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CHARACTERIZATION/QUANTIFICATION OF THE FACTORS INVOLVED IN THE IMPARTING A MAGNETOPHORETIC MOBILITY ON CELLS AND PARTICLES

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INTRODUCTION: Numerous published studies indicate the direct relationship between cell surface antigen expression level and cell function. Thus, it is important to obtain quantitative information on certain cell surface antigen expression level. In our laboratory, we have developed an instrument named Cell Tracking Velocimetry (CTV) based on the concept of magnetophoresis. The CTV system has been used to measure the "degree to which" a cell is immunomagnetically labeled, which is referred to as magnetophoretic mobility, m_c [1].

Popular magnetic reagents in immunomagnetic cell labeling are of particle (such as Dynal™ beads, Dynal AS, Oslo, Norway) and colloidal (such as MACS™ beads, Miltenyi Biotec, Germany) sizes. In our application, colloid-sized magnetic beads are used because the number of beads bound to the cell surface is directly proportional to cell surface antigen expression level due to their small size.

The magnetic force acting on a labeled cell in a magnetic field can be expressed as:

$$F_m = (n_1 q_1 l_1)(n_2 q_2 l_2) n_3 F_b \equiv N_b \cdot F_b \qquad (1)$$

where n_1 is the total number of antigen molecules per cell, q_1 is the fraction of antigen molecules on the cell surface bound by primary antibodies, and l_1 is the valence of the primary antibody. This pattern is repeated when n_2 is the total number of secondary antibody binding sites on the primary antibody, q_2 is the fraction of binding sites on the primary antibodies that are occupied by the secondary antibodies, l_2 is the valence of secondary antibody, and finally, n_3 is the number of magnetic beads conjugated to a secondary antibody. When antibodies are used, $n_1 q_1 l_1$ corresponds to **Antibody Binding Capacity** (ABC), and when $q_1 = q_{\max}$ it corresponds to the maximum number of primary antibodies bound to the cell surface. Parameters $n_2 q_2 l_2 n_3$ can also be combined into one term β, and β represents the number of magnetic beads bound to one primary antibody. b can be thought of as an amplification factor. Alternatively, the term N_b can be used which corresponds to the amount of magnetic beads bound to the cell (or particle) surface [2].

The magnetic force acting on a magnetic bead is expressed as:

$$F_b = \Delta c V \cdot \nabla\left(\frac{B^2}{2m_0}\right) \equiv k \cdot \nabla\left(\frac{B^2}{2m_0}\right) \qquad (2)$$

where B is the imposed magnetic field induction; m_0 is the magnetic permeability of free space; Δc is the difference in volumetric magnetic susceptibility between the magnetic bead c_b and the medium c_f , V is the volume of a magnetic particle; $k \equiv \Delta c \cdot V$ is defined as "the magnetic bead-field interaction parameter".

If the labeled cell moves relatively slowly in the medium, Stoke's law can be applied, and the drag force is given as:

$$F_d = 3pu_c D_c h \qquad (3)$$

where D_c is the diameter of the cell, η is the viscosity of the medium and u_c is the induced velocity in the field.

By applying force balance in the magnetic field direction, we can solve the induced velocity u_c as expressed in equation (4).

$$u_C = \frac{n_1 q_1 l_1 \cdot b \cdot k}{3p D_C h} \cdot \nabla\left(\frac{B^2}{2m_0}\right) \qquad (4)$$

We can further normalize u_C into magnetic mobility m_C, by dividing u_C by magnetic energy density S_m, which is defined as $S_m \equiv \nabla\left(\frac{B^2}{2m_0}\right)$, so that:

$$m_C = \frac{N_b \cdot b \cdot k}{3p D_c h} \qquad (5)$$

where N_b is a function of the amount of magnetic beads used in labeling process.

Magnetic bead-field interaction parameter k is a parameter of the magnetic nanobeads.

In the content of this paper, our work is composed of two parts:

- Studying the relationship between mobility and amount of labeling reagents;
- Determination of k value

METHODS:

The CTV measures magnetophoretic mobility m_c of a particle or cell, using a well-defined magnetic field [3,4]. The diagram of our CTV system is shown in Figure 1.

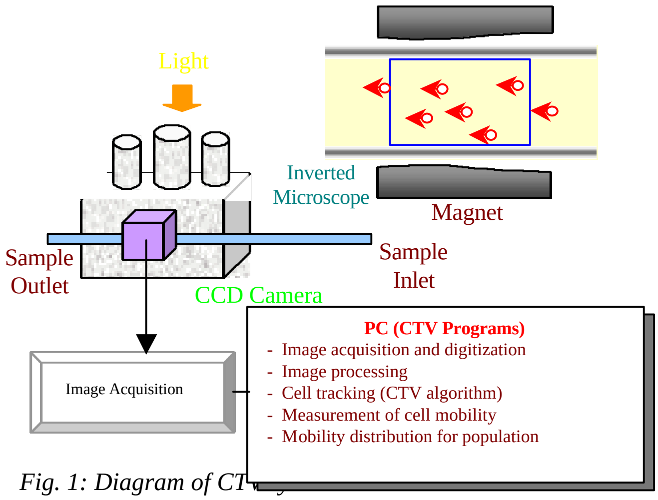

Fig. 1: Diagram of CT

ProActive® Biotin-coated microspheres (Bangs Laboratories, IN, USA) and Streptavidin MACS™ beads (Miltenyi Biotec, CA, USA) were used in our study. Biotin-coated microspheres are uniformly sized polystyrene particles (5.12 μm) with certain amount of biotin molecules bound to the surface. Streptavidin MACS™ beads are colloidal super-paramagnetic nano-beads (50 nm) conjugated to streptavidin. Biotin-streptavidin interaction is one of the strongest non-covalent bonds (Ka =10^{15}/M vs. 10^{7}-10^{11}/M for antibody-antigen interactions).

A water solution of Gadolinium (Gd^{3+}) salt (Optimark®, Mallinckrodt Inc.) was used as suspending fluid. Gadolinium salt is a paramagnetic chemical, and it was used to change the volumetric magnetic susceptibility of the medium, c_f , to study the induced change in mobility.

In our work, we labeled 10^6 biotin-coated microsphere with certain amount of streptavidin MACS™ beads at 4°C for 30 minutes. Then washed with x20 water three times by centrifuge. After discarding the supernatant, we added gadolinium-containing medium and measured mobility using CTV.

RESULTS:

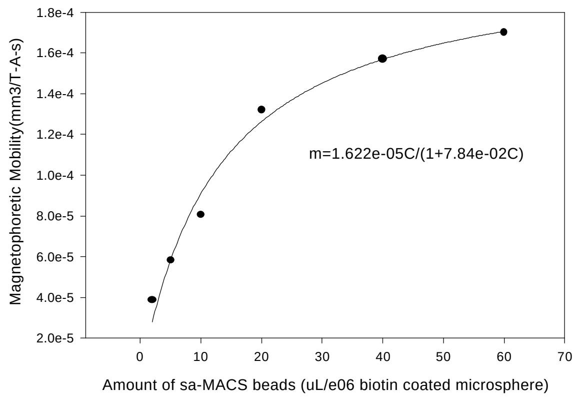

Fig. 2. Saturation curve of magnetophoretic mobility versus amount of labeling agents.

By changing the concentration of gadolinium salt in the solution, magnetophoretic mobility will change. When $c_f = c_b$, microspheres will not move in the magnetic field (zero mobility). Based on Figure 3, we can determine c_b value as shown.

DISCUSSION & CONCLUSIONS:

Following the concept of antibody-antigen interaction, we can deduct equation (6).

$$q_1 = \frac{K_a C}{1 + K_a \cdot C} \qquad (6)$$

where Ka is the association constant between the receptor (usually proteins) and antibody, and C is the free concentration of antibodies in the solution.

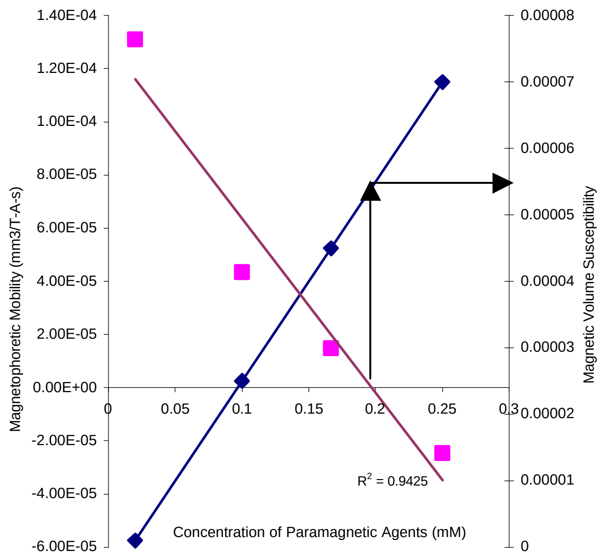

Fig. 3: Relation between mobility and concentration of paramagnetic agents

By combining equation (5) and (6), we obtain the following expression:

$$m_c = \frac{k b l_1}{3p D_c h} \cdot \frac{n_1 \cdot K_a \cdot C}{1 + K_a \cdot C} \qquad (7)$$

In Figure 3, we used above equation to fit the data. We used the total amount of labeling reagents instead of free concentration of labeling reagents in equation (7) by assuming the total amount of labeling reagents is a reasonably large value. Significant agreement between the data and equation (7) is observed.

Future work will focus on further verification of equation (7). If this equation is the appropriate expression of the relation between mobility, surface antigen, and labeling agents, work will continue to use the CTV system to measure the **true** surface antigen expression level, n_1 in equation (7).

Measuring magnetic bead-field interaction parameter k is a necessary step to quantitate cell surface antigen using our CTV system. However, It is rather challenging to measure the volumetric magnetic susceptibility of nano-paramagnetic particles c_b . Figure 4 shows when $c_f = 0.00007$, some microspheres have the positive mobility while others have negative mobility, which implies that there is a concentration gradient of paramagnetic salts in the monitored area.

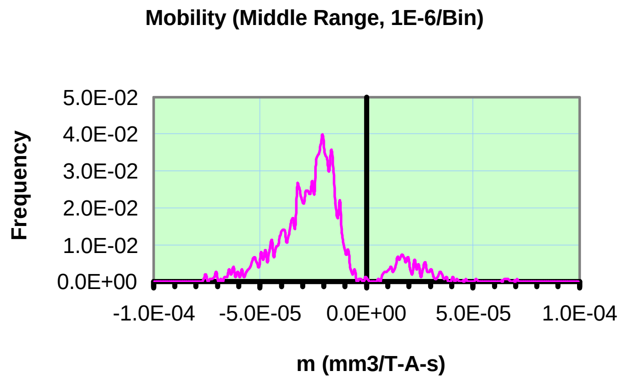

Fig. 4: Output of CTV (when $c_f = 0.00007$ *).*

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