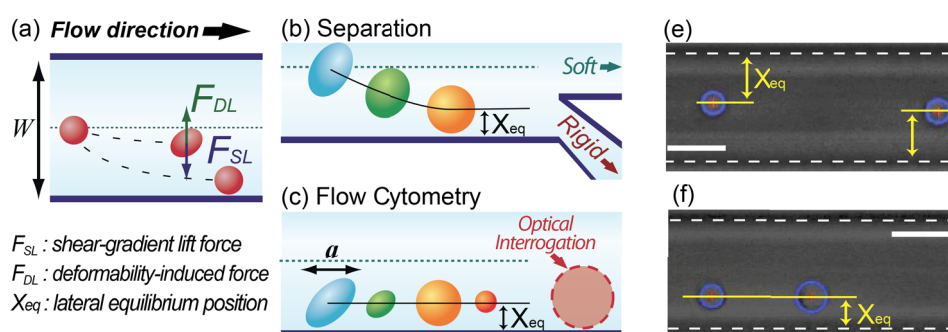


Figure 1 (a) Schematic of lift forces acting on flowing deformable cells in inertial microflows. (b) At moderate flow rate ($R_c \leq 21$), F_{DL} is dominant in determining X_{eq} of flowing cells, but (c) F_{DL} becomes less significant at higher flow rate ($R_c > 21$). Here, the channel Reynolds number, R_c , is defined as $R_c = \rho V D_h / \mu$ where ρ , V , μ are the density, the maximum velocity, and the viscosity of fluid and D_h is the channel hydraulic diameter. High-speed microscopic images of undifferentiated hESCs at (e) low ($R_c=12$) and (f) high ($R_c=79$) flow rates. The diameter (blue circle) and the center (red cross) of individual cells were measured using a custom-built MATLAB script. Scale bar is $50 \mu\text{m}$.

Non-uniform focusing positions resulting from single-cell physical properties (e.g. size and deformability) can potentially be used to discriminate between cell populations; however, focusing position differences would not be desirable for FC applications in which a larger optical interrogation area would be required leading to the increased possibility of coincident detection of multiple cells and thus dilution and lower analysis rates. Thus, it is essential to identify the flow conditions, for which flowing cells can be differentially or uniformly positioned, in order to satisfy conflicting conditions that deformability induced cell separation and compact flow cytometry require.

EXPERIMENTAL

We systematically investigated the effect of fluid inertia on lateral migration of deformable cells, including normal, benign and invasive breast epithelial cell lines as well as undifferentiated human embryonic stem cells (undiff-hESCs) and their differentiated counterparts (diff-hESCs). Undiff-hESC colonies were maintained as previously described [3]. hESCs were differentiated following previously described protocols without RA treatment [4] and the differentiated state of hESCs was assessed by monitoring the expression level of pluripotency markers (e.g. SSEA4 and OCT4), using immunofluorescence detection of the surface antigen as well as quantitative RT-PCR (see Figure 3 (a-c)).

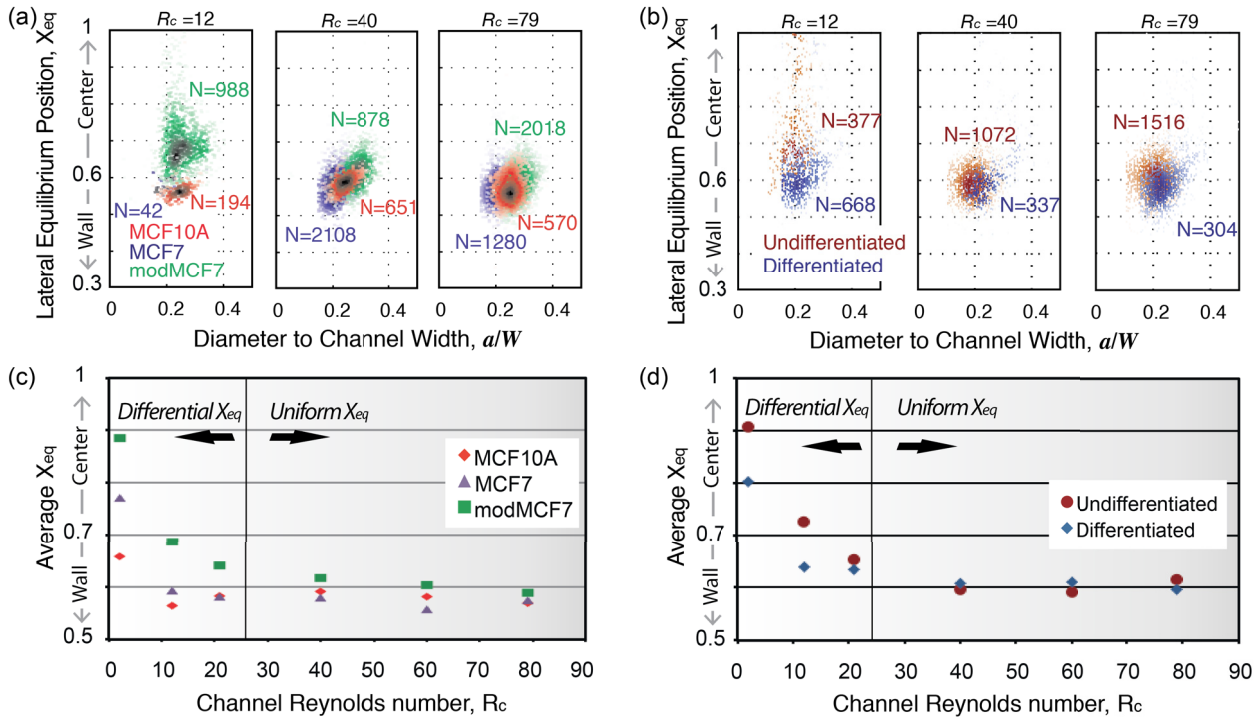


Figure 2 Phenotype-dependent and independent inertial focusing of living cells. Lateral equilibrium position, X_{eq} , of (a) breast epithelial cells (MCF10A) and benign (MCF7) and invasive breast carcinoma cells (modMCF7, chemically modified [5]) and (b) undifferentiated and 14-day differentiated hESCs varies as flow rate increases. Different mean X_{eq} observed for (c) breast-origin cells with varying disease states and (d) hESCs with varying states of pluripotency at low R_c while all tested cell lines were tightly focused at uniform X_{eq} at higher R_c .

For flow cytometry application, we used a straight high aspect ratio channel ($W = 65 \mu\text{m}$, $H = 143 \mu\text{m}$ and $L = 4.5 \text{ cm}$), in which flowing cells are inertially focused at two distinct lateral locations and a uniform height. Data analysis of massive data sets of inertially focused cells flowing through the microchannel using a custom-built MATLAB image analysis code was performed to determine the diameter and the center position of flowing cells with statistical significance (Figure 2). Experimental flow conditions varied from a channel Reynolds number, R_c , of 2 to 80 while cell diameters ranged between 9 and $28 \mu\text{m}$. For cell enrichment, we implemented a single-layered microfluidic device, consisting of one inlet with coarse filters, a straight focusing channel ($W = 70 \mu\text{m}$, $H = 180 \mu\text{m}$ and $L = 4.5 \text{ cm}$), a gradually expanding region, and 7 branched outlets with fluidic resistors (Figure 3 (d)). The cell suspension was injected through the device at $R_c = 20$ and OCT4 expression level of cells collected from each fraction was measured and normalized by that of cells that were not flown through the device.

RESULTS AND DISCUSSION

As shown in Figure 2, at relatively low flow rate ($R_c \leq 21$), the lateral equilibrium positions of more deformable invasive cancer cells and undiff-hESCs were found to be closer to the channel centerline than those for less deformable healthy phenotype and diff-hESCs, respectively, in agreement with previous biomechanical measurements [2, 5, 6]. In contrast, as the inertia of the fluid increases with R_c , all tested cells were tightly focused at a uniform lateral equilibrium position. This suggests that the deformability-induced lift, F_{DL} , and shear-gradient lift, F_{SL} , interact over several regimes. At low Reynolds number, F_{DL} has a significant effect on cell focusing position while with increasing inertia of the fluid this force becomes less significant. This intrinsically high flow rate, required for locating flowing cells at uniform focusing positions regardless of their deformability variation, should be particularly beneficial for flow cytometry applications as an increase in overall processing speed can be expected with inertial focusing.

Furthermore, as a proof of concept towards the cell-based stem cell therapy, we performed the enrichment of undiff-hESCs from undesired impurity cells (e.g. murine feeder cells and differentiated cells). In an agreement with X_{eq} measurements, more deformable undiff-hESCs were slightly enriched in the inner outlets (outlet 2 through 4), as evidenced by the higher expression of a pluripotency marker (Figure 3 (e)). The highest enrichment of undiff-hESCs was observed from cells collected at outlet 2 compared to outlet 3 and 4. This can be attributed to the heterogeneity in cell size distributions, potentially resulting from unsynchronized cell cycles. Further investigation will be required in order to improve the enrichment efficiency.

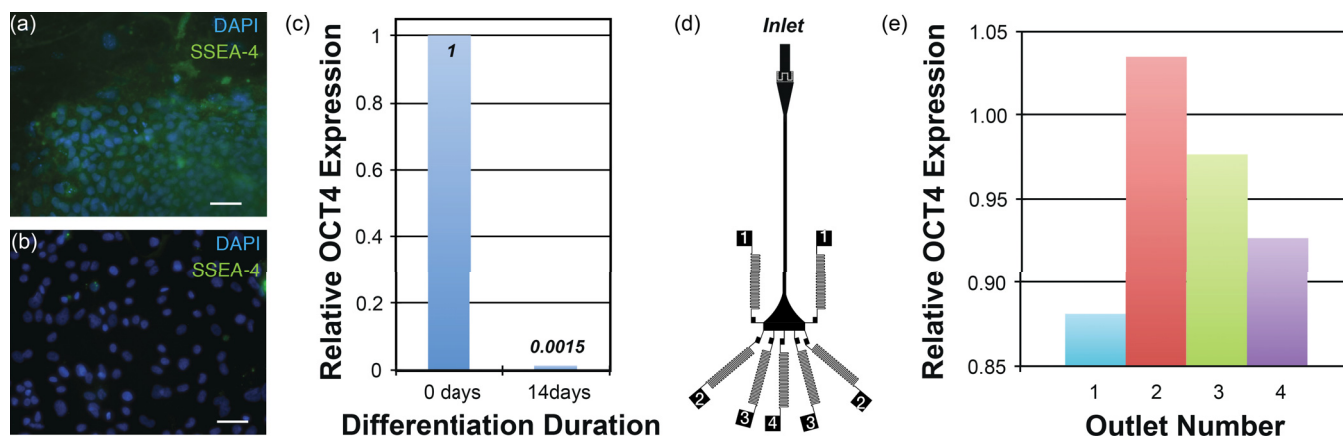


Figure 3 Fluorescent microscopic images of (a) undifferentiated and (b) 14-day differentiated hESCs stained with DAPI and SSEA-4-FITC. Scale bar is 50 μm . (c) Relative gene expression level of a pluripotency marker for undifferentiated and 14-day differentiated hESCs. Both immunofluorescence detection and gene expression measurements confirmed that hESCs have lost pluripotency after 2 weeks of differentiation. (d) Device design used for label-free cell separation based on differential X_{eq} and (e) the relative OCT4 expression level of cells collected from each fraction of the device.

CONCLUSION

Overall, our results suggest that there exist optimum ranges of flow rates for different applications. A relatively low flow rate can be utilized for cell separation and classification (e.g., deformability activated cell separation [2]) whereas a high flow rate is required for uniform positioning (e.g., Flow cytometry). It was also demonstrated that more deformable undifferentiated human embryonic stem cells could be enriched and collected at inner outlets. A systematic understanding of how flow conditions affect the lateral equilibrium positions of deformable living cells will provide a concrete basis for the development of novel microfluidic systems that identify or separate target cells.

ACKNOWLEDGEMENTS

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CONTACT

*D. Di Carlo, tel: +1-310-9833235; dicarlo@seas.ucla.edu