Microfluidic high gradient magnetic cell separation

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Separation of blood cells by native susceptibility and by the selective attachment of magnetic beads has recently been demonstrated on microfluidic devices. We discuss the basic principles of how forces are generated via the magnetic susceptibility of an object and how microfluidics can be combined with micron-scale magnetic field gradients to greatly enhance in principle the fractionating power of magnetic fields. We discuss our efforts and those of others to build practical microfluidic devices for the magnetic separation of blood cells. We also discuss our attempts to integrate magnetic separation with other microfluidic features for developing handheld medical diagnostic tools. © *2006 American Institute of Physics*. [DOI: 10.1063/1.2165782]

INTRODUCTION

Cell separation by centrifuge is performed nearly every time blood is extracted from the body. In a centrifuge blood is separated along a density gradient. Despite 30 years of research into other means of blood separation the centrifuge remains the workhorse of hematology laboratories. Microfluidic cell separation and lab on a chip technology may one day change that.

Microfluidics technology has been touted as a revolutionary force in biology. One can now imagine a handheld diagnostic device that would require only a drop of blood. It would pass the fluid around to multiple areas within itself and measure everything from salt concentration to cell size, type, and number; then, by comparing that information to a databank of healthy and sick profiles, could pinpoint any problems and prescribe a treatment plan.

One key component of the lab on a chip approach is a cell separator. Cell separators are required for counting and collecting various cell types. The field of cell separation is not new, and attempts to miniaturize it have begun by modifying existing techniques.

Modern cell separation begins with flow cytometry. Flow cytometry is the analysis and separation of single cells by dropping them past an optical detector. A computer makes a decision, based on input from the detector, whether or not to alter, using an electric field, the trajectory of the falling cell into one of a number of bins. Some notable applications of flow cytometry are: obtaining helper T lymphocyte counts for monitoring HIV treatment, analysis of malignant tumor cells and identification and separation of rare stem cells.[1] Flow cytometry is an emerging diagnostic field, but it is in limited use because the apparatus, involving a number of lasers and detectors, is large and expensive. Magnetic separation is very inexpensive in comparison and many of the tasks for which flow cytometry is used, such as stem cell, T-cell, and tumor cell isolation can be performed using magnetic separators.[2–4]

Magnetic cell separation is easily divided into two classes: using magnetic beads to select cell types, and using the native magnetic susceptibility of cells to select cell types. The use of magnetic beads coated with cell-specific antibodies to separate certain cell types is only about 15 years old, but has blossomed recently as an affordable way of isolating rare cells. Once the magnetic beads are bound to the cells, a magnetic field gradient is all that is required to separate them from the bulk. The magnetic beads, ranging in size from 10 nm to 10 μm, are typically a mixture of polymer and iron oxide particles, Fe_2O_3 and Fe_3O_4.

The separation of red blood cells from whole blood by native susceptibility was shown in 1975 by Melville *et al.*[5] While most biological matter is composed of water, a diamagnetic substance, certain cells contain paramagnetic material. The most paramagnetic cell in the body is the deoxygenated red blood cell. Each red blood cell contains around 250 million hemoglobin proteins, each protein molecule carries four iron atoms, each with up to four unpaired electrons, giving the cell a paramagnetic component not present in other cells. This unique property of red blood cells allows for their removal from whole blood using a magnetic field.

The magnetic force on any paramagnetic body in vacuum, either a cell or a bead is

$$\mathbf{F}_M = \frac{\chi V}{\mu_o} B \nabla B. \tag{1}$$

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For a cell in fluid we replace χ with $\Delta\chi=\chi_{\text{cell}}-\chi_{\text{plasma}}$, the difference in susceptibility between the cell and surrounding medium. Takayasu *et al.*[7] measured the susceptibility of red blood cells, $\chi_{\text{rbc}}=4\times10^{-6}$, and blood plasma, $\chi_{\text{plasma}}=-8\times10^{-6}$ (SI). The susceptibility of water is -8×10^{-6}.

The linear response, [Eq. (1)] is generally assumed to be valid for biological materials. When working with magnetic beads the response is generally not linear. It is important to know the saturation field for the particular beads being used. Beads less than 100 nm in diameter are usually considered superparamagnetic, having a single domain per bead, and thus attain saturation at very low magnetic fields. In our experience MACS® microbeads, while being around 50 nm in diameter, saturate at an applied field of 0.02 T. Larger beads may saturate at higher magnetic fields.

For beads that have saturated in the applied field, i.e., superparamagnetic beads, the magnetic moment, $\mathbf{m}_s$, is a vector quantity of constant magnitude, but parallel to $\mathbf{B}$. Because the magnetization is constant and not proportional to the applied field, the force on the bead is proportional to the gradient of $\mathbf{B}$, not the gradient of $\mathbf{B}^2$. The force on the bead in this case can be written

$$\mathbf{F}_{\text{mag}} = m_s \nabla B. \tag{2}$$

To compute the net force on a cell coated with magnetic beads that have reached their saturation magnetization we must know the saturation moment of each bead and how many beads are on the cell. For magnetic field gradients that change significantly over the size of the cell we must also know how the beads are distributed throughout the cell.

In the first case the net force on the cell is proportional to $B\nabla B$. In the second case the force on the cell is proportional to ∇B. A large magnetic field gradient is useful in both cases. Magnetic field gradients of 10^4-10^5 T/m have been generated in high gradient magnetic (HGM) filters by using loosely packed micrometer to millimeter sized ferromagnetic particles. Microfabrication provides the tools to specify the geometry, size, and arrangement of features. This ability provides new ways of controlling the interaction between the cell and magnetic field.

MICROFLUIDIC SEPARATION BY NATIVE SUSCEPTIBILITY

High gradient magnetic separation has been shown to be effective at separating red blood cells from whole blood.[5,8,9,30] Magnetic changes in red blood cells have also been used to separate diseased cells and cells with congenital abnormalities.[10,11] These devices typically use small (24 μm diam) ferromagnetic wire loosely packed into a region near the poles of a large magnet, creating a magnetic filter. The small wires generate high magnetic field gradients when magnetized and trap the paramagnetic cells. Initially the red blood cells are retained in the high gradient region, while the other cells and plasma are rinsed out. When the external magnet is removed or switched off, the red blood cells are flushed out.

Han *et al.*[12] recently showed the continuous separation of red blood cells in a microfluidic device by passing whole blood around a thick magnetized (50 μm) nickel stripe of approximately 2.5 cm in length (Fig. 1). The stripe was magnetized in the z direction and consequently the magnetic field gradient is directed away from the nickel stripe throughout most of the fluid region. The researchers observed that red blood cells were concentrated into the high magnetic flux regions after 20 min. Remarkably this effect was observed using whole blood that had not been reduced to its deoxygenated, paramagnetic state. In their oxygenated state, red blood cells are slightly diamagnetic, but less so than the surrounding plasma. The researchers measured a maximum magnetic force of about 30 fN at a field of 0.2 T. This corresponds to gradients of about 200 T/m.

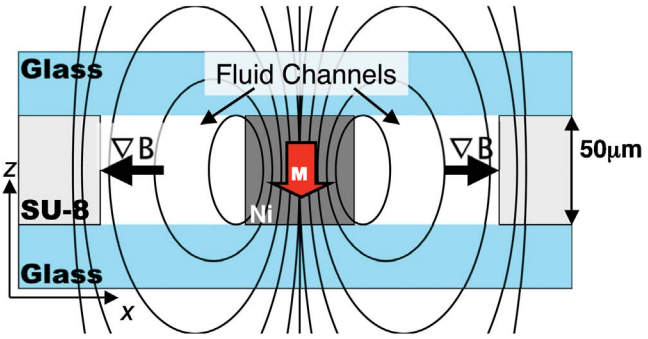

FIG. 1. (Color online) Cross section of the magnetic separation device described by Han *et al.* The nickel stripe (the dark grey object in the center) is magnetized by an externally applied field of 0.2 T. Fluid flows out of the plane in the white areas. The magnetic field gradient is directed away from the nickel stripe throughout most of the fluid region. Red blood cells were observed to move away from the nickel stripe at approximately 0.5 μm/s.

MICROFLUIDIC SEPARATION VIA MAGNETIC BEADS

Immunomagnetic cell separation, in which specific cells are separated by the selective attachment of magnetic particles, has become a common technique in cell biology.[3,13–16] When large (greater than two micrometers) beads are used a rare earth permanent magnet of a few centimeters in size is capable of holding the labeled cells while the unlabeled cells are washed away. When smaller beads are used, some form of magnetic gradient intensifier, such as the packed ferromagnetic wire described above, is generally required. A number of recent publications have described microfluidic devices for the separation of magnetic beads,[6,17–22] but fewer have successfully sorted cells.[23,24,26]

The device described by Inglis *et al.*[23] used magnetic stripes recessed into a silicon substrate to alter the flow of magnetically labeled cells. The stripes were magnetized by an externally applied field of 0.08 T. These stripes were placed at a small angle to the direction of fluid flow and a narrow stream of cells was carried by the flow over the stripes. Magnetically labeled cells (CD45 microbeads from Miltenyi Biotech, Auburn, CA) were attracted to the stripes and tended to follow the stripe direction, while unlabeled cells did not interact with the stripes and followed the direction of fluid flow (Fig. 2). The magnetic force on the cells was estimated to be about 6 pN, with maximum field gradients of around 5000 T/m. For comparison, the Stokes drag on a sphere in a flow of 100 μm/s is 9.5 pN. The greatest

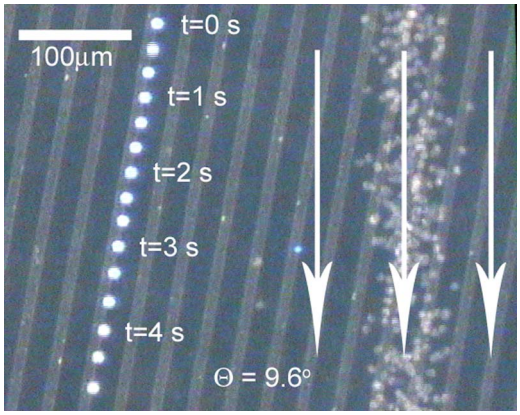

FIG. 2. (Color online) Image from the cell separation device described by Inglis *et al.* Time lapse image showing a single tagged fluorescing leukocyte at different times tracking a recessed magnetic stripe at an angle of 9.6° to the fluid velocity, (110 μm/s) indicated by white arrows. Red blood cells on the right are from a single image. All cells entered the chip at the same point approximately 1.5 mm above the field of view.

challenge with this design is preventing the magnetically labeled cells from permanent sticking to the stripes. A significant number ($\sim$50%) of magnetically labeled cells either stuck permanently to the nickel stripes or were not sufficiently attracted to the stripes to be separated.

DISCUSSION

The two methods of HGM cell separation discussed here each have advantages and disadvantages. Separation by native susceptibility leaves cells largely unaffected, an asset for any later analysis. It is also a simple way of isolating red blood cells without plasma, platelets or any white blood cells. The advantage of using cell-specific magnetic beads to extract certain cell types is that nearly any cell type can be separated, and there are dozens of different blood cell groups, each representing potentially useful information.

Truly useful separation devices will likely have to combine multiple separation techniques, of which magnetic separation may be just one part. Attempts at integrating multiple functions onto a single chip are being made in every field of microfluidics. We are currently testing a device that combines bead-based magnetic cell separation with cell size profiling using deterministic lateral displacement.[25] The goal of that device is to obtain a size histogram of specific cell types and look for changes in response to infection.

CONCLUSION

The big picture of magnetic separation and microfluidics is in making useful devices. What improvements can be made by learning how to microfabricate? The microfluidic devices described here are a step on the road to handheld rapid medical diagnostic tools. They have the potential to operate better than conventional magnetic separators and to do some of the same things that elaborate flow cytometers can do, but at a fraction of the space and cost.[27–29]

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