SEPARATION AND DETECTION OF RARE CELLS VIA MULTISTAGE MAGNETIC GRADIENT IN A MICROFLUIDIC DISK
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ABSTRACT
Cyto-analysis of rare cells requires separation and detection with either procedure possesses substantial challenge. This paper presents a disk-based microfluidic platform for both procedures via immunomagnetic negative selection process. MCF7 are represented target rare cells and spiked into Jurkat non-target cells. The microfluidic platform’s unique features include external multistage magnetic gradient to trap labeled Jurkats via on-disk double trapping areas, drainage of fluid to substantially shorten detection time, and bin-like regions to capture target cells to facilitate seamless enumeration process. The yield of detected MCF7 is 60±10% even over a wide range of concentrations from 10⁻³ to 10⁻⁶.

KEYWORDS: Disk, Rare cells separation, Immunomagnetic method

INTRODUCTION
Detection and quantification of metastatic cancer cells in blood is an important tool for cancer diagnosis and disease prognosis. For example, the quantity of circulating tumor cells (CTC) in peripheral blood can serve as an indicator for monitoring the response of adjuvant therapy and disease stage forecasting1-3. Methodologically, several clinical techniques have been useful for enumeration of cells, such as flow cytometry, immunomagnetic cell separation, size-based or density-based enumeration approaches. However, immunomagnetic separation is currently the common technology. Positive or negative selection is a key strategic decision in isolation of rare cells. This binding efficacy is more crucial for positive selection than for negative selection. When immunological phenotype of the rare cells is unclear, negative selection might be a better strategy than positive selection. Negative selection captures non-target cells while leaving target rare cells un-labeled. This factor seems to be one of the overarching considerations for its usage to maximize the possibility of obtaining viable target cells for culturing and further analysis. In light of the discussions above, there is still a need to leverage upon various advantages of microfluidics to separate and enumerate rare cells to perform negative selection, allowing harvest of unharmed rare cells for subsequent molecular analysis. Therefore, we present a device that integrates immunomagnetic negative-selection approach in a compact disk (CD)-based microfluidic platform that is capable of separation and enumeration of rare cells with rarity up to 10⁻⁶.

DESIGN FEATURES
There are three dominant reasons for using the disk-based approach: (1) the need of handling large sample volume in order to isolate rare cells, (2) centrifugal force is useful for isolation purpose as well as other cellular processing steps, and (3) the “batch” process appears attractive since it allows one disposable unit to contain all steps involved. Leverage upon these, several unique design features of this CD platform are as follow. First, external magnetic field gradients – induced by multiple concentric-circular magnets – are coupled with on-disk double trapping areas enabling effective capture of magnetically labeled non-target cells. Non-target cells not captured by the first trapping area can have a second chance to be retained. Computational results on the magnetic gradients over the trapping areas are demonstrated. Through these designs, they could decrease large of non-target cells into detection zone, which could interfere with the detection, and increase the purity.

The second on-disk feature relates to efficient enumeration of rare cells. By nature of this process, a large sample volume is needed hence a long detection time is required. To wit, common process employs a centrifugation step to collect rare target cells, re-suspending the cells in a small fluid volume (100µl–500µl) and then transferring to flow cytometry or microscope for cell detection or enumeration. However, these steps require sample handling and transfer, which causes highly undesirable cell loss. In this work, a microfluidic structure is utilized to deplete roughly 95% of cell-containing fluid, substantially increasing volume fraction of cells, all within one platform – thus minimizing cell loss.

EXPERIMENTAL
The experimental setup and the disk’s microfluidic design are shown in Figure 1. A composite magnet is placed in contact with the top of the disk and the entire assembly is mounted on a DC motor, as shown in Figure 1a. Figure 1b shows the microfluidic pattern of one sector. Figure 2 illustrates the microfluidic process of separation and collection of target cells. Figure 3 shows the composite magnet has a much stronger effect of the $(\vec{B} \cdot \vec{V})\vec{B}$ term with large contribution near...
each edges, suggesting the multistage field gradient enhances efficacy of immunomagnetic trapping. The three-layer CD disk was fabricated as two PMMA (1 mm thick), laminating adhesives and a protective tape. A CO₂ laser engraver was used to cut through the PMMA with microfluidic features. Two cell lines were used to interrogate the efficacy of the disk-based platform: MCF7 as target rare cells and Jurkat as non-target background cells. To interrogate the performance of the disk, results from two commercial cell separation products - AutoMACS separator and BD IMagnet separator - were used as comparison.

Figure 1: Design of the disk-based microfluidic device. (a) Rotating assembly of the experimental setup. (b) Features in the disk: (A) inlet reservoir (first trapping area), (B) second trapping area, (C) outer reservoir, (D) collection bins, (E) waste reservoir, (F) extraction channel, (G) connecting channels; also serve as capillary valves, and (H) air vents.

Results show non-linear magnetic coupling effect of the multistage magnet substantially increase trapping efficacy. Figure 4a shows both the inlet reservoir (region A) and second trapping area (region B) after a particular test. Most Jurkats were trapped in the region A. Figure 4b and Figure 4c shows fluorescent image of Jurkats, labeled with CD45-PE, captured in the inlet reservoir (region A) and the second trapping area (region B). Comparison of fluorescent images between Figure 4b with Figure 4c suggests that the second trapping area enhanced the depletion rate about 9%. Detection is enhanced by depletion of 95% of non-cell-containing fluid in the collection area, substantially reducing the detection time needed to enumerate target cells. Figure 5 shows the fluorescent signal and morphology of cells in three collection bins. The average yield of detected MCF7 is proved to be near-constant 60±10% even over a wide range of concentrations from 10⁻³ to 10⁻⁶. Figure 6 presents detected 10~1000 MCF7 cells in 1×10⁶ Jurkats via three different immunomagnetic cell separators: disk platform, AutoMACS separator, and BD IMagnet separator. Comparison from AutoMACS and BD IMagnet separators revealed the average yield from the disk (60%) is superior to that from AutoMACS (37.3%) and BD IMagnet (48.3%).

Figure 2. The separation process through the disk’s microfluidic network.

Figure 3. Computation of the term $\frac{\langle \hat{B} \cdot \nabla \rangle}{\hat{B}}$ at the plane 100μm below the bottom face of the magnet.

RESULTS AND DISCUSSION

Results show non-linear magnetic coupling effect of the multistage magnet substantially increase trapping efficacy. Figure 4a shows both the inlet reservoir (region A) and second trapping area (region B) after a particular test. Most Jurkats were trapped in the region A. Figure 4b and Figure 4c shows fluorescent image of Jurkats, labeled with CD45-PE, captured in the inlet reservoir (region A) and the second trapping area (region B). Comparison of fluorescent images between Figure 4b with Figure 4c suggests that the second trapping area enhanced the depletion rate about 9%. Detection is enhanced by depletion of 95% of non-cell-containing fluid in the collection area, substantially reducing the detection time needed to enumerate target cells. Figure 5 shows the fluorescent signal and morphology of cells in three collection bins. The average yield of detected MCF7 is proved to be near-constant 60±10% even over a wide range of concentrations from 10⁻³ to 10⁻⁶. Figure 6 presents detected 10~1000 MCF7 cells in 1×10⁶ Jurkats via three different immunomagnetic cell separators: disk platform, AutoMACS separator, and BD IMagnet separator. Comparison from AutoMACS and BD IMagnet separators revealed the average yield from the disk (60%) is superior to that from AutoMACS (37.3%) and BD IMagnet (48.3%).
Figure 4 Jurkats trapped in the two trapping regions and their characterization with CD45 labeling. (a) The four-stage (M1 to M4) magnet was put on top of the trapping areas (regions A and B). After immunomagnetic separation, most Jurkat (brown colored region) remained in the inlet reservoir (A). (b) Fluorescent image of Jurkat captured in the inlet reservoir (region A), where most were trapped. (c) Fluorescent image of Jurkat captured in the second trapping area (region B), in case they were not trapped in region A.

Figure 5. Micrographs for cell identification in the collection bins. MCF7 cells labeled with CK are shown in green dots in the left photo. Jurkats are labeled with CD45, indicated by the gray arrow green dots in the left photo. Jurkats are labeled with CD45, indicated by the gray arrows.

Figure 6. Numbers of detected MCF7 (in $10^6$ Jurkats) with spiked number of MCF7 from $10$ to $10^6$. Results from separation using disk, AutoMACS, and BD IMagnet are shown.

CONCLUSION
This work presents an economical disk-based microfluidic platform to detect rare cells. Multistage magnetic gradient and double trapping regions serve to enhance the capture of immunomagnetically bound cells in the disk’s inner region, while label-free cells are collected near the outer rim. Depletion of non-cell containing fluid prior to fluorescence scanning reduced the detection time required to 1/20 of that without this process. The most important result of this work is the fact that the 60% yield – higher than that of AutoMACS and BD IMagnet separation – is independent of the spiked cell number, even over a large range of rarity from $10^3$ to $10^6$. Advantages of near-constant yield, roughly 30 minutes of operation, an acceptable level of cell loss, and potentially low cost system should aid in cyto-analysis of rare cells. This disk would also be applicable to many rare cell detection scenarios with distinct immunological features, such as stem cell detection and prenatal diagnosis.

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REFERENCES

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