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Rapid Characterization of Magnetic Moment of Cells for Magnetic Separation

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Abstract

NCI-H1650 lung cancer cell lines labeled with magnetic nanoparticles via the Epithelial Cell Adhesion Molecule (EpCAM) antigen were previously shown to be captured at high efficiencies by a microfabricated magnetic sifter. If fine control and optimization of the magnetic separation process is to be achieved, it is vital to be able to characterize the labeled cells' magnetic moment rapidly. We have thus adapted a rapid prototyping method to obtain the saturation magnetic moment of these cells. This method utilizes a cross-correlation algorithm to analyze the cells' motion in a simple fluidic channel to obtain their magnetophoretic velocity, and is effective even when the magnetic moments of cells are small. This rapid characterization is proven useful in optimizing our microfabricated magnetic sifter procedures for magnetic cell capture.

Keywords

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

I. INTRODUCTION

MANY CELL BIOLOGY EXPERIMENTS REQUIRE CELL separation or purification as a pre-processing step for a specific subset of cells from a heterogeneous population. While some separations discriminate on entirely physical cellular characteristics, such as density or size, cell surface antigen expression has become an especially important criterion [1], [2]. Numerous technologies for cell sorting have thus been developed, most notably, fluorescence-activated cell sorting (FACS), where cells are labeled with fluorophores conjugated to various antibodies of interest [3].

Magnetic separation is also frequently used in cell separation as most biological systems are non-magnetic, thus allowing for extremely specific manipulation of cell subpopulations. Additionally, the conventional superparamagnetic particle consists of iron oxide in a polymer matrix, and does not typically exhibit cyto-toxicity. Magnetic cell separation kits

are thus easily available from various companies such as Miltenyi Biotec and Invitrogen Life Technologies. Numerous research groups have also produced a variety of magnetic separation devices [4]-[9].

In this context, our lab has microfabricated a magnetic separation device for high throughput cell purification [10], [11]. The magnetic sifter is a $7\text{ mm} \times 7\text{ mm}$ silicon die that contains an array of $40\text{ }\mu\text{m} \times 40\text{ }\mu\text{m}$ slots. Being covered with a soft magnetic material, large magnetic field gradients are set up within the slots when an external field is applied and magnetically labeled cells will be retained as they traverse the magnetic sifter. Upon removal of the external field, these captured cells can then be easily eluted.

In order for magnetic separation to be utilized, it is often helpful if researchers are able to rapidly characterize the magnetic moment of the desired cells after magnetic labeling. Such information is helpful in optimization of the magnetic separation protocol for maximal capture efficiency. While other methods exist for characterizing this parameter, many focus on precise single cell measurement such as in single particle tracking, which can be very accurate but more challenging to implement computationally, or require large cell numbers in order to make a bulk measurement, such as via an alternating gradient magnetometer (AGM) [12], [13].

In the rest of this paper, we present a simple and rapidly fabricated fluidic device for measuring the magnetic moment of cells after magnetic labeling is complete, and the cross-correlation algorithm used in the data analysis. We then illustrate how this can guide downstream cell purification with the magnetic sifter for optimal processing efficiency.

II. EXPERIMENTAL METHODS

In the following sections, we will outline the magnetic moment characterization and cell purification processes.

A. Magnetic Moment Characterization

1) Magnetic Labeling of Cells—NCI-H1650 lung cancer tumor cells were first incubated with $1\text{ }\mu\text{g/ml}$ of biotinylated anti-human Epithelial Cell Adhesion Molecule antibody (9C4 clone from Biolegend, Inc) and $0.25\text{ }\mu\text{g/ml}$ of fluorescein isothiocyanate (FITC)-conjugated anti-human Human Leukocyte Antigen (HLA-A,B,C) antibody (W6/32 clone from Biolegend, Inc) for 45 minutes. The cells were then incubated with magnetic nanoparticles (MagCollect Streptavidin Ferrofluid from R&D Systems) for an hour. The cells were then further incubated with $8\text{ }\mu\text{g/ml}$ of biotin-conjugated R-phycoerythrin (Invitrogen Life Technologies) for 15 minutes. The cells are washed after each incubation step to remove excess unbound antibodies, magnetic particles and fluorescent molecules. 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 .

Two unique labeling conditions are used in the subsequent experiments. In the first, the magnetic nanoparticles were added at a dilution of $10\times$ while the second condition had the

magnetic nanoparticles at a dilution of 100 $\times$. This thus creates cells with two distinct levels of magnetic labeling.

2) Alternating Gradient Magnetometer Characterization—After magnetic labeling, the cells are spun down into a small 100 μ l volume at a density of approximately 10^7 cells/ml which is drop-cast onto a 3 mm $\times$ 3 mm silicon wafer. A small aliquot is taken to determine the actual cell density via a hemocytometer count, thus providing the total number of cells present. The magnetic moment per cell can then be obtained via an AGM (Princeton Measurements Corp) measurement of the drop-cast suspension on the wafer.

3) Fluidic Channel Magnetic Moment Characterization—A 7.5 cm $\times$ 2.5 cm microscope glass slide (Erie Scientific Company) is first cleaned in a plasma cleaner unit (PDC-32G, Harrick Plasma) for 15 minutes. A permanent marker is then used to delineate a 1 cm $\times$ 2 cm channel on the glass slide. This is a rapid prototyping method commonly used in lab-on-chip devices to create a hydrophobic barrier preventing the spread of fluid outside of the delimited area [14].

25 μ l of the cell suspension is then added into the channel and the slide is put under a microscope. A 1.9 cm square NdFeB magnet of thickness 1.3 cm in a holder is then set at a distance of 3 mm from the glass slide (Fig. 1). The holder was previously tested to ensure the glass slide's position would be centered relative to the NdFeB magnets to reduce vertical components of the magnetic field gradient. A video of the motion of the H1650 cells under this external magnetic field gradient is then recorded for 30 seconds. Longer durations are not used as cell sedimentation was typically observed after a minute. The video is subsequently loaded into Matlab, and individual frames are analyzed for a background signal, which is subtracted, before a cross-correlation algorithm is used to obtain the cells' magnetophoretic velocity.

B. Cell Purification

1) Cell Capture and Elution—The magnetic sifter is loaded onto a custom-made acrylic holder with laser-cut ports acting as fluid inlet and outlet (Fig. 1). Polyethylene tubing is used to connect the holder to a syringe pump (New Era Pump Systems). 100 μ l of the cell suspension is pumped through the sifter while an external field across the sifter of approximately 2×10^5 A/m as measured by a Hall probe is applied via a pair of 5.1 cm $\times$ 2.5 cm rectangular NdFeB permanent magnets (K&J Magnetics) of 1.6 cm thickness. 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).

III. RESULTS

A. Magnetic Moment Characterization

The cells present in the fluidic channel are essentially exposed to three main forces, a horizontal magnetic force due to the magnetic field gradient from the applied NdFeB magnets, a vertical gravitational force, and a fluidic drag force opposing the cells' motion.

Since no flow is being applied across the fluidic channel, and the fluidic channel dimensions are 3 orders of magnitude larger than the H1650 cells' diameter, channel boundary effects can be ignored and the Reynolds number can be computed by the following expression for a sphere in a fluid:

$$\text{Re} = \frac{\rho \nu D}{\mu} \quad (1)$$

where D and ν are the diameter and velocity of the cell, and μ and ρ are the dynamic viscosity and density of the fluid. Typical values of 10^{-3} Pa · s for viscosity, 10^3 kg/m³ for density, 17 µm for diameter and 1 mm/s for velocity yield $\text{Re} \approx 0.017$. This places the system in the laminar flow regime and yields the following expression for Stokes drag on the cells:

$$\vec{F}_d = 3\pi\mu\nu\vec{v}D \quad (2)$$

The magnetic forces experienced by the cell are in turn given by the following expression:

$$\vec{F}_m = (\vec{m} \cdot \nabla) \vec{B} \quad (3)$$

where $\vec{m}$ represents the magnetic moment of the cell and $\vec{B}$ represents the magnetic field. Prior AGM measurements indicate the nanoparticles approach saturation at fields above 8×10^4 A/m. Since the average field over the fluidic channel is measured by a Hall probe to be approximately 1.2×10^5 A/m, Eq. 3 can be further simplified:

$$\vec{F}_m = m_s \cdot \nabla (|\vec{B}|) \quad (4)$$

where m_s represents the cell's saturation magnetic moment.

Additionally, since the magnetic and gravitational forces are in orthogonal vertical and horizontal directions, the force balance at equilibrium in the horizontal direction yields the following expression for the m_s of the cell:

$$m_s = \frac{3\pi\mu\vec{v}D}{\nabla (|\vec{B}|)} \quad (5)$$

Determination of the velocity at which the cells are moving towards the external NdFeB magnets in the fluidic channel thus yields the saturation magnetic moment of the cell.

In order to obtain the magnetophoretic velocity, a cross-correlation algorithm was used in the analysis of the videos. Cross-correlation is a widely used technique in signal processing for assessing the similarity between two inputs as a function of a displacement or time delay. When the videos are loaded into Matlab, each frame yields an $m \times n$ matrix of the intensity of each pixel. The cross-correlation matrix between frames f_1 and f_2 can then be computed as:

$$C_{1,2}(i, j) = \sum_{a=0}^m \sum_{b=0}^n f_1(a, b) \cdot f_2^*(a+i, b+j) \quad (6)$$

The indices range from $-(m-1)$ to $(m-1)$ for i and from $-(n-1)$ to $(n-1)$ for j , and the specific value of i and j which corresponds to the maximum value of $C_{1,2}$ also indicates the displacement for which the two frames overlap most, and thus, provide information as to the distance traveled by the cells in the time between frames. As the video is recorded over the same area of the fluidic channel, stationary objects in the field of view will contribute a strong signal to the cross-correlation matrix at the point $(0, 0)$. It is thus useful to further subtract the background from the images prior to computation of the cross-correlation (Fig. 2). Magnetophoretic velocity of the cells can then be obtained by relating the displacement to the frame rate of the video.

1) Parameter Measurements—In order to obtain m_s from Eqn. 5, the diameter of the H1650 cells and the magnitude of the magnetic field gradient need to be determined. The average diameter of the H1650 cells was measured to be $17 \mu\text{m}$ via a Scepter automated cell counter (Millipore Inc). Dynal beads (Invitrogen Life Technologies) were also put on the setup at an identical displacement from the external NdFeB magnets so as to obtain their magnetophoretic velocity. Using the magnetic moment obtained from AGM measurement of the beads, the ratio of the field gradient to the fluid viscosity can be obtained for this specific external applied magnetic field gradient and buffer:

$$\frac{\mu}{\nabla(|\vec{B}|)} = 6.25 \times 10^{-5} \text{ kg}/(\text{T s})$$

2) Comparison with AGM—In these experiments, cells labeled with the R&D magnetic nanoparticles under 1:10 and 1:100 dilution conditions had their magnetic moment measured via AGM. Similarly labeled cells were also put onto the fluidic channel as described above and their saturation magnetic moment was obtained by measuring their magnetophoretic velocity. The m_s per cell for both incubation conditions are presented in Table I.

3) Effect of Different Magnetic Moment of Cells—In the following experiments, cells magnetically labeled under the two conditions described were passed through the sifter at flow rates between 5 ml/hr and 40 ml/hr. In order to quantify the sifter's performance, we defined the metric:

$$\text{Harvest Efficiency} = \frac{E}{I} \quad (7)$$

where I is the total number of magnetically labeled cells in the initial sample, and E is the number of magnetically labeled cells in the eluted sample. Results are presented in Fig. 3.

The harvest efficiency in both cases experience a gradual decrease with flow rate. Since retention of magnetically labeled cells is determined by a balance between fluidic drag forces through the sifter which are proportional to flow rate, and magnetic forces to the edges of the sifter, such an inverse correlation is expected. Reducing the extent of magnetic labeling, as evidenced by the lower magnetic moment measured for incubation with a 100× dilution, thus impacts negatively on the capture performance of the sifter, with harvest efficiencies for the 100× dilution condition being consistently lower than those for the 10× dilution condition.

IV. DISCUSSION

In previous work, the magnetic sifter has demonstrated its effectiveness in cell capture and release when the cells are well labeled with magnetic nanoparticles. As demonstrated above, different labeling extents will result in different harvest efficiency profiles across flow rates. While it is possible to ensure high harvest efficiencies by processing the samples only at low flow rates, this adds unnecessarily to processing time when the cells of interest are well-labeled with magnetic nanoparticles. For example, when the H1650 cells are incubated with magnetic nanoparticles at a 10× dilution, harvest efficiency remains relatively constant between 5 ml/hr and 20 ml/hr. This means the sample could have been processed at 4 times the speed without a noticeable loss in performance. A rapid characterization method like the one presented here will thus be useful as a simple means of assessing the optimal conditions for running subsequent magnetic cell separations, especially since only a small fraction of the sample is needed for the measurement.

This might be especially critical in real biological experiments with small cell populations. Due to the low magnetic moment of the cells, in order for AGM to be viable, the samples processed here were typically on the order of 10^6 cells. This would be impractical in rare cell populations. In contrast, measurements for magnetophoretic velocity only required 25 μl samples ($\approx 10^3$ cells). Additionally, the current channel size (1 cm $\times$ 2 cm) is much larger than the actual imaged area. The sample volume needed can thus be further reduced via the use of smaller fluidic channels.

Interestingly, the saturation magnetic moment obtained via the magnetophoretic velocity measurement was consistently higher than the AGM measurements. A possible reason is that early apoptotic cells which may not be labeled might look intact on the hemocytometer, and will be included in the cell count during the AGM measurement, thus reducing the average magnetic moment per cell. However, these unlabeled cells will not move, and will be removed during background subtraction. This suggests that the m_s value from the magnetophoresis measurements might be more accurate. The standard deviation from AGM measurements is consistently smaller however, and this is probably a consequence of the

size of the samples since standard deviation typically decreases with increased sample numbers in a $1/\sqrt{N}$ manner.

Also, it should be noted that the cross-correlation algorithm actually provides the mode values for magnetophoretic velocity. In a bulk measurement for a single cell population like the H1650 cell line, the central limit theorem dictates that the mode measurement will approach the mean. However, in mixed cell populations with variable labeling effectiveness, different magnetophoretic velocities should manifest as distinct peaks and this could be useful in magnetic separations of heterogeneous samples, although this was not shown here.

V. CONCLUSION

In this work, we presented a method for rapid characterization of the magnetic moment of cells. It was shown that results are consistent with the magnetic moment obtained via AGM measurements, although there are certain subtle differences in the quantities being measured. The rapid prototyping and simple analysis makes the method conducive for implementation prior to any magnetic separation and we demonstrate how knowledge of the magnetic moment can be useful in optimizing magnetic separation processes.

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Fig. 1.

(a) Photograph of the fluidic channel setup under the microscope. (b) Photograph of the magnetic sifter setup with NdFeB magnets providing a horizontal field. Cell suspensions are loaded in at the top while polyethylene tubing connects the outlet below to a syringe pump.

Fig. 2.

- (a) The image is the output from the cross-correlation computation, with the peaks being the most common velocities of particles in the images processed. The strong peak at (0,0) in (a) is a result of stationary objects in the background of the images. (b) is the plot of the same data when the cross-correlation is computed after background subtraction. The cells have moved 64 μm in the y-direction.

Fig. 3.

Plot of harvest efficiency of the magnetic sifter with flow rate for two separate incubation conditions for the cells.

Table I

Comparison of saturation magnetic moment obtained via AGM measurements and cross-correlation calculations.

	$m_s (\times 10^{-13} \text{ Am}^2)$	
	AGM	Cross-correlation
100× Dilution	0.99±0.09	1.15±0.28
10× Dilution	2.25±0.23	2.78±0.77