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Effect of Magnetic Field Gradient on Effectiveness of the Magnetic Sifter for Cell Purification

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Abstract

In our experiments with NCI-H1650 lung cancer cell lines labeled with magnetic nanoparticles via the Epithelial Cell Adhesion Molecule (EpCAM) antigen, we demonstrate capture efficiencies above 90% even at sample flow rates of 5 ml/h through our microfabricated magnetic sifter. We also improve the elution efficiencies from between 50% and 60% to close to 90% via optimization of the permanent magnet size and position used to magnetize the sifter. We then explain our observations via the use of finite element software for magnetic field and field gradient distributions, and a particle tracing algorithm, illustrating the impact of magnetic field gradients on the performance of the magnetic sifter. The high capture and elution efficiencies observed here is especially significant for magnetic separation of biologically interesting but rare moieties such as cancer stem cells for downstream analysis.

Index Terms

Cell separation; magnetic devices; magnetic forces; magnetic microspheres; magnetic nanoparticles; magnetic separation

I. Introduction

Cell separations and purifications are a vital part of many biological experiments. In order to carry out further biological studies, researchers often have to identify and isolate a specific subset of cells from a heterogeneous population as part of their sample preparation. This separation process can be based on various cellular characteristics, such as density, size or cell surface antigen expression [1], [2]. This demand for enhanced purity has in turn led to the development of numerous technologies for cell sorting, most notably, fluorescence-activated cell sorting (FACS), where cells are labeled with fluorophores conjugated to various antibodies of interest [3].

Magnetic separation is particularly useful for cell separation as most biological systems are nonmagnetic, thus allowing for extremely fine manipulation of cell subpopulations via specific antigen-antibody conjugations. Additionally, these paramagnetic particles are typically composed of iron oxide in a sugar or polymer matrix, and are nontoxic. Magnetic techniques for cell separation have thus become widely adopted, with various companies

such as Miltenyi Biotec, R&D Systems and Invitrogen providing kits for cell purification that employ such paramagnetic particles. Numerous research groups have also produced a variety of magnetic separation devices [4]–[8].

In recent years, there has also been increasingly strong interest in various single cell analysis techniques for different biological systems. This trend has been born out of an increasing awareness of the heterogeneity present in biology. A prominent example of this is the cancer stem cell hypothesis, where researchers postulate that only a rare fraction of the cells present in tumors are responsible for tumor growth and propagation [9]. Bulk properties measured from such tumors without first selecting for specific fractions are thus uninformative.

In this context, our lab has previously pioneered a microfabricated magnetic separation device that can be used for high throughput cell purification (Fig. 1) [10], [11]. The magnetic sifter is a 7 mm $\times$ 7 mm silicon die that contains an array of 40 μm $\times$ 40 μm slots supported on a silicon nitride membrane. The device is covered with a soft magnetic material (Permalloy), thus it responds to the application of an external magnetic field (applied via NdFeB permanent magnets) and large magnetic field gradients are set up within the slots that can capture magnetically labeled cells that are being pumped through the magnetic sifter. Upon the removal of the NdFeB magnets, these magnetically labeled cells will no longer be attracted to the device, and can be easily eluted from the device. In the rest of this paper, we present our cell purification results and an analysis of the magnetic field gradients present within the device and its impact on the magnetic sifter's performance.

II. Experimental Methods

In the following sections, we will outline the device fabrication process, the cell purification process, and conclude with a description of the finite element simulations done to explain the device performance.

A. Magnetic Sifter Fabrication

1) Die Fabrication—A double polished silicon wafer is first coated with 2 μm of silicon nitride via low pressure chemical vapor deposition. Photolithography is then used to pattern an array of 40 μm $\times$ 40 μm square slots onto the membrane. The back of the wafer is patterned with an array of large hexagonal holes which are etched through the silicon wafer via a deep reactive ion etching process. This creates an array of pores that permits fluid flow through the device.

2) Magnetic Film Deposition—A 12 μm thick Ni₈₀Fe₂₀ (numbers represent the atomic percentage) film was deposited onto the sifter die after fabrication using a Perkin-Elmer high vacuum rf-sputtering system. A 30 nm layer of silicon dioxide is subsequently sputtered onto the magnetic layer via the same sputtering system to passivate the surface.

B. Cell Purification

1) Magnetic Labeling of Cells—NCI-H1650 lung cancer tumor cells were first incubated with 1 $\mu\text{g}/\text{ml}$ of biotinylated anti-human Epithelial Cell Adhesion Molecule antibody (9C4 clone from Biolegend, Inc) and 0.25 $\mu\text{g}/\text{ml}$ of fluorescein isothiocyanate (FITC)-conjugated anti-human Human Leukocyte Antigen (HLA-A, B, C) antibody (W6/32 clone from Biolegend, Inc) for 45 min. The cells were then incubated with magnetic nanoparticles (MagCollect Streptavidin Ferrofluid from R&D Systems) at a dilution of 1:10 for an hour. The cells are washed after each incubation step to remove excess unbound antibodies and magnetic particles. After labeling is complete, the cells are resuspended in

buffer and a hemocytometer is used to adjust the final cell suspension concentration to approximately 10^5 cells/ml.

2) Cell Capture and Elution—The magnetic sifter is first loaded onto a custom-made acrylic holder with laser-cut ports acting as fluid inlet and outlet (Fig. 2). O-rings are used to prevent any leaks, and polyethylene tubing is used to connect the holder to a syringe pump (New Era Pump Systems). 100 μ l of the cell suspension is then pumped through the sifter while an external field is applied via a pair of NdFeB permanent magnets (K&J Magnetics). A flow-through fraction is then collected for further analysis. After the entire sample has passed through the sifter, the permanent magnets are removed, and the captured cells are eluted from the device with fresh buffer. The flow-through fraction and the eluted fraction are subsequently brought to a FACS facility in Stanford where flow cytometry is used to quantify the total amount of cells present in both fractions (BD LSRFortessa, BD Bioscience).

C. Finite Element Simulations

In our simulations, the entire device is separated into two distinct segments: 1) the magnetic field gradients generated within the slots of the sifter is used to assess the potential capture performance; and 2) the peripheral magnetic field gradients caused by the NdFeB permanent magnets external to the device are also simulated to better describe the cells' trajectories prior to the cells coming into contact with the sifter.

While the H1650 cells do display visible heterogeneity in size, it is assumed in the following simulations that the cells are of a constant density (1080 kg/m^3), diameter ($20 \text{ }\mu\text{m}$), and have similar magnetic moments ($2.5 \times 10^{-13} \text{ Am}^2$) Am after magnetic labeling has been completed. In order to obtain the magnetic moment for simulation, H1650 cells were first labeled with magnetic particles as per the actual purification process. The magnetic moment was then measured with an alternating gradient magnetometer (AGM, Princeton Measurements Corp) by drop-casting a 100 μ l volume of the cell suspension on a $3 \text{ mm} \times 3 \text{ mm}$ silicon wafer (Fig. 3).

1) Pore Array Simulations—A single pore array was drawn in Ansoft Maxwell 3-D, and a 2-D field map was generated for the plane of the sifter for a $12 \text{ }\mu\text{m}$ thick permalloy film. A uniform external field of $2 \times 10^5 \text{ A/m}$ was applied across the film as a boundary condition. Since the array is repeated across the sifter, symmetry conditions apply and only a single pore array is simulated. Due to concerns over variations in flow across the sifter, Comsol Multiphysics was also used to simulate the trajectory of particles through the sifter, incorporating both computational fluid dynamics and the magnetostatic conditions on the sifter (Fig. 4).

2) Field Gradients From Permanent Magnets—The permanent NdFeB setups were also drawn in Ansoft Maxwell 3-D, and the magnetic fields in the volume between each pair of magnet was solved. The results were then exported to Matlab, where a particle tracing algorithm was implemented to determine the amount of cells that were being lost due to magnetic field gradients prior to them reaching the magnetic sifter. 4 commercially available permanent magnet sizes were chosen to represent a range of sizes: 1.3 cm diameter circular magnets of 1.3 cm thickness; 2.5 cm square magnets of 1.6 cm thickness; 5.1 cm $\times$ 2.5 cm rectangular magnets of 1.6 cm thickness; 7.6 cm $\times$ 3.8 cm rectangular magnets of 1.3 cm thickness. The spacing between the magnet pairs was chosen to provide a consistent magnetic field across the sifter of approximately $2 \times 10^5 \text{ A/m}$ as measured by a Hall probe. In the simulations, the NdFeB permanent magnets were set to have uniform axial polarization of 1.4 T.

III. Results

A. Cell Purification

In order to quantify the performance of the sifter, we defined three separate metrics

$$\text{Recovery Ratio} = \frac{F+E}{I} \quad (1)$$

$$\text{Capture Efficiency} = 1 - \frac{F}{I} \quad (2)$$

$$\text{Harvest Efficiency} = \frac{E}{I} \quad (3)$$

where I is the total number of magnetically labeled cells in the initial sample, F is the number of magnetically labeled cells in the flow-through sample, and E is the number of magnetically labeled cells in the eluted sample.

The recovery ratio thus quantifies the amount of unidentified losses occurring in the device due to factors such as nonspecific adhesion; the capture efficiency measures how well magnetically labeled particles are being retained in the device; and the harvest efficiency describes how well captured cells can be eluted for further downstream characterization and analysis.

1) Effect of Flow-Rate—In these experiments, magnetically labeled cells were passed through the standard setup utilizing 1.3 cm diameter cylindrical NdFeB magnets of 1.3 cm thickness at flow rates between 2 ml/h and 15 ml/h. The recovery ratios, harvest efficiencies and capture efficiencies were thus calculated and are presented in Fig. 5.

2) Effect of Permanent Magnet Setup—In the following experiments, magnetically labeled cells were passed through the sifter at 5 ml/h. 4 separate permanent magnet pairs were used during the flow-through phase, as previously described in the simulation, in order to determine their impact on losses of magnetically labeled cells prior to reaching the sifter. The results are presented in Fig. 6.

B. Finite Element Simulations

1) Pore Array Simulations—From the Maxwell simulation results, we noticed that the magnetic field gradients within the pores were extremely high, with field gradient magnitudes within the slots of between 2×10^3 T/m and 2×10^5 T/m (Fig. 7). In addition, upon applying the parameters from the sifter system into Comsol, we obtained close to 100% capture for flow rates corresponding to 5 ml/h. Representative cell trajectories passing through the sifter as obtained from these simulations are presented in Fig. 8.

2) Field Gradients From Permanent Magnets—While the system can be solved deterministically across the whole volume of the sample, it is more computationally efficient to use a Monte Carlo approach to get an estimate for the amount of magnetically labeled cells that will be captured by the magnetic field gradients from the permanent magnets prior to reaching the sifter. In this approach, we randomize the release of cells within the sample well attached to the sifter, and trace the trajectory of the particles incorporating laminar flow conditions and the magnetic fields and field gradients obtained from Maxwell. Cells which

reach the bottom of the sample well are considered as having reached the sifter, while the rest are considered losses due to the magnetic field gradients from the permanent NdFeB magnets. This then resembles a series of Bernoulli trials where p represents the probability that a cell passing through the entire device will reach the sifter. With a sufficiently large number of trials, the central limit theorem allows us to use a normal approximation. We can then compute an estimate for p (the fraction of cells loaded into the device which will reach the sifter) along with its corresponding confidence interval via the following expression:

$$\hat{p} \pm z_{1-\alpha/2} \sqrt{\frac{\hat{p}(1-\hat{p})}{n}}$$

where $\hat{p}$ is the estimate for p obtained from the Monte Carlo simulations, $Z_{1-\alpha/2}$ represents the $1 - \alpha/2$ th quartile of a normal distribution, and n is the number of trials in the simulation [12].

The losses thus calculated via simulation are qualitatively similar to those obtained experimentally, with an obvious improvement in recovery ratio as the permanent magnet setup is changed from the 1.3 cm diameter cylindrical magnet to the other larger magnets. The simulation results further confirm our hypothesis that the change in permanent magnet setup decreases the stray magnetic field gradients encountered by the labeled cells in the sample well prior to the sifter, thus improving the sifter's harvest efficiency and recovery ratio. While qualitatively similar, the simulations consistently overestimate the losses incurred across the different permanent magnet setups, suggesting the magnetic forces are being over-estimated, the fluidic forces are being underestimated or both. This could be due to the use of a simplified fluidic model in the simulations, where a parabolic laminar flow profile was assumed, and all inter-cell hydrodynamic and magnetic interactions were ignored.

IV. Discussion

In our experiments, the magnetic sifter demonstrates its effectiveness in cell capture and release with high capture efficiencies of >90% at flow rates between 2 ml/h and 15 ml/h. Since the magnetically labeled cells are essentially facing a competition between fluidic drag forces pulling them through the sifter and magnetic forces attracting them to the edges of the sifter, an inverse correlation between flow rates and capture efficiency is expected, and our results do show a slight decrease in capture efficiency and harvest efficiency as the flow rate increases, although this relationship has not been fully explored or quantified in this work. The simulations suggest the presence of large magnetic field gradients within the individual sifter slots, and thus further explain the high capture efficiencies noticed empirically.

The flow rates presented here between 2 ml/h and 15 ml/h are likely to be suitable for most cell isolation requirements, with only 4 min of separation time required for 1 ml of sample at a flow rate of 15 ml/h. While higher flow rates are possible, we would expect a decrease in capture performance to eventually manifest. Significantly though, while we expected the magnetic sifter to perform better at lower flow rates such as 1 ml/h and below, we noticed during our experiments that the density of the cells would cause them to settle through the solution and this becomes an issue at slower flow rates. This does set a lower limit as to the operating range of the magnetic sifter.

Another interesting observation we made over the course of our experiments was that while we chose the permanent magnets and custom-made the holders initially in an effort to ensure

a uniform external field across the magnetic sifter itself as measured by the use of a Hall probe, magnetic field gradients were still being experienced by the sample volume in the well prior to entrance into the sifter. This also led to a significant decrease in the harvest efficiency of the sifter, as these cells, which are mostly pulled to the sides of the sample well, are not easily eluted due to the laminar flow profile generating negligible fluid drag forces near the channel walls. A judicious choice of permanent magnets and analysis of any stray magnetic field gradients that might be present in the setup outside of the device is thus critical to preventing cell losses in any magnetic separation device. Such losses which occur in addition to nonspecific cell adhesion would further reduce the performance of any cell separation device.

A feature of the magnetic sifter that will be of particular interest to potential users is the high harvest efficiencies observed in the separation experiments, especially after the change in permanent magnet setups. In rare cell separations or instances where the biological sample is extremely precious, it becomes critical for the separation process to minimize losses. In addition, after separation with the sifter, a dye exclusion assay was done with the samples, and the majority of the cells remain viable postprocessing. This suggests that the entire separation and elution process is effective, yet sufficiently gentle that the cells are not exposed to excessive mechanical damage and retain intact cell membranes. While no further biological analysis was conducted with the cells here, it suggests the possibility for subsequent culture and growth of cell subpopulations separated via the magnetic sifter.

V. Conclusion

In this work, we presented the high efficiency capture and elution of magnetically labeled cells via the microfabricated magnetic sifter. In addition, we showed how a consideration of the magnetic field gradients from both the device and the application of any external magnetic field allows further minimization of cell losses within the device to approximately 10%. The high capture and elution efficiencies at high flow rates on the order of 5 ml/h could be very useful in rare cell enrichment and sorting applications, where large sample volumes have to be processed rapidly and losses are critical due to the rarity of the cells of interest.

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Fig. 1.
 (a) Microfabricated magnetic sifter. (b) Bright-field microscope image of tessellation of many pore arrays on the sifter. (c) Magnified image of a single pore array consisting of 7 $40 \mu\text{m} \times 40 \mu\text{m}$ slots.

Fig. 2. Photograph of the custom-made acrylic holder with the sifter loaded and NdFeB magnets attached. Cell suspension solutions are loaded via the inlet on the top and the bottom tube is attached to a syringe pump.

Fig. 3.

Plot of magnetic hysteresis loop of 5.6×10^8 cells (concentration determined by a hemocytometer) after magnetic labeling is complete. The magnetic moment per cell measured via the AGM is thus used as a parameter in subsequent simulations.

Fig. 4.

(a) Snapshot of simulated geometry in Ansoft Maxwell 3-D. (b) Snapshot of simulated geometry in Comsol Multiphysics. In both snapshots, a single pore array of seven $40\ \mu\text{m} \times 40\ \mu\text{m}$ slots are drawn in a hexagonal arrangement as per the actual fabricated sifter. The simulated geometry in (b) includes a channel both before and after the sifter so there is adequate length for the flow profile through the sifter to develop. Dimensions of geometry in (b) are given in μm .

Fig. 5.
Plot of variations in recovery ratios, capture efficiencies and harvest efficiencies across different flow rates through the sifter.

Fig. 6.

Above plots illustrate how the recovery ratios and harvest efficiencies are much lower for the 1.3 cm cylindrical magnet than for the other setups, while the capture efficiencies for all setups are above 90%. This suggests that a small fraction of captured magnetically labeled cells are not eluted.

Fig. 7. Two-dimensional plot of the magnitude of the magnetic field gradients in the plane of the sifter in the slots etched through the sifter. The magnitude is plotted on a logarithmic scale, and indicates the strong field gradients generated within the sifter slots.

Fig. 8.

Plot of particle trajectories obtained from Comsol as cells are released at $75\ \mu\text{m}$ above the sifter surface. For clarity, the cells have been released in a single line. At flow rates of $5\ \text{ml/h}$, almost all the cells released curve towards the sifter slot edges as predicted from the large field gradients. None of the cells manage to flow through the sifter without getting captured by the slot edges for the simulation where cells are assigned magnetic moments of $2.5 \times 10^{-13}\ \text{Am}^2$.

TABLE I

Comparison of Recovery Ratios Obtained Experimentally and Calculated via Monte Carlo Simulations

	Magnet Sizes			
	1.3 cm Cylinder	2.54 cm Square	5.1 × 2.5 cm Rectangle	7.6 × 3.8 cm Rectangle
Recovery Ratio	0.71±0.06	0.86±0.02	0.90±0.04	0.89±0.05
Simulation	0.57±0.05	0.75±0.05	0.78±0.06	0.80±0.05

Values for recovery ratio are provided with the standard deviation for the experiments, and the 90% confidence interval for the simulations.