STRESS-FREE CENTRIFUGO-MAGNETIC 2D-SEPARATION OF CANCER CELLS IN A STOPPED-FLOW MODE

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ABSTRACT

This paper presents a refined centrifugo-magnetophoretic platform enabling 2-dimensional separation of cancer cells from background cells [1]. The multi-force, stopped-flow separation combines centrifugal sedimentation with lateral magnetic deflection. In this way, hydrodynamic stresses and variations typical in pressure-driven microfluidic platforms [2][3] are minimized, and collisions between cells and chamber walls, a common disadvantage of stand-alone magnetic separation systems, is avoided. In this work, basic separation of magnetic particles from a background of non-magnetic particles is shown first. Then, magnetic particles functionalized with anti-EpCAM antibodies are used to successfully capture and separate MCF-7 breast cancer cells from background HeLa cells.

KEYWORDS: Centrifugal, magnetophoresis, microfluidics, separation

INTRODUCTION

The interplay between magnetic forces and the unique conditions found in microfluidic systems has been intensively investigated [2]-[4]. Magnetophoretic systems have potential for real-world applications, for example, in systems biology and diagnostics, especially for separation purposes (*e.g.*, for concentration and purification of cells, proteins, or DNA). The development of a microfluidic separation system would have many uses, especially as related to biological cell sorting. The development of a centrifugal microfluidic platform, known for its strengths in sample preparation [5]-[7], would enable coupling with previously established sample preparation functions to enable an integrated cell analysis system.

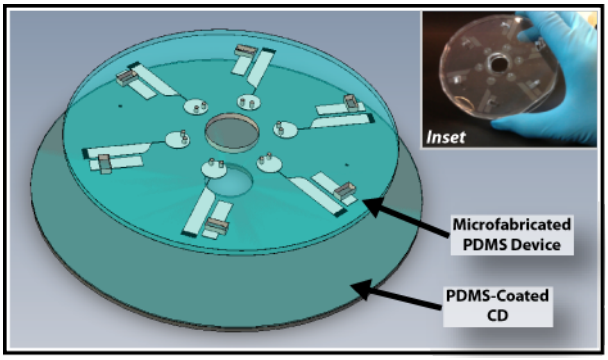

Figure 1: Schematic and photograph (inset) of the centrifugo-magnetophoretic microfluidic device for cell separation.

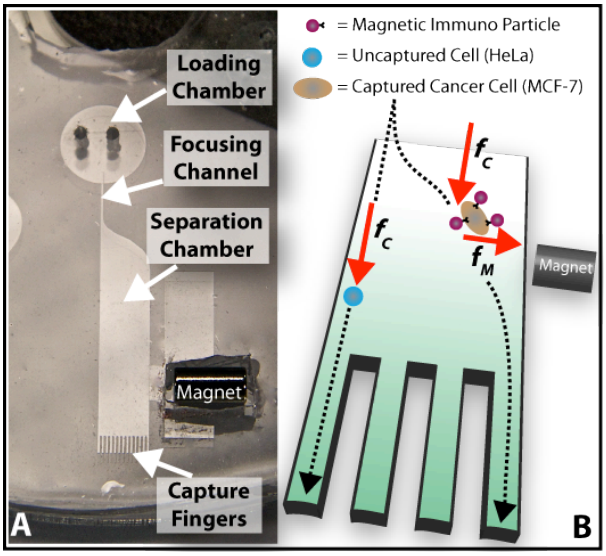

Figure 2: (A) Photograph showing the relevant features of each device, and (B) Schematic showing the separation principle. While all cells are subject to the centrifugal force (f_C), only the magnetic force (f_M) deflects immuno-captured cells on a smooth trajectory.

THEORY

The main theory of this work is based on two principles as follows: 1) paramagnetic particles experience different degrees of magnetic force depending on their size, and 2) MCF-7 cells bind to beads functionalized with anti-EpCAM antibodies, while background HeLa cells do not. The forces present on particles in the microfluidic chamber include the centrifugal force (F_c), the magnetic force (F_m), and the Stokes drag (F_s). The centrifugal force is calculated as follows:

$$\boldsymbol{F}_c = m\, R\, \omega^2 \qquad (1)$$

where m is the particle mass, R is the radial distance from the center of rotation, and ω is the angular frequency of rotation. The magnetic force can be calculated as follows:

$$\boldsymbol{F}_m = \frac{\Delta\chi \cdot V_p}{\mu_m} (\nabla \cdot \boldsymbol{B}) \cdot \boldsymbol{B} \quad (2)$$

where χ is the magnetic susceptibility of the particle, V_p is the volume of the particle, μ_m is the permeability of the liquid, and $\boldsymbol{B}$ is the magnetic field strength at a given point. Finally, the hydrodynamic Stokes drag force can be calculated as follows:

$$\boldsymbol{F}_s = 6\pi\eta r v \quad (3)$$

where η is the viscosity of the fluid, r is the radius of the particle, and v is the velocity of the particle. The combination of each of these forces on the particles in the separation chamber determines their trajectory and final separation position.

EXPERIMENTAL

A compact-disc sized polydimethylsiloxane (PDMS) disc (Fig. 1) was fabricated, primed with liquid using degas-driven flow, and then placed on a spindle-motor. Permanent magnets were mounted on the disc and microparticles or cells were then loaded and centrifugally sedimented through the system. The particles tested included polystyrene particles and iron-core, paramagnetic, polystyrene particles. For cell separation, streptavidin-coated, magnetic particles were functionalized with a biotinylated, goat-derived, anti-EpCAM IgG antibody; a secondary, FITC-labeled, anti-goat IgG was used for validation of functionalization. The cells used in this experiment were EpCAM-expressing MCF-7 breast cancer cells while HeLa cells were the control (known to surface-express little or no EpCAM). Cells were incubated with the magnetic capture particles at room temperature for 1 hour and then processed using the magnetophoretic system. Trajectories of the particles/cells were monitored during centrifugation using a stroboscopic imaging system [8]. Figure 2 highlights a separation element on the disc showing relevant features (Fig. 2A) and the cell separation principle (Fig. 2B).

RESULTS & DISCUSSION

The initial proof-of-concept experiments clearly demonstrated separation of magnetic particles from non-magnetic particles with a high separation efficiency of over 90% (Fig. 3). As expected, non-magnetic particles were sedimented in capture fingers well-aligned with the outlet of the focusing channel; a distribution of the non-magnetic particles was seen across the first three capture fingers. This spread is partially due to the fact that the large, 300-µm wide focusing channel exceeds the width of a single capture finger (170 µm wide). Under the influence of the magnetic field, over 95% of the magnetic particles were routed into capture finger 13 on the opposite side of the separation chamber, close to the magnet (Fig. 3); the majority of the remaining magnetic particles were routed into the neighboring capture finger 12. These results establish basic magnetophoretic functionality and exhibit good reproducibility of the prototype system

The next experiments showed that the streptavidin-coated magnetic particles were successfully and specifically functionalized with the capture antibody (anti-EpCAM IgG) (Fig. 4), and, after incubation with cells, specific capture of MCF-7 cells (and little or no capture of HeLa cells) was observed (Fig. 5A). Finally, magnetophoretic separation of the cell mixture showed specific deflection of particle-tagged MCF-7 cells by the magnetic field towards capture fingers on the opposite side closest to the permanent magnet. In contrast, the HeLa control cells not captured by the IgG-functionalized magnetic particles sedimented in capture fingers well-aligned with the focusing outlet channel (Fig. 5B), thus achieving separation. Separation was shown to be sensitive to the ratio of magnetic capture particles to cancer cells.

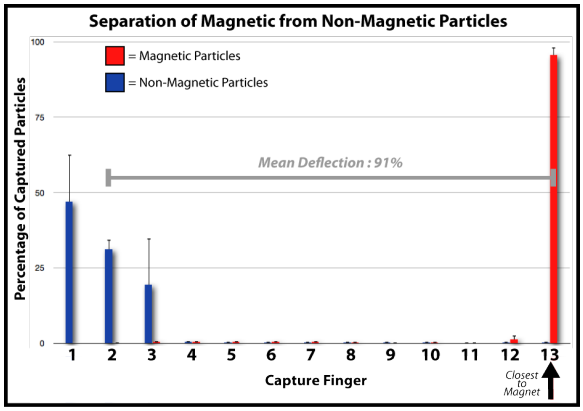

Figure 3: Data showing a 91% separation of magnetic and non-magnetic particles across the capture fingers (n=3, error bars are +1 std. deviation).

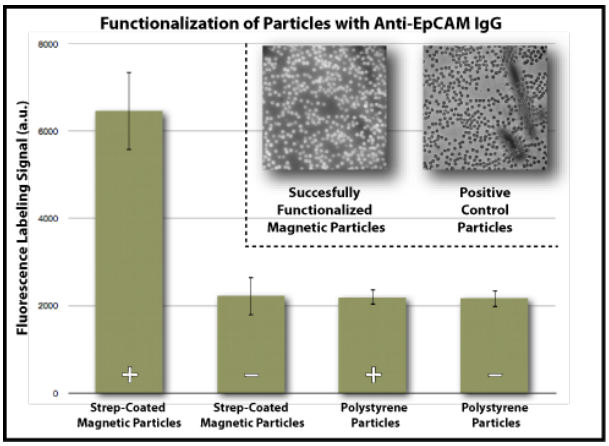

Figure 4: Validation data showing successful and specific functionalization of biotinylated, anti-EpCAM IgG on streptavidin-coated magnetic microparticles. Plain polystyrene particles act as a control. The + and – indicate incubation with and without the capture IgG, respectively.

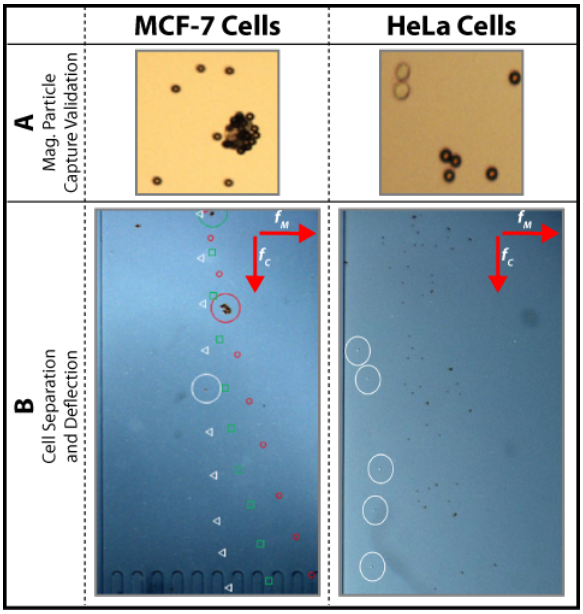

Figure 5: (A) Specific binding between the magnetic capture particles and MCF-7 cells and no binding between the capture particles and HeLa cells, and (B) Deflection and separation of a single magnetic particle (white, triangle), an MCF-7 cell bound by several magnetic capture particles (green, square), and an MCF-7 cell bound by many magnetic capture particles (red, circle); HeLa cells (white circles, right) show no capture/deflection.

CONCLUSION

This work successfully demonstrated the feasibility of a novel, centrifugo-magnetic separation of MCF-7 cells from HeLa cells according to their surface-protein expression. The main advantages of the simple and robust hardware platform are derived from the very stable hydrodynamic conditions in the centrifugally enabled, pulse-free, stopped-flow mode for stress-free cell handling. Future work will be focused on achieving improved resolution towards the development of higher-order multiplexed cell separation systems as well as on post-separation investigation of the gently separated cells.

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