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Magnetic needles and superparamagnetic cells

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Abstract

Superparamagnetic nanoparticles can be attached in great numbers to pathogenic cells using specific antibodies so that the magnetically-labeled cells themselves become superparamagnets. The cells can then be manipulated and drawn out of biological fluids, as in a biopsy, very selectively using a magnetic needle. We examine the origins and uncertainties in the forces exerted on magnetic nanoparticles by static magnetic fields, leading to a model for trajectories and collection times of dilute superparamagnetic cells in biological fluids. We discuss the design and application of such magnetic needles and the theory of collection times. We compare the mathematical model to measurements in a variety of media including blood.

1. Introduction

Superparamagnetic particles have found a number of applications in biomedical science (Pankhurst *et al* 2003). The *in vivo* detection of very small clusters of cancer cells using sensitive superconducting quantum interference devices (SQUIDs) appears to be one such promising application (Flynn and Bryant 2005, Flynn *et al* 2007). We describe here another application in which a magnetic biopsy needle may be used to collect only specific cells labeled with magnetic nanoparticles, *in vivo*, while ignoring unlabeled cells. In this paper we discuss the magnetic needle method, outline a mathematical model of cell collection and present some preliminary *in vitro* data on collection times for cancer cells in various media.

A superparamagnetic particle (Berkov (1998), p 66), consisting of a single-domain core of ferromagnetic or ferrimagnetic material, presents a very large permanent magnetic moment relative to atoms or molecules of ordinary paramagnetic matter. For example, a single-domain spherical core⁵ of pure magnetite (Fe₃O₄) 25 nm in diameter will have an effective magnetic moment of approximately 4.5×10^5 Bohr magnetons, or 4×10^{-18} J T⁻¹. When an external field is applied to a collection of such particles, the large individual magnetic moments of the single-domain cores align with the field more readily than do normal paramagnetic substances, whose magnetic moments are only a few Bohr magnetons⁶.

⁵Magnetite crystals are generally octohedral, but also may occur as cubic or dodecahedral.

⁶We distinguish between single-domain cores and multi-domain cores. The particles we have been using are claimed by the manufacturer to have 'multi-domain cores' containing between 15 and 20 'single-domain cores'. At present we can measure the average number of single-domain cores decorating a sample of cells, but we cannot measure the number of nanoparticles. Because of the ambiguity, unless otherwise indicated, we will use a model in which each nanoparticle has one single-domain core detectable by the SQUID system. The structure of these nanoparticles is presently under investigation.

For biological applications, the magnetite particles are encased in biocompatible material, e.g. silica with a carboxyl coating, to which antibodies or other ligands can be attached. The surface of a cell, bearing antigens specific to the antibodies attached to the nanoparticles, can be labeled or 'decorated' with $\sim 10^4$ of these particles, causing the decorated cell itself to behave as a superparamagnet. The cell then develops an overall magnetic moment as the individual magnetic moments of the attached nanoparticles align with an applied field. When the field is switched off, the decorated cell loses its overall magnetic moment as the moments of the nanoparticle single-domain cores relax into a random distribution of orientations.

Superparamagnetic cells may be collected with specially designed magnetic fields leading to the applications mentioned above. Pioneering work in the mobility of such cells has been published by Zborowski (1997) and also McClosky *et al* (2000) who present a study of decorated cells and microbeads. Fang *et al* (1999) detected magnetically-labeled cancer cells using magnetic deposition. Rotariu *et al* (2004) and Iacob *et al* (2004) have discussed the use of magnetizable needles and wires to target magnetic microspheres *in vivo*. Recently, Furlani and Furlani (2007) have developed a model for predicting magnetic targeting of multifunctional particles. Here, we shall examine further the magnetic forces on individual nanoparticles and cells, and explore mathematical models for collection of decorated cells by magnetic needles. Data on collection times of decorated cancer and T-cells *in vitro* are presented.

2. Force on a superparamagnetic particle in a magnetic field gradient

In this section we review the basic physics of the force on a magnetic nanoparticle and a decorated cell in a magnetic field gradient in a thermal bath. The potential energy (coupling energy) of a particle with magnetic dipole moment $\vec{\mu}$ in a magnetic field $\vec{B}$ is

$$U = - \vec{\mu} \cdot \vec{B}. \quad (1)$$

The expression for an energy-conservative force is

$$\vec{F} = - \nabla U = \vec{\nabla} (\vec{\mu} \cdot \vec{B}). \quad (2)$$

In the absence of appreciable currents and time-dependent fields (Stratton 1941), the force may also be written as

$$\vec{F} = (\vec{\mu} \cdot \vec{\nabla}) \vec{B}. \quad (3)$$

The force on the dipole suspended in a fluid medium varies with time as the moment orients toward the field direction, after the field B is applied, modulated by random fluctuations from Brownian motion. If the particle is hindered in its ability to rotate as a rigid body because it is tethered to a cell, for example, the orientation of the dipole moment of its ferrimagnetic core can still move toward the applied field via the Néel (1949) mechanism. The characteristic time in this mechanism depends on the applied magnetic field as well as its size and magnetic properties.

The average force on a dipole⁷ is then

$$\vec{F}_{av} = \mu (\cos \theta) \vec{\nabla} B \quad (4)$$

An ensemble of magnetic nanoparticles may be treated using classical Boltzmann statistics (Kittel 1953). In this case, the average value of $\cos \theta$ is

⁷It is incorrect to consider the force on the dipole in its average energy state, which would give a larger force, rather than the average force on the dipole.

$$\overline{\cos \theta} \equiv \int e^{-U/kT} \cos \theta d\Omega / \int e^{-U/kT} d\Omega = L(x), \quad (5)$$

where $L(x)$ is the Langevin function,

$$L(x) = \coth(x) - \frac{1}{x}, \quad (6)$$

and $x = \mu B/kT$.

The RMS variation in $\overline{\cos \theta}$ due to thermal fluctuations is

$$(\delta \overline{\cos \theta})^2 \equiv \int e^{-U/kT} (\cos \theta - \overline{\cos \theta})^2 d\Omega / \int e^{-U/kT} d\Omega = L'(x), \quad (7)$$

where $L'(x)$ is the derivative of the Langevin function,

$$L'(x) = \frac{1}{x^2} - \operatorname{cosech}^2 x. \quad (8)$$

From this it follows that the force on a cell decorated with n nanoparticles each with a moment μ is given by

$$\vec{F}_{\text{cell}} = \mu \vec{\nabla} B \{nL(x) \pm \sqrt{nL'(x)}\}. \quad (9)$$

One can see from figure 1, displaying $L(x)$ and $\sqrt{L'(x)}$, that, especially in weak fields, the single nanoparticle ($n = 1$) undergoes large excursions in orientation (as well as spatial Brownian motion) and consequently large drift-velocity fluctuations, whereas, since fluctuations go as $1/\sqrt{n}$, a more massive decorated cell with n large (equation (9)) will have a more steady drift velocity as derived below.

3. Drift velocity in a divergent magnetic field

3.1. Cells decorated with nanoparticles

We model the cell as a sphere of (hydrodynamic) radius R with n nanoparticles tethered to its surface. The nanoparticles are fixed to the cell via the attachment of antibodies to the specific antigens on the cell's surface (Chosy *et al* 2003). The Stokes viscous drag on the spherical cell is given by

$$\vec{F} = 6\pi\eta R\vec{v}, \quad (10)$$

where η is the viscosity of the suspending fluid and $\vec{v}$ is the relative velocity of the cell with respect to the fluid. We assume for now the fluid is at rest with respect to the magnetic needle.

Considering the cell at terminal velocity, we equate the drag to the magnetic force to obtain

$$\vec{v} = n\mu \vec{\nabla} B \{L(x) \pm \sqrt{L'(x)/n}\} / 6\pi\eta R. \quad (11)$$

Since the force is along the gradient of the magnitude of the field B , the cell in general does not move in the direction of $\vec{B}$ even though for maximum force the moment must align with it.

3.2. Single nanoparticles

The drift velocity of a single nanoparticle can be obtained from equation (11) by putting $n = 1$ and replacing the cell radius R by a , the hydrodynamic radius of the nanoparticle, which includes the inert coating and attached antibodies. The drift velocity of a nanoparticle is much

smaller than that of a decorated cell under the same conditions. For example, the ratio of velocity of the cell to that of the nanoparticle, based on equation (11), is

$$\frac{v_{\text{cell}}}{v_{\text{nanoparticle}}} = \frac{na}{R} \approx 100, \quad (12)$$

where we have taken the hydrodynamic radius of the nanoparticle as 50 nm and that of the cell decorated with $n = 10^4$ nanoparticles as 5 μm . As we shall show later the collection rates go roughly like the square root of the velocity. Decorated cells therefore can be typically collected of the order of ten times faster than free unagglomerated nanoparticles.

The above results are based on the premise that rotation of magnetic moments of the single-domain nanoparticle cores is essentially unhindered on the time scales of the collection times.

3.3. Hindered rotation

We have assumed that the single-domain nanoparticle cores are free to orient in the direction of the applied magnetic field ('extrinsic superparamagnetism'). By use of the classical Boltzmann statistics above, we have shown that $\cos \theta$ can be estimated for a given value of x , the coupling energy to the magnetic field divided by the thermal energy kT . The characteristic time that is required for the particle to approach equilibrium, such that its alignment fluctuates about $\cos \theta$ with amplitude variation of $\sqrt{(\delta \cos \theta)^2}$ after a change in B has been made, is called the 'Brownian relaxation time', and is given by

$$\tau_B = \frac{4\pi\eta a^3}{kT}. \quad (13)$$

This time is relatively short: 310 μs for a radius of 50 nm in water at room temperature, for example.

A free magnetic nanoparticle can be pulled through its fluid environment in accord with the formulas above. However, if the magnetic nanoparticle is bound to the surface of a cell so that the nanoparticle cores cannot rotate freely (hindered), then the orientation of the cell's magnetic moment could not follow the magnetic field direction along a path in which the direction of the applied field is varying. Brownian relaxation would not be available. Such hindered particles are said to exhibit 'intrinsic superparamagnetism' (Rosensweig 1985). The coherent collective electron moments of a single domain in a nanocrystal can orient in the direction of the applied field without a change in the orientation of the crystal itself, the so-called Néel mechanism. The relaxation time for the Néel (1955) mechanism is

$$\tau_N = \tau_0 \exp\{(KV/kT)(1 - B/B_K)^2\}, \quad (14)$$

where τ_N is the time for the system to make its way by thermal fluctuations over an energy barrier of $E = KV$, where K is a magnetocrystalline anisotropy energy per unit volume (Landau and Lifshitz 1960) and V is the volume of the ferrimagnetic domain. B_K , the anisotropy field, is $2K/M_S$. Usually τ_0 is taken to be 1 ns (Bean and Livingston 1959). Because of the exponential nature of the expression for τ_N , its value changes quite rapidly as one increases the (spherical) particle's radius. The 'blocking radius' is reached when τ_N exceeds the 'observation time', the interval during which we require a response. Analogously, one may speak of a 'blocking temperature'. Romanus *et al* (2003) have determined distributions of E in nanoparticle samples from the temperature dependence of the remanent field measured with a SQUID gradiometer.

For weak fields such that $B/B_K \ll 1$, when the relaxation time for particles decorating cells exceeds several seconds, the cell might not in general follow the path of the field. Although particles with τ_N much larger than collection times might not be very effective in moving cells about in the fluid, a broad range of particle sizes can contribute to pulling cells through magnetic gradients. In the experiments described below, a SQUID (superconducting quantum interference device) array (Flynn and Bryant 2005) was used to detect the particles collected on the needle. The detected particle sizes are limited to a narrow range because of the requirements that the remanence times fit within the time window of the data acquisition.

Equation (14) may be rewritten in terms of the nanoparticle's magnetic moment,

$$\tau_N = \tau_0 \exp \langle (\mu_p B_K / 2kT) (1 - B/B_K)^2 \rangle. \quad (15)$$

Figure 2 is a representative plot of τ_N in equation (15) as a function of the magnetic moment of the single-domain magnetite core. As mentioned above, in studying cell collection rates with magnetic needles, as we shall describe later, we measure the remanence fields from cells on the needle with a SQUID system (Flynn and Bryant 2005), detecting those particles for which τ_N lies between 50 ms and about 1 s. By fitting an excitation curve of a sample of fixed (rotationally hindered) nanoparticles to the Langevin function we extracted an average magnetic moment of the particle of $3.85 \times 10^{-18} \text{ J T}^{-1}$. The corresponding τ_N must be in our measurement time window, implying that B_K should be around 445 Gauss (0.0445 T). Using the bulk properties of magnetite listed in table 1 (anisotropy energy density of $K = 1.35 \times 10^4 \text{ J m}^{-3}$ and a spontaneous magnetization, $M_S = 4.72 \times 10^5 \text{ J T}^{-1} \text{ m}^{-3}$) we find $B_K = 572$ Gauss. These numbers are in a reasonable agreement in view of the uncertainties, especially in K (Cullity (1972), p 233). We see from figure 2 that τ_N , for times of practical interest in many medical applications, occurs in a narrow range of magnetic moments. When the magnetic needles collect cells in biopsies, for example, for microscopic examination, the optimal particle size would still be limited by the collection times, in view of the rapid onset of blocking. The optimal particle size distribution must be determined empirically for each application with differing observation times, guided by considerations we discuss here.

When even a modest magnetic field is present, the relaxation time is dramatically reduced, and the particles with larger moments may be able to follow the gradient and thereby help pull the cell into the strongest field region. Clearly one wishes to use nanoparticles with the largest possible moments consistent with relaxation times sufficiently small compared with collection times.

4. Collection times

An important biomedical application of superparamagnetic mobility is the collection of labeled cancer cells in the bone marrow or in peritoneal fluid, for example, with a magnetic biopsy needle. This 'smart needle' would increase the efficiency of such collection, thereby minimizing the need for multiple biopsies. Ideally only the targeted cells would have nanoparticles attached and are attracted to the needle, making the detection of cancer, such as leukemia or wandering metastasized cells, much simpler to analyze under the microscope since many fewer normal cells would be collected. The effectiveness of this novel technique depends upon the length of time required to collect cancer cells located in an accessible volume around the needle. This time is related to the strength of the magnetic field from the needle, the viscosity of the medium and the number of nanoparticle cores per cell and their characteristics. We will discuss below an application of the formulas we have discussed above and show that these provide a *lower limit* to the rate at which cells can be collected. Our results here apply to solitary cells; as we shall see, agglomeration and density-dependent collective effects could enhance the collection rates (Rosenzweig 1985).

5. Magnetic needle prototypes

5.1. Small permanent-magnet needle

We have experimented with thin permanent-magnetic needle prototypes using tiny, 2 mm long cylindrical NdFeB (rare earth) permanent magnets of 1 mm diameter⁸, silver plated, in various combinations. The individual permanent magnets are placed inside a polyimide tube⁹ of length about 16 cm, along with lengths of nonmagnetic stainless-steel rods¹⁰ of the same diameter for stiffening (see figure 3). The needles are equipped with a collecting sheath about 5 cm long made of a larger bore polyimide tube that is sealed on one end with a trimmed drop of epoxy. The sheath slips over the magnet end of the needle, covering the portion inserted into the suspension of magnetic cells, so that magnetic material collected by the needle can easily be removed and studied (using, for example, SQUID detection or microscopic examination) by slipping off the sheath after the exposure to the sample is completed. The diameter of this needle arrangement is approximately that of a standard biopsy needle.

We have examined the collection efficiency of several different arrangements of these magnets, ranging from just a single magnet on the tip of the needle up to 20 contiguous magnets. The needle we shall discuss in more detail below has just two such magnets on the tip of the needle, separated from one another by a 2 mm gap consisting of a 2 mm long machined piece of 1 mm diameter non-magnetic stainless-steel rod. This arrangement provides higher collection efficiency in the near field than three such contiguous magnets or the 20-magnet string, in the geometries we have so far considered. Other geometries can be modeled with the computational codes we have devised, for example, to determine the optimum gap size, etc.

5.2. Calibration

The fields along the axis, leading from the tip, of the various magnet configurations were mapped and fitted with numerical models to check the dependence predicted on distance from the tip as well as to calibrate the overall strength of the configuration (see figure 4). The mathematical model of the magnetic fields of the needle and the collection efficiency is discussed below, using the two-magnets-plus-gap configuration as an example.

6. Model

6.1. The field of the magnetic needle

The magnetic needle under current investigation in general consists of an arrangement of N cylindrical permanent magnets each 1 mm in diameter and 2 mm long, with appropriate stiffening rods, spacers and sheaths. Each individual permanent magnet, with magnetic field running parallel to its axis of symmetry, is modeled as two very thin discs, covering the two ends of the magnet, a north and south pole, separated by the length (2 mm) of the magnet. The strength of the disc ω is determined from the calibration curves shown in figure 4, and is taken to be 750 Gauss. This means that the magnetic field at the surface of the disc at its center is $2\pi\omega$ or 4712 Gauss along the z direction (at $z = 0$ in figure 6). A single-point dipole sitting at the center of the 2 mm long single magnet and producing 4712 Gauss at its surface would have a magnetic moment of $2.36 \times 10^{-3} \text{ J T}^{-1}$ (or A m^2). Figure 5 shows the computed magnitude B in cylindrical geometry in the upper right-hand quadrant for the needle constructed from two magnets coaxial and aligned (i.e. not opposed), separated by a gap of 2mm. A mirror image of this plot reflected along the z -axis about zero would represent the field near the other dipole magnet in this pair. The dip at $z = 0$ and $\rho = 2.58 \text{ mm}$ encircles the needle and represents an

⁸Supplied by American Magnetic Engineering, Inc. (www.amerimagnet@yahoo.com), Kevin Yao.

⁹Polyimide tube SWPT-040-30, ST WL 0.0403 'ID, 0.0423' OD, Small Parts, Inc. (www.smallparts.com).

¹⁰Stainless steel type 316LVM, GWXX-0400, 0.0400+/-0.0002 diameter, Small Parts, Inc.

‘equatorial singularity’. The presence of this interesting feature can be noted in the cell trajectories, but it has little effect on collection efficiencies.

6.2. The range of the needle

The trajectories of superparamagnetic cells in the field of the magnetic needle can be calculated using the magnetic field model. We numerically integrate the expression for the drift velocity given by equation (11) to find the position as a function of time:

$$\vec{r}(t) = \int_0^t \vec{v} dt' = \int_0^t \left\{ \mu \vec{\nabla} BL(x) / 6\pi R \right\} dt' \quad (16)$$

For convenience in our calculations, we use a time-reversed scenario, in which we reverse the sign on the velocity vector. We choose equally-spaced starting locations on the surface of the needle magnet and plot the computed trajectories in cylindrical coordinates, ρ and z . The axis of the needle, z (axis of rotational symmetry), is the locus of $\rho = 0$. The trajectories are coplanar and do not depend on the azimuthal angle ϕ around the z -axis. The travel times to the needle from various points along the trajectories are marked at equally-spaced time intervals. Cells found in the regions between two adjacent trajectories will eventually be collected on the needle between the two ‘starting’ (actually ‘landing’) points, so that it is easy to visualize how they will be distributed along the needle after capture. The paths of cells as a function of time to capture points on the needle are shown in figure 6. The rings of collected cells on the sheath shown in figure 3 illustrate the distribution of landing sites on the magnet.

The volumes around the needle from which the decorated cells are swept out and collected on the needle sheath in the given times are determined by these calculations. Curves are fit to each set of time markers corresponding to each of the eight drift times shown in figure 6, as well as to a symmetric set of times for $z < 0$, not shown in figure 6. These curves of constant drift time to the needle sheath are plotted in figure 7. The corresponding volumes are calculated from the figures of revolution that these curves generate when rotated 360° about the z -axis. The volume of the needle itself is of course excluded from the volume in which cells are collected.

The volume of fluid surrounding the needle from which our model predicts all decorated cells will be collected is shown in figure 8 (‘calc $F = 1$ ’), which includes additional points to those given by figure 7 (1–8 min) for times of 16, 32 and 48 min. The parameters used in the computation are those given in table 1.

7. Scaling

Since the numerical calculation described in section 6 is involved and tedious, we have prepared a two-parameter approximating formula, fitted to the results discussed above, that can be scaled for cases involving parameter values different from those given in table 1. The plausibility of our formula is based on simple considerations as follows. Regard the needle as a point dipole and ignore the angular dependence; the radial drift velocity can be written as

$$\frac{dr}{dt} = \kappa \frac{G}{r^\beta}, \quad (17)$$

where κ contains lumped constants, independent of r . Although both G and β are functions of r , we conjecture that they may be used as fitting constants over the region of interest, allowing us to separate variables and integrate equation (17),

$$\int_0^r u^\beta du = \kappa G \int_0^t d\tau. \quad (18)$$

Consequently, we find the volume swept out in time t goes as

$$V \propto (Gt)^\alpha, \quad (19)$$

where $\alpha = 3/(\beta + 1)$, so that $\frac{3}{8} < \alpha < \frac{3}{5}$, the far- and near-field limits, respectively. That is, when two dipoles are very near one another the drift velocity goes as $\frac{1}{r^4}$, whereas for large separations it goes as $\frac{1}{r^7}$. Remarkably this form (19) fits our results for the two-magnet plus gap model, as shown in figure 8, very well, with $\alpha \approx 1/2$, so that our scaled formula for the volume collected as a function of time, for the time intervals we have chosen, is best fit by

$$V(t) = (226.6 Ft)^{0.4681}, \quad (20)$$

where t is in seconds and V is in microliters. F is a scaling factor for G , so that $F = G/G_0$, where $G_0 (= n/\eta R)$ are the lumped parameters as given in table 1. Thus F is unity for the example shown in figure 7. The collection is $F^{0.4681}$, its same dependence with t . For example, if the viscosity of the fluid in which the cells are suspended is increased to three times that of water, then $F = 1/3$ and $f = 0.60$, so that our collection rate will drop to 60% of the rate in water.

8. Comparison with measurements

We have made a number of measurements of the collection rates for decorated cells using magnetic needles. To assure specificity as well as to closely approximate the actual conditions under which the needle will be used, we used live leukemia T-cells. We show results from a specific immortal leukemia cell line, SupT1, that can be targeted by a specific antibody, CD2. Nanoparticles with a carboxyl coating were conjugated with CD2 antibodies (Jaeto *et al* 2007). They were subsequently incubated with the leukemia cells for 1 h, resulting in the tethering of approximately 10 000 nanoparticle cores to each cell, so that each cell on the average has a magnetic moment of 3.85×10^{-14} A m², as seen by the SQUID. A cell-cytometry count was made to determine the number of cells in a given sample. The cell sample was then placed under the SQUID sensor array and the remanence field was measured after applying a short pulsed field. The resulting magnetic field distribution emanating from the sample was then compared to a theoretical dipole calculation and the total magnetic moment of the sample determined. This further confirmed the number of nanoparticles per cell in the sample using the previously measured magnetic moment μ of a single-nanoparticle core. Important to note is that the actual magnetic moment on the cell could well be larger than that measured because the nanoparticle core size distribution probably is appreciably broader than the relaxation observation window of the SQUID system. Even though many of the cores maybe invisible to the SQUIDS, they will still contribute to the magnetic tug on the cell, perhaps accounting at least in part for collection rates that are larger than one might have otherwise expected. At present we do not know the polydispersity of our nanoparticles.

In order to measure the fraction of cells collected by the needle, and to determine the dependence on viscosity of the suspension medium, the SupT1 cells, as well as Jurkat cells targeted with CD3, were suspended in various fluids including PBS, EDTA, Ficoll and blood. The magnetic needle is then inserted into the suspension, as would be the case in a biopsy measurement, for a measured time. Although our measurements covered a large range of times, actual biopsy measurements *in vivo* would probably be limited to about 2 min. After removal from the fluid, the sheath, to which the magnetically collected cells are attached, is slipped off the needle and placed under the SQUID array, and, as described above, the net magnetic moment of the collected cells is measured¹². From the ratio of the number of cells collected

to the number of cells determined to be in the suspension, and from the total volume of fluid in the vial, we find the volume sampled by the needle. These data are plotted in figure 9. Also shown are the theoretical-model-determined volumes described above for the two-magnet with gap using the parameters in table 1.

To illustrate the usefulness of equation (20) we have made scaled predictions for the Ficoll and blood measurements with $F = 2$ and $F = 30$. The comparison is shown in figure 10.

9. Discussion

The measured collection rate of cells is much more rapid than the model predicts. This discrepancy may arise at least in part from the possibility that the cores we detect with our SQUID arrays are a small subset of a broad distribution of cores present. Members of this broad distribution could all contribute to the magnetic tug on the cell, but would be largely invisible to the SQUIDS because their relaxation times would span a range much broader than the relaxation-time window for SQUID detection. Unfortunately we do not know our actual size distribution. A movie of the cell collection process using a digital microscope reveals two effects that may also contribute to this accelerated collection process. One is the visual observation of large agglomerations of cells moving rapidly to the needle. The other is that the suspending fluid seems to exhibit convective behavior, in which currents appear to carry fluid containing cells toward the needle, while currents depleted of cells flow away. Qualitatively it appears that the paramagnetic cell or nanoparticle densities in the suspensions studied are large enough so that the coupling of the cells to the fluid by Newton's third law (the force of the fluid on the cell is equal to the force of the cell on the fluid) stimulates the formation of convecting currents of fluid. We do not know what role aggregates of nanoparticles, as discussed by Eberbeck *et al* 2005, unattached to cells, play in our observations.

The importance of agglomeration in these data varies with the preparation. Its effect on collection rate may depend on the form the agglomerate takes. For example, if the cells tend to couple in a long string, yielding a relatively small effective hydrodynamic radius, the viscous drag is much smaller than if the same number of cells formed a sphere. The magnetic tug on the agglomerate would be proportional to the total number of nanoparticles that it contains, but the viscous drag, and thus its drift velocity, depends on the shape. One expects that the viscous drag has a much weaker dependence on the number of cells in an agglomeration (which even in the case of a spherical blob goes like the cube root of the number of cells) than the magnetic attraction that is proportional to the number of decorated cells. The cells themselves could be distorted by the magnetic and viscous drag forces acting on them as well.

In view of the time dependence of the experimental data, a large distribution in sizes of agglomerates may be indicated. The larger sizes would arrive at the tip first and, as they are cleared out, the smaller ones would drift in more slowly. The theoretical analysis and experimental data may represent a novel method for determining the agglomeration numbers for cell collections of this type.

Measurements are underway to determine the role of the number density of superparamagnetic cells, as well as the nanoparticle-core-size distribution, in the action of the magnetic needle. The theoretical results shown in figure 9 represent a conservative lower limit to the rate at which decorated cells can be collected, corresponding to monodisperse core size distributions and sufficiently dilute suspensions where magnetically-induced convection and interactions between the decorated cells are unimportant and the suspending fluid remains static. In figure 10 the differences in the collection rates between decorated cells in blood and Ficoll are

¹²Agglomerations of nanoparticles can also be picked up in the SQUID measurements; we are currently investigating this effect.

probably due primarily to differences in viscosity, but also may reflect other collective interactions that evolve differently in the two fluids. The ratio of F values can be used to gauge the relative strength of the forces.

10. Conclusion

We have examined the physical principles involved in the mobility of cells decorated with magnetic nanoparticles in viscous fluids. We have presented theoretical results that represent the lower limit to be expected in the collection rates, corresponding to suspensions of monodisperse superparamagnetic cells sufficiently dilute that inter-cell interactions and collective fluid-cell behavior can be neglected. Medical applications of this technique will involve superparamagnetic cell densities for which our results should apply and serve as a guide. The model predictions can become more realistic as we learn more about the properties of our nanoparticles.

When free rotation of the dipole moments of magnetic nanoparticles becomes hindered we have considered the Néel mechanism and the associated blocking phenomenon. For reasonable collection times, the ferrimagnetic cores for particles to be used in moving cells through magnetic gradients in viscous fluids must have sizes below the region of the blocking radius. On the other hand, for remote detection of such cells, using SQUIDs, for example, the nanoparticle core radii must be only in a narrow region near the blocking radius. Thus, the population of nanoparticles used for moving cells and the population of nanoparticles tethered to cells detectable with SQUIDs could be distinctly different, although using the same distribution is an attractive option. If one wishes to tow and detect the same cells, the selection of the optimal nanoparticle type and size distribution must be done with these considerations in mind. Although SQUID sensors have been used here to measure relative collection efficiencies, the ultimate goal of this technique is microscopic examination of collected cells to identify the nature of disease targeted by the attached antibodies. Substantially fewer cells need be collected in this case than are needed to obtain an adequate SQUID signal, currently approximately 50 000 cells.

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Figure 1.

The Langevin function $L(x)$ and RMS variation $\sqrt{L'(x)}$ as a function of $x = \mu B/kT$. At body temperature (310 K) for magnetic moments typical for our application ($3.85 \times 10^{-18} \text{ J T}^{-1}$), a field of 100 Gauss corresponds to $x = 9$.

Figure 2.

Plot of equation (15) showing the dependence of τ_N on magnetic moment of the superparamagnetic nanoparticle for the applied magnetic field B in steps of 10 Gauss. We have taken B_K as 455 Gauss and T as 310 K. Taking the region between τ_N of 0.1 s and 1 s as the interval where the particles can be detected with our technique, we can estimate that the Chemicell particles (SiMAG-1/1411), magnetite, have single-domain core diameters 24–25 nm.

Figure 3.

Photo of the tip of a magnetic needle, left, showing the two 2 mm long, 1 mm diameter cylindrical magnets separated by a nonmagnetic steel spacer 2 mm long. A nonmagnetic stainless-steel stiffening rod 17 cm long abuts the lower magnet and the ensemble is encased in a polyimide tube. On the right is a 5 cm long polyimide sheath that slips over the tip of the needle. The needle, with its sheath slipped on, was inserted for 15 s into a vial containing a suspension of 3.6 million Jurkat T-cells decorated with CD3-coupled SiMAG TCL magnetic nanoparticles (Chemicell Berlin) in 3.5 ml of PBS. The dark bands are superparamagnetic cells collected by the needle, coincident with the ends of the tiny magnets where the gradient is highest. The cells can then be transferred to microscope slides, for example, for analysis.

Figure 4.

Calibration curves for tiny magnets to form needles, showing measurements¹¹ of the magnetic field directed along the axis of the needle as a function of the distance from the tip of the needle. The predictions from our mathematical models are also shown. The field falls off faster with z as fewer magnets are used. The fields from all three configurations extrapolate to the same field at the tip of the needle, about 4700 Gauss (0.47 T).

¹¹F W Bell, Gauss/Teslameter Model 5070. The diameter of the nominal active area of the probe (STH57-0404), quoted as 0.015 (0.4 mm), is inconsequential for the measurements presented.

Figure 5. Log B in the upper half ($z > 0$) of the magnetic region of the needle. Note the cusps on the corners of the magnet and the equatorial singularity where B goes to zero.

Figure 6.

Tip end of the needle composed of two tiny magnets separated by a gap. The tiny silver-plated Nd cylindrical magnet, 2 mm long, radius 0.5 mm, extends from $z = 1$ to $z = 3$ mm. A non-magnetic stainless-steel spacer of the same radius extends from $z = 1$ to $z = -1$ mm, with another tiny magnet from $z = -1$ to $z = -3$ mm (not shown). The small circles mark the starting points for decorated cells to drift into contact with the magnet's sheath, in increments of 1 min. The circles closest to the needle are cells 1 min away from the sheath; the most distant circles mark locations of cells that will arrive at the sheath 8 min later. Also shown are contour lines on a logarithmic scale of the magnitude of the B field. Note the circles around the equatorial singularity. The 1 mm diameter stainless-steel stiffening rod with its polyimide covering extends along the z -axis to $z = -167$ mm on this plot (not shown).

Figure 7.

Magnet range in 1 min increments. Boundaries of the volumes (surfaces of revolution about the z -axis) from which decorated cells (under conditions discussed in the text) are swept corresponding to drift times ranging in 1 min increments from 1 min (innermost curve) to 8 min (outermost curve). Darker regions denote the two tiny magnets aligned along the z -axis. Lighter regions denote the stainless-steel spacer and stiffening rod. The overall length of the needle is about 17 cm. Please note that the scaling is different along the two coordinate axes. The suspending fluid is assumed to be stationary with no convection or other mixing mechanisms.

Figure 8.

Power law fit to the model calculation of volume swept versus elapsed time, using parameters listed in table 1 in the velocity coefficient. Formula (20) with $F = 1$ is the result of the fit. For $F = 1/5$ and $F = 5$ the computations made using the model are simply compared with the results of the scaling prediction (20) with very good agreement.

Figure 9.

Comparison of our model prediction ($F = 1$ calculation) with collection time courses for live decorated T-cells using the two-magnet-with-gap needle shows that the model is a lower limit on the amount of cells collected in a given time. Other effects, such as convection and agglomeration, as well as magnetite domains invisible to the SQUID, enhance the transport of the cells in the range of the needle.

Figure 10.

Similar to figure 9 with equation (20) scaled, putting $F = 2$ and $F = 30$ to visually agree with the data with differing suspending fluids, blood and Ficoll. From this comparison one concludes that the difference in the lumped constants is about a factor of 15 and probably arises from differences in viscosity. Even though the model is a lower limit, the shapes of the curves are concordant, except for the initial draws, which we suspect include very ‘magnetically-attractive’ agglomerations of superparamagnetic cells.

Table 1

Parameters used in examples. The nanoparticles used in this work were SiMAG-1/1411 (Chemicell) each of which the manufacturer believes has 15–20 single-domain cores. The value of μ corresponds to a single-domain core that is detectable by the SQUID system.

Nanoparticle: magnetite Fe_3O_4	Symbol	Value	Units (all SI)
Core (single domain) radius	r	12.5	nm
Hydrodynamic radius	a	50	nm
Magnetocrystalline anisotropy constant	K	1.35×10^4	J m^{-3}
Spontaneous magnetization	M_S	4.72×10^5	$\text{J T}^{-1} \text{m}^{-3}$
Anisotropy field	B_K	0.0572	T
Magnetic moment	μ	3.85×10^{-18}	J T^{-1} or A m^2
Decorated cell			
Hydrodynamic radius	R	5	μm
Nanoparticles per cell	n	10^4	
Temperature	T	310	K
Viscosity	η	1×10^{-3}	Pa s
Boltzmann constant	k	1.38×10^{-23}	J K^{-1}
Permeability of free space	μ_0	$4\pi \times 10^{-7}$	N A^{-2}