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Application of magnetic carriers to two examples of quantitative cell analysis

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ABSTRACT

The use of magnetophoretic mobility as a surrogate for fluorescence intensity in quantitative cell analysis was investigated. The objectives of quantitative fluorescence flow cytometry include establishing a level of labeling for the setting of parameters in fluorescence activated cell sorters (FACS) and the determination of levels of uptake of fluorescently labeled substrates by living cells. Likewise, the objectives of quantitative magnetic cytometry include establishing a level of labeling for the setting of parameters in flowing magnetic cell sorters and the determination of levels of uptake of magnetically labeled substrates by living cells. The magnetic counterpart to fluorescence intensity is *magnetophoretic mobility*, defined as the velocity imparted to a suspended cell per unit of magnetic ponderomotive force. A commercial velocimeter available for making this measurement was used to demonstrate both applications. Cultured *Gallus* lymphoma cells were immunolabeled with commercial magnetic beads and shown to have adequate magnetophoretic mobility to be separated by a novel flowing magnetic separator. Phagocytosis of starch nanoparticles having magnetic cores by cultured Chinese hamster ovary cells, a CHO line, was quantified on the basis of magnetophoretic mobility.

1. Introduction

When cells are to be separated by fluorescence activated cell sorters (FACS) it is customary to determine the distribution of fluorescence intensity in a fluorophore-labeled population of cells and to set flow parameters that select the desired cell population [1]. Likewise the measurement of magnetophoretic mobility has been used historically to set flow parameters in a quadrupole magnetic cell sorter [2–5] and a very early version of a magnetic flow sorter [6,7]. This approach does not appear to have been applied to a wider variety of magnetic cell sorters. The magnetophoretic mobility requirements for almost any magnetic separation can be determined by computational fluid dynamic analysis [8,9]. In a separation with flow-rate requirements, for example, a minimum required mobility can be calculated. Tumor cells were chosen as an example of cells to be labeled for magnetic separation owing to interest in magnetically separating tumor cells from circulating blood [10,11].

Fluorescence flow cytometry is also used to determine levels of uptake of fluorescently labeled substrates by living cells. Fluorescent substrates are usually antibodies identifying cell surface markers and may or may not be internalized by receptor-mediated endocytosis and/or due to cell-membrane regeneration. The determination of levels of uptake of magnetically labeled substrates by living cells can be assessed by the measurement of magnetophoretic mobility [12]. There is considerable interest in the phagocytosis of nanomaterials [13], and nanomaterials used in MRI have magnetic cores [14]. Micro- and nanoparticles are ingested by cells by mechanisms dependent on particle size and surface composition including targeting moieties such as antibody labels [15] and can be ingested by a plethora of cell uptake mechanisms (phagocytosis, pinocytosis, receptor and non-receptor mediated endocytosis). Fluorescent labels modify the surface properties of most types of particles (with the possible exception of particles coated with fluorescent antibodies), whereas magnetic cores do not necessarily modify particle surface chemistry. One unintended consequence of labeling of blood and bone marrow with beads is the nonspecific ingestion of labeling particles by phagocytic cells in the environment either by direct uptake or by released endocytotic vesicles [16]. In this study a commercial velocimeter was used to measure magnetophoretic mobility distributions in two example applications: flowing magnetic cell separation and nanoparticle phagocytosis. The adequacy of tumor cell labeling to meet the requirements of a particular flowing separator was established, and the kinetics of starch-particle phagocytosis were characterized.

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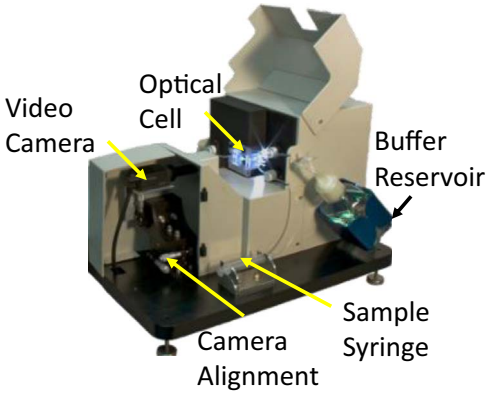

Fig. 1. Labeled photograph of the Hyperflux™ magnetic velocimeter demonstrated in this study.

2. Experimental

2.1. Cells

The tumor cell line used in all tests is CRL-211, DT40, obtained from ATCC, a chicken B-cell lymphoma cell line. These cells were maintained in suspension culture by twice-weekly passage in culture medium consisting of 69% Dulbecco's modified eagle's medium (DMEM), 10% tryptose phosphate broth solution, 5% chicken serum, and 1% ABAM (Antibiotic-Antimycotic mixture, all produced by SIGMA™, St. Louis, MO, USA, plus 10% fetal bovine serum (FBS) produced by ATCC. Cells were counted by hemacytometer and diluted in Dulbecco's phosphate-buffered saline (PBS) to about 5×10^4 cells/mL for reaction with bead reagent and evaluation in the Hyperflux™ velocimeter.

CHO Cells (Chinese Hamster Ovary cells, line CHO-K1) were maintained in monolayer culture in T-75 flasks at a passage ratio of about 1:8 every two days. For endocytosis experiments cells were trypsinized and counted for plating at about 1×10^6 cells per well in 6-well plates and incubated at 37 C for one day. They were then switched to complete medium containing various concentrations of magnetic nanoparticles for various times from 1 to 24 h. They were then trypsinized and suspended in Hanks' Balanced Salts Solution for analysis using materials and protocols as previously described [17].

2.2. Particles

Medium to high mobility particles were required for tumor cell labeling for the separator proposed for use. Beads were magnetically selected according to manufacturer's instructions before and after antibody labeling. The antibody used is Mouse monoclonal M-1 Anti-Chicken IgM mu chain (Biotin), Abcam™ product id ab99719. Labeling of beads with this antibody was achieved before mixing particles with cells following manufacturers' instructions. Magnetic beads used in the testing are 2.8 μm diameter Dynabeads® Biotin Binder (InVitrogen/Dynal) with measured magnetophoretic mobility range of $1.3–2.0\times10^{-11}$ m^3/TAS, the concentration of beads is 4×10^8 beads/mL. Nonspecific particle internalization was avoided by reacting label with cells at 8 C or 23 C. Phagocytosis (deliberate internalization) studies utilized 50 and 100 nm superparamagnetic iron-oxide nanoparticles with magnetite core and starch matrix and coating (Chemicell FluidMAG-D, Berlin, Germany, Article Number: 4101-1 (1 mL)) [16]. The concentration of nanoparticles is expressed as μg/mL of iron.

2.3. Magnetophoretic mobility measurement

Magnetophoretic mobility is the ratio of the terminal velocity of the particle, v_m, to the gradient of the magnetic energy, $\frac{\nabla B_0^2}{2\mu_0}$, with B being the local magnetic flux density at the point of the particle or cell:

$$U_m = v_m / \frac{\nabla B_0^2}{2\mu_0} \quad (1)$$

The units of U_m are m s^{-1}/T A m^{-2} or m^3T^{-1} A^{-1} s^{-1} (meters cubed per Tesla-Ampere-second), expressed in this work as m^3/TAs. The Hyperflux™ velocimeter (IKOTECH, LLC, New Albany IN, USA) measures v_m by image velocimetry and divides it by the denominator in Eq. (1), which is an adjustable constant in the velocimeter software. The Hyperflux™ image velocimeter, in brief, consists of a stopped-flow sample cell connected to sample, supply and waste fluid reservoirs and served by an automated pump, which transfers a fresh volume of sample into the optical cell after each "set" of a specified number of video frames has been recorded by a high-resolution camera. Raw video frames are maintained in a file that can then be analyzed using operator-selected parameters, especially including an intensity threshold setting that is adjusted interactively on the basis of simultaneous image and graphical display. For every recorded event at least 20 parameters are calculated and stored including velocity, magnetophoretic mobility, size, shape and image processing parameters. Additional details are given in [18], and a view of the Hyperflux™ velocimeter is given in Fig. 1. An example of a data display screen is shown in Fig. 2.

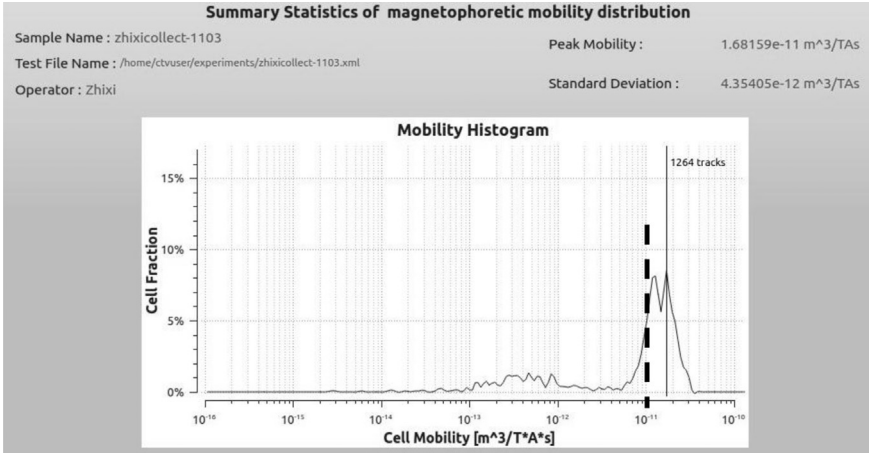

Fig. 2. Screen shot of mobility histogram generated automatically by the Hyperflux™ velocimeter for magnetically labeled chicken lymphoma cells. Vertical solid line indicates peak mobility. Vertical dashed line indicates minimum magnetophoretic mobility (1.3×10^{-11} m^3/TAs) for 100% capture of cells in a modeled cell separator flowing at 1.0 mL/min.

3. Results and Discussion

3.1. Magnetophoretic mobility of tumor cells labeled for flowing separation

Magnetophoretic mobility distributions were determined on the basis of several thousand analyzed cell tracks, and an example is given in Fig. 2, a screen shot of the Hyperflux™ velocimeter output. For the flowing magnetic separator in question, a compact multistage capture device with a desired flow rate of 1.0 mL/min, the minimum required magnetophoretic mobility for 100% cell capture was calculated to be 1.3×10^{-11} m^3/TAs. This is marked as a dashed vertical line in Fig. 2. From the mobility data set it may be calculated that the separator in question would capture about 90% of the labeled cells.

3.2. Magnetophoretic mobility and nanoparticle phagocytosis

In order to use magnetophoretic mobility as a robust indicator of particle ingestion, instrument settings that provide reproducible results were established. The most significant operator-controlled setting is a threshold intensity value used by the Hyperflux™ image analysis package to accept or reject imaged objects for calculation of their average magnetophoretic mobilities. The range of intensity values is 0–255. In Fig. 3 it is seen that mid-range values, 130 and 190 for example, provide essentially reproducible mobility distributions for magnetically labeled CHO cells.

Cells were fed several concentrations (based on μg/mL Fe) of 100 nm starch-coated Chemicell Fluid MAG-D magnetic particles for 24 h in kinetic studies, and mobility histograms were determined on the basis of velocities calculated from several thousand tracks. Histograms of cells' magnetophoretic mobilities are given in Fig. 4. The clear trend to higher mobility is seen by visual comparison of the five histograms, and peak mobilities plotted vs. particle concentration in Fig. 5 follow a monotonic trend up to 200 μg/mL Fe. These observations using magnetophoretic mobility as a measurement of phagocytosis are consistent with quantifications using other, traditional chemical and cytological methods [17].

4. Conclusions

Labeled tumor cells have been magnetically characterized, and phagocytosis kinetics studies have been performed in a user laboratory by measuring magnetophoretic mobility distributions using the Hyperflux™ magnetic velocimeters. The ability of labeled tumor cells to be captured by a flowing cell separator was predicted. The kinetics of starch nanoparticle phagocytosis was characterized quantitatively, providing data suitable for theoretical model fitting. Such measurements can now be achieved on a rapid, convenient and routine basis using commercial instrumentation.

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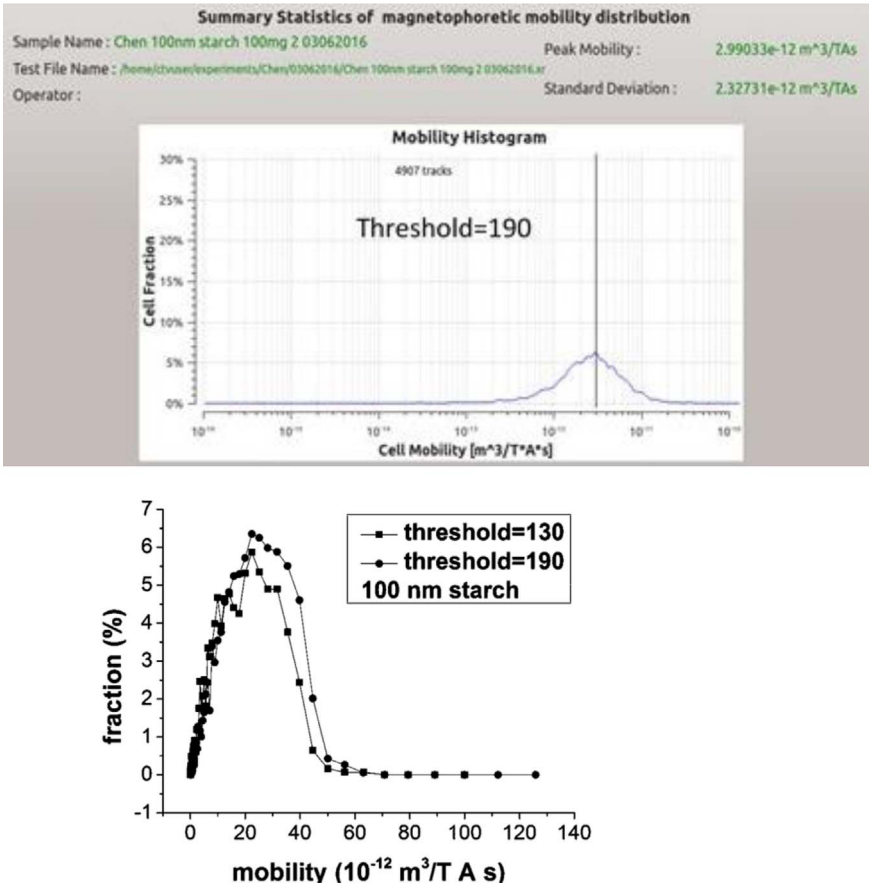

Fig. 3. Magnetophoretic mobility distributions of CHO cells labeled for 24 h with 100 nm starch-coated Chemicell Fluid MAG-D magnetic particles measured using two intensity threshold settings of the Hyperflux™ velocimeter. Top: Screen shot of image analysis data at Threshold=190. Lower: Mobility distributions at threshold =130 and 190 on a linear mobility scale.

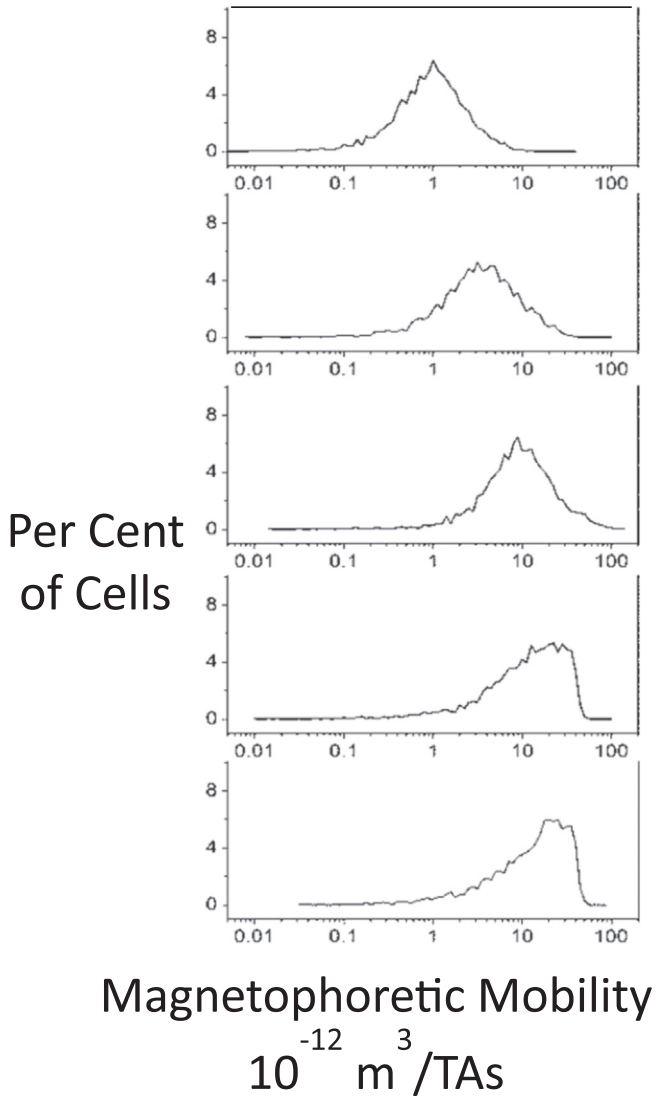

Fig. 4. Magnetophoretic mobility distributions of CHO cells labeled for 24 h with five concentrations of 100 nm starch-coated beads. There is a 15-fold increase in beads/cell over this concentration range. Unlabeled cells have no magnetophoretic mobility.

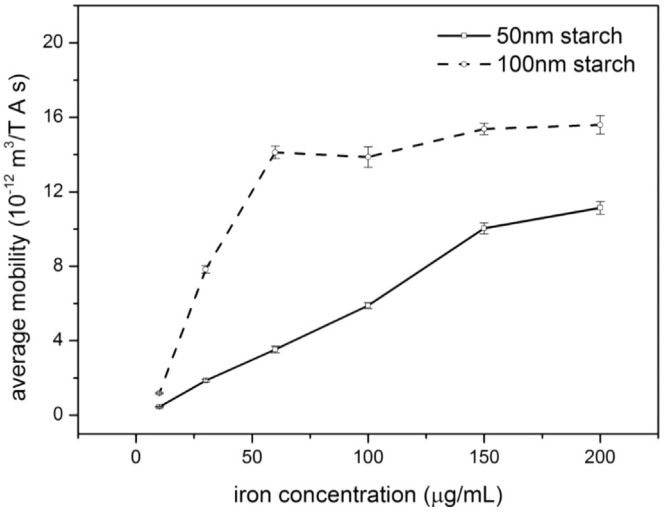

Fig. 5. Average magnetophoretic mobility of CHO cells that ingested five concentrations of 50 nm or 100 nm starch-coated beads vs. concentration of beads as measured by iron content.

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