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Nanobiomagnetics

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Chapter 15

NANOBIOMAGNETICS

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Abstract

The application of nanomagnetic materials to biological systems has produced significant advances in research, diagnosis, and treatment of numerous pathologies. This chapter summarizes the major applications of magnetic materials: magnetic targeting, drug and gene delivery, magnetic separation, the use of magnetic beads in manipulating single molecules, as contrast agents in magnetic resonance imaging, and for hyperthermia. Biocompatibility requirements for magnetic materials used in these applications are reviewed.

1. INTRODUCTION

"Nanobiomagnetism" is the intersection of nanomagnetism and medicine that focuses on biological systems and/or processes. Magnetism is an inherent facet of life, from iron in blood to the ability of magnetotactic bacteria, birds, honeybees and other creatures to navigate by the Earth's magnetic field. Iron plays a critical part in many aspects of human neurophysiology. Naturally occurring iron in the body usually is stored within ferritin, which are

12-nm hollow spherical shells that each can hold up to 2,500 iron atoms in the form of mineralized ferrihydrite. Anomalous amounts of iron—possibly in nanoscale form—are associated with many neurodegenerative disorders such as Alzheimer's, Parkinson's, and Huntington's diseases [1].

The ability of magnets to act on objects at a distance makes them valuable medical tools. A 1624 report described the extraction of an iron splinter from an eye using a magnet [2]. Safety pins, bullets and grenade splinters were removed using magnets [3–5]. Grazing cows are fed magnets to prevent sharp metallic objects they eat from damaging the intestines. The invention of stronger, smaller permanent magnets made possible more delicate applications, such as temporarily fixing prosthesis in dentistry, guiding catheters through the body, and navigating within the brain [6–8].

Nanoscale materials have a special relevance to biomedical applications due to their size compatibility with cells (10–100 μm), viruses (20–450 nm), proteins (5–50 nm) and genes (2 nm wide by 10–100 nm long). Nanoparticles are small enough to move inside the body without disrupting normal functions, and can access spaces inaccessible by other means. Cells react to the topography of their environment on size scales as small as 5 nm - up to 1000 times smaller than their own size [9, 10]. Changes in response to topography literally can induce growth or death. Nanostructured materials allow study of these critical processes on a single-cell level [11].

2. MATERIALS

Magnetic biomaterials have different constraints than materials used for other applications. *In vivo* (in the body) applications require strict biocompatibility. *In vitro* (outside of the body) applications have less strict requirements, but techniques involving living cells still must consider the effect of the materials on the sample under study. In addition to biocompatibility, materials must be capable of being functionalized with one or more molecules, must retain their magnetic properties for a reasonable period of time in aqueous media with varying pH, must not be cleared too quickly from the bloodstream, and must form stable, non-aggregating dispersions [12, 13].

2.1. Biotoxicity

Cells can be killed by external agents or can be induced to "commit suicide" via a pattern of events called programmed cell death or *apoptosis.* Injuries from external agents include mechanical damage and exposure to toxic chemicals (e.g. chemotherapy). Substances toxic to cells are called cytotoxic, and different types of cells can have different responses to the same material.

The vast majority of research in this area involves iron-oxide particles, as they are highly biocompatible, cheap, can be made in a variety of ways and sizes, and can be made as superparamagnets or ferrimagnets. Iron oxides are metabolized into elemental iron and oxygen by hydrolytic enzymes in the body. The iron joins the normal body stores and the body compensates by taking up less iron from the stomach. Intravenous injections of up to 250 mg iron/kg body weight does not produce chronic or acute hepatotoxicity in rats [14], while 1–3 mg/kg has been used clinically in humans [15].

The disadvantage of iron oxides, however, is their low magnetic susceptibilities. Iron nanoparticles offer an order of magnitude greater susceptibility than iron oxides at room temperature, but are easily oxidized and are not as biocompatible as their oxides. Fe-C composite particles made by mechanical milling, chemical reduction, or plasmochemical recon-densation have been used in clinical trials for hyperthermia and drug delivery [16-18]. These particles are biocompatible and the carbon in the particles may assist in reversing drug-induced toxicities via physical adsorption. The need for monodisperse particles for magnetic recording has stimulated a many chemical methods that produce a broad variety of element and alloy nanoparticles, including Co, Ni, Cu, FePt, CoPt, Fe, $CoFe_2O_4$, $MnFe_2O_4$, $SmCo_5$ and even core-shell particles such as $FePt/Fe_3O_4$ [13, 19–27]. Physical deposition techniques such as inert gas condensation [28–31] and laser ablation [32–35] also are used.

2.2. Coatings

Coatings can improve oxidation resistance, colloidal stability, the ability to functionalize, phagocyte resistance, mechanical stability, and biocompatibility. Unfortunately, coating a magnetic nanoparticle with a biocompatible material does not necessarily render the nanoparticle biocompatible [36]. The chemistry for functionalizing gold is well established and gold coating offers corrosion resistance; however, biotoxicity issues may need to be resolved [37, 38]. Polysaccharide coatings such as dextran, starch, and chitosan are biocompatible and offer a range of functionalization options [39–41]; however, they can be structurally weak and can be dissolved by highly acidic environments. Silicon-based coatings are used to protect particles from lysomal enzymatic digestion, and improve mechanical properties and chemical stability [42–45]. Silica-coating can improve chemical stability, but a porous coating may allow the contents inside to be dissolved or oxidized [46, 47].

Many polymers are biocompatible and may be used as coatings for metallic or ceramic particles, or can serve as hosts by either capturing nanopar-

ticles inside a larger polymer particle or attaching nanoparticles to their surfaces. Polyethylene Glycol (PEG) and related polymers covalently bond to surfaces or are adsorbed on magnetic nanoparticles and can prolong the circulation time in the bloodstream [39, 40].

2.3. Clearance Time

Particles introduced into the bloodstream are covered rapidly by components of the circulation, such as plasma proteins, in a process called *opsonization.* Opsonization makes the particles recognizable to the body's major defense system, the reticuloendothelial system (RES). The RES comprises a diffuse system of phagocytic cells (which engulf inert material) that are primarily associated with the connective tissues in the liver, spleen, and lymph nodes. Macrophage (Kupffer) cells in the liver and macrophages of the spleen and circulation are important in removing particles identified by opsonization. A significant fraction of nanoparticles can be cleared from the circulation system in as little as 15 minutes [48, 49].

The clearance rate is dependent on size, charge, surface hydrophobicity and the number and nature of functional groups on the surface [48, 50]. These variables are interdependent, making understanding the role of each one independently challenging. Some of these variables also may affect the magnetic properties. For example, smaller particles more easily evade the RES; however, the smaller size usually results in a smaller moment.

Anionic particles with negative surface charge have a high affinity for the cell membrane and are typically taken up by the endocytic process [51]. Cationic magnetite particles show significantly lower cell-survival rates but their toxicity depends highly on the magnitude of the surface charge. More highly cationic particles tend to be more toxic [52]. Hydrophilic surfaces such as dextran, polyethylene glycol, polyethylene oxide, poloxamers, polysorbates and polyoxamines provide a dynamic "cloud" of hydrophilic and neutral chains at the particle surface that repel plasma proteins and prevent rapid removal of particles from circulation [53–55].

Related to clearance time is the manner in which the nanoparticles attach to cells. Nanoparticles can be internalized or remain adhered to the surface. The mechanism is determined by the surface charge, adhesion properties, and chemical functionality of the cells with respect to the nanoparticle.

2.4. Magnetic Fluids

The delivery of nanoparticles in the human body usually requires suspending the nanoparticles in a water-based fluid. *In-vitro* applications also usually require an aqueous environment. Magnetic nanoparticles must remain suspended in fluid (or be easily re-dispersed when needed) and cannot form aggregates due to van der Waals or magnetic interactions.

Magnetic particles in a solution undergo two types of relaxation:

Brownian relaxation, in which the entire particle rotates, and Néel relaxation, in which the moment rotates while the particle remains still. The Brownian relaxation time τ_B is

$$\tau_B = \frac{3\eta V_H}{kT} \tag{1}$$

where V_H is the hydrodynamic volume of the particle, k is the Boltzmann constant, T is the temperature, and η is the dynamic viscosity of the carrier liquid. The hydrodynamic diameter characterizes how a particle moves through the fluid in which it is suspended and may be different than the magnetic size due to agglomeration, coating, or interactions between the fluid and the nanoparticle surface [56, 57].

The Néel relaxation time τ_N is defined by [58, 59]

$$\tau_{N} = \tau_o \exp(\Delta E/kT) \tag{2}$$

where ΔE is the energy barrier over which the magnetization must reverse, and $\tau_o \sim 10^{-9}$ s. ΔE usually is determined by the product of the anisotropy K and the magnetic volume V: $\Delta E = KV$. Particles with relaxation times faster than 100 s are called superparamagnetic. Since ΔE depends on the nanoparticle size as the radius cubed, τ_N can range easily over 9–10 orders of magnitude. The hydrodynamic and magnetic diameters of the particle can be determined by the peak frequency of the ac susceptibility [57].

The relaxation rates are affected differently by the immobilization of the nanoparticles. The Brownian relaxation time can be changed by changing the viscosity of the carrier while the Néel relaxation should be independent of the carrier liquid. The relaxation rates also are affected differently by phenomena such as immobilization of a nanoparticle due to endocytosis.

3. TARGETING

Some materials are taken up easily by all types of cells, while others are preferentially taken up by specific types of cells. A disadvantage to systemic treatment is that healthy cells can be negatively affected, as in chemotherapy. Often, a specific type of molecule needs to be separated for further study, so the ability to differentiate between different types of cells is very impor-

tant. Nanoparticles can be localized by physical, chemical, and/or magnetic targeting.

Physical or passive targeting uses surface features of nanoparticles, such as hydrophobicity, charge or pH, to induce reactions that cause the nanoparticles to stick to or enter the cells [60]. This mechanism is highly non-specific, but may be used and to take advantage of the natural clearance of the RES if the targeted organs are within the RES [61]. Nanoparticles can be targeted to certain pathologies such as tumor or inflamed tissue because of leaky vasculature. This phenomenon is known as the enhanced permeation retention (EPR) effect [62].

Chemical targeting uses functionalization of particles to increase the specificity of binding [63]. Functionalization is the physical or chemical association of ligands—targeting agents, therapeutics, surfactants, etc.—with a magnetic nanoparticle. Functional groups may be incorporated using covalent or non-covalent bonding, and/or physical adsorption. Binding to a receptor of interest is called specific binding, while binding to that *and* other sites is called non-specific binding. Antibodies, for example, specifically bind to their antigen, providing an effective means of tagging. Molecules used for targeting include proteins, oligonucleotides, antibodies and their fragments, lectins, hormones, charged molecules, nucleic acids, peptides, and receptor ligands.

Magnetic targeting is used when a therapy has limited ability to be chemically targeted to specific types of cells or tissues due to high nonspecific binding. The force felt by a magnetic moment of value m in a gradient field is

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

The magnetic targeting force must compete with the force due to linear blood-flow rates of about 0.05 cm/s in capillaries to 10 cm/s in arteries and 50 cm/s in the aorta. Iron-oxide nanoparticles require flux densities at the target site on the order of 0.1 to 1.0 T with field gradients ranging from 8 T/m (femoral arteries) to over 100 T/m for carotid arteries [39, 64].

The accuracy of magnetic targeting also is dependent on the depth of the target tissue within the body: organs such as the liver and the lungs are harder to target than organs closer to the surface or in the extremities. The primary challenges are producing a focused field of sufficient magnitude and gradient, and fabricating nanoparticles with sufficiently high moment [65]. As an example, magnetic targeting was useful for treating subcutaneous mouse lymphoma models, but was not effective in intraspinal glioblastoma models [64]. Similarly, permanent magnets are more effective on surface tumors and tumors in extremities, while electromagnets or superconducting magnets (such as in clinical MRI machines) can be used in locations requiring higher gradients.

Magnetic targeting has been shown to reduce significantly the movement of particles to undesired organs and tissues during the time the magnetic field is applied [64]. Micron-sized FeC nanoparticles have been targeted successfully to various organs, including the liver, lungs, and the brain [66, 67]. A complication is that, in some cases, the particles are retained only while the magnetic field is applied. Some studies indicate that the magnetic field promotes extravasation (movement of nanoparticles out of blood vessels) and the particles remain in the target area after removal of the field; however, other studies show that nanoparticles migrate after the targeting field is removed [66, 68–70].

One relatively new approach proposed for magnetic targeting is "ferromagnetic seeding" [71–73]. Nano-sized ferromagnetic objects ("dockers") could be inserted by catheter. The docker would reinforce the magnetic gradient of the external magnetic field, decreasing the need for externally applied high-gradient fields. A similar idea has been suggested for stents in treatment of vascular disease [74–76].

4. MAGNETIC SEPARATION

The detection of specific molecules is critical for diagnosis, treatment, and prevention of disease. The development of fast, handheld analysis units capable of detecting multiple species is made more urgent by fears of biological and chemical terrorism. Magnetic separation has been applied to everything from separating tin from stainless steel at recycling centers to separating pure natural diamonds from diamonds with inclusions of other (magnetic) minerals.

Few cells are naturally magnetic enough to be separated due using their own inherent magnetism, so the cells must be attached to a magnetic nano- or micro-particle with a detectable magnetic moment. Magnetic cell sorting first was proposed using surface markers for cell receptors [77]. Magnetic cell separation allows separation of target cells directly from blood, bone marrow and other fluids in short times due to the fast reaction kinetics. The limiting factor for magnetic separation is identifying a linking molecule with high specificity for the desired cell.

Magnetic sorting may be accomplished with micron-sized or nanometer-sized particles [78]. Smaller nanoparticles produce suspensions that are stable against sedimentation due to gravity or an applied magnetic field, while larger particles can be used to take advantage of sedimentation as part of the separation process [79].

Applications include purging malignant cells from autologous stem cell products [80], water purification [81], minimizing and recycling nuclear waste [82, 83] and recovering heavy metals [84]. Blood purification using magnetic carriers has been used to treat autoimmune and inflammatory diseases, including myasthenia gravis, lupus and Guillain-Barré syndrome [85]. Gram-negative bacteria, such as *E. coli* can be detected at concentrations of 15 cfu/mL, while gram-positive bacteria such as vancomycin-resistant enterococci can be detected at even lower concentrations [86, 87]. T4 and T8 cells in HIV-infected patients have been isolated using magnetic separation, thus allowing study of the effect of different drugs on specific types of cells [88]. Isolation of rare cell populations such as endothelial cells in blood down to 10 cells/ml has been accomplished [89].

Magnetic separation consists of three parts: tagging or labeling the desired cells with a magnetic marker as described earlier, separating magnetically labeled cells from unlabeled cells, and measuring the magnetic properties to quantify the number of cells present.

4.1. Separation

Separation may be done in batch or flow configurations, depending on the specific application. In batch processing, the magnetic beads and the analyte material are mixed. The reaction kinetics determine the amount of time necessary to wait for a sufficient amount of binding to occur. A magnet is used to separate the magnetically targeted cells from the non-targeted cells, as shown in Fig. 1. The most commonly used (and commercially available) materials for cell separations are micron-sized polymer beads into which a magnetic material — usually maghemite — has been embedded.

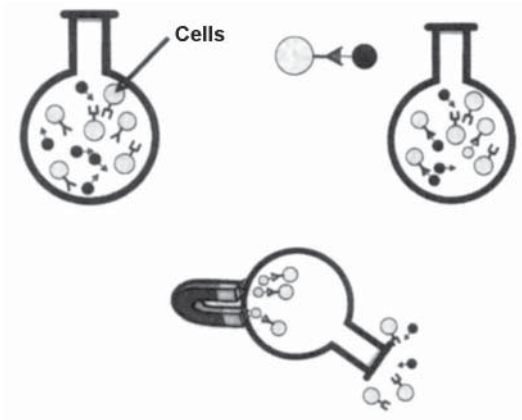

Figure 1. A schematic illustration of the magnetic cell separation process.

Fluid-flow techniques allow continuous processing and are advancing rapidly due to microfabrication capabilities [89, 90]. A permanent magnet can be used to either deflect or collect magnetically labeled particles. Several flow channels may be used in parallel to increase throughput. The magnetic force acting on a magnetic carrier is given by [91]:

$$F_b = \frac{1}{2\mu_o} \Delta\chi V_H \nabla B^2 \tag{4}$$

where F_b is the force on a single magnetic carrier, ∇B^2 is the magnetic energy gradient, V_H is the hydrodynamic volume of the magnetic carrier, and $\Delta\chi$ is the difference in magnetic susceptibility between the carrier and the suspending medium. This deflective force competes with the drag force F_d of the fluid on the particle.

$$F_d = 3\pi v_m D_H \eta \tag{5}$$

where D_H is the hydrodynamic diameter of the magnetic carrier, v_m, is the velocity of the magnetic carrier and η is the fluid viscosity.

4.2. Detection

Magnetic sorting techniques have high potential for real-time detection and monitoring of bacterial, viral and other pathogenic contamination [92]. Integrated structures utilizing nanolithography can perform sorting and quantitative analysis in a single device. Magnetic transducers have low interference, low background signal, do not require sample pre-treatment, and can be small enough to be portable. Magnetoresistive techniques have an advantage over techniques that use, for example, MFM or AFM tips to manipulate magnetic beads attached to molecules, in that they are much faster and have the potential to detect more than one molecule at a time.

Spin-valve and other magnetoresistive devices detect the stray field from a magnetic micro- or nanobead, as illustrated by **Fig. 2**. Lithographically fabricated microcircuits [93–96] may be used to manipulate the magnetic particles. Detection limits in the 10^2 nM can be achieved, and detection of single particles is theoretically possible.

Detection of multiple species on a single chip is possible by fixing a probe molecule (often DNA) to a polymer layer covering the sensor, as shown in **Fig. 3**. The analyte DNA is a single strand complementary to the probe DNA and is labeled (often with biotin). Magnetic microspheres functionalized with streptavidin (which attaches to biotin) are then introduced; the microspheres bind to the biotin, which is present only on the successfully trapped DNA.

The signal measured by sensor can be used to quantify the amount of analyte present. The response to the sensor is determined by the in-plane component of the stray fields induced by the magnetized microspheres. Concentrations as low as 3.2 pg/ml have been detected [98]. The introduction of tunneling magnetoresistance (TMR) sensing elements and smaller magnetic markers will increase the sensitivity of the method.

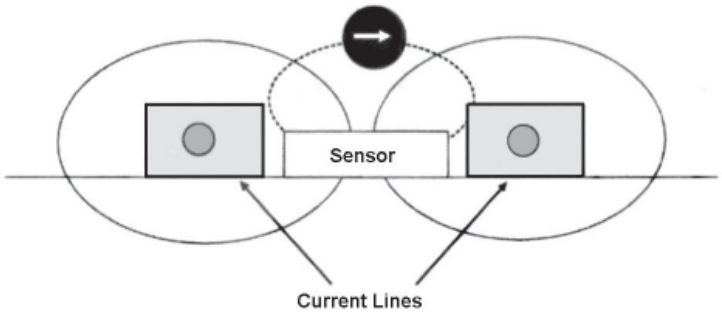

Figure 2. Sensing the stray field of a magnetic bead using a magnetoresistive sensor [93].

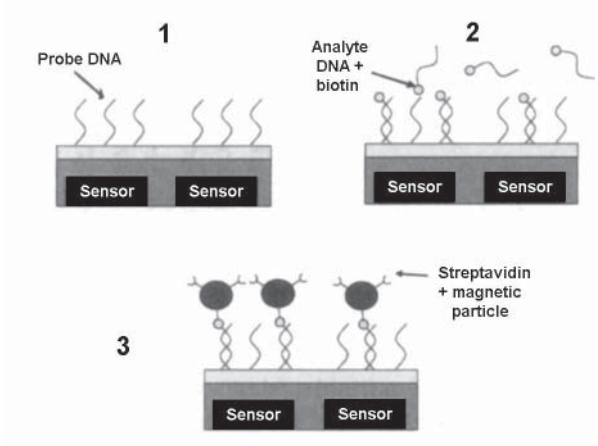

Figure 3. Process of detecting DNA via a magnetoresistive device. (After [97]) The probe DNA is fixed to a polymer layer on the chip (1), the analyte DNA tagged with a molecule such as biotin in introduced and allowed to bind (2). The excess analyte is removed and a molecule that binds to the biotin (streptavidin) is attached to the magnetic label and introduced (3).

The Bead Array Counter (BARC) was one of the first sensors to measure DNA-DNA, antibody-antigen and ligand-receptor pairs at the level of single molecules [99–101]. Adapting technology developed for MRAM allows the possibility of testing for a large number of different molecules with a single chip because different sensors can share circuitry.

A primary challenge in "lab on a chip" devices is to integrate the fluidics and the sensing devices on a scale that allows a handheld device [102]. A microfabricated chip comprising a compact electromagnet, a GMR sensor and a microfluidic flow cell that can simultaneously detect eight different analytes and is small enough to sit on a table top already has been developed [103]. More recently, a planar Hall effect magnetic sensor using exchange-biased Permalloy has been shown capable of detecting a single 2-micron magnetic bead, which corresponds to a 300 nV signal at 10 mA and 15 Oe applied field [104]. The advantage of planar Hall sensors is that the entire active surface is used for bead detection, in contrast to GMR or spin-valve sensors. A higher signal-to-noise ratio is expected, and the lower noise of the planar Hall sensors is expected to make detection of single nanobeads possible [105]. Magnetic tunnel junctions and anisotropic magnetoresistive rings are also possible candidates.

An alternative detection technique for biological molecules is based on changes in the Brownian relaxation due to binding [106, 107]. The relaxation frequency of a nanoparticle changes when it binds to another molecule, as the binding increases its hydrodynamic size. The shift is proportional to the hydrodynamic size of the nanoparticle, allowing discrimination between target molecules with different sizes (although molecules with different functionalities, but similar sizes cannot be independently detected). An advantage of this technique is a signal is present both before and after binding, which allows for reliability checks.

Superconducting Interference Device (SQUID) sensors are more sensitive than GMR devices, but require low temperatures and magnetic shielding. RF Squids have been used to measure magnetic markers to which monoclonal antibodies have been attached [108]. SQUID sensors also can be used to detect changes in relaxation due to binding or changes in local environment. Magnetic nanoparticles immobilized *in vivo,* homogeneously distributed over a total volume of 0.1 ml, could be detected at a limit of 0.3 nmol using a SQUID gradiometer [109, 110].

5. MAGNETIC TWEEZERS

Magnetic beads can provide a "handle" that can be used to manipulate molecules and investigate intermolecular interactions [111]. DNA strands can be attached to a glass surface on one end and a micron-sized magnetic bead on

the other. Magnetic fields can be used to apply linear or torsional force to the DNA, allowing investigation of the extension and elasticity of the molecule using forces from fN to a few tens of pN [112–115].

Mechanical manipulation is interesting because cell morphology regulates many functions, including cell growth, proliferation, protein synthesis and gene expression; however, there is much to be understood about how mechanical signals are translated into biological processes. Magnetic twisting cytometry can be used to apply controlled mechanical stresses directly to specific cell-surface receptors *via* ligand-coated particles. Cellular mechanical properties such as cell stiffness and viscosity can be measured in this manner [116–118]. The reliability of these measurements, however, can be affected by how the magnetic bead binds to the cell. Although nanoparticles may localize at a cell-membrane receptor and remain external to the cell, most nanoparticles are taken into the cell via receptor-mediated endocytosis, which can produce an inhomogeneous cell shape. Applied forces and torques may not be as evenly distributed across these regions and a shear stress may result. The internalization of magnetic nanoparticles by cells can, however, allow probes of the cell interior. Anionic ferromagnetic nanoparticles can be internalized by endosomes via endocytosis, producing a superparamagnetic endosome. The magnetized endosomes elongate in the field, allowing study of the rheological properties of the cell interior [119,120].

6. DRUG AND GENE DELIVERY

Controlled drug delivery has the potential to improve drug efficacy, as well as patient convenience and compliance [121, 122]. Although overall drug dosage can be reduced by 50–80%, dosage at the target site is increased and systemic uptake is decreased. Local drug delivery also reduces the patient-to-patient pharmacokinetic variability inherent in oral and intravenous applications. Protecting the drugs until they reach their target area increases the usability of drugs that have a short half-life in the body. Allowing the release of a drug over a prolonged period of time maximizes the effect of drugs such as chemotherapeutics that are effective only during a specific part of a cell's life cycle.

Nanoparticles were first used for drug delivery starting around 1970, when they were developed as carriers for vaccines and anticancer drugs, and are now widely used [123–125]. The ideal nanoparticle for drug delivery must be able to efficiently incorporate a reasonably high weight fraction (loading) of the drug, must form a stable suspension in an aqueous medium, must be biocompatible and biodegradable, and must not be cleared too rapidly from the bloodstream. In addition, nanoparticles should be able to be made in a range

of sizes, but with uniform size distribution, and should be able to be further functionalized. The main attraction of magnetic nanoparticles from the perspective of drug delivery is the ability to use the magnetic properties to either limit the drug to a particular region using magnetic targeting, or to release the drug remotely.

Drug may be encapsulated in, conjugated to, or adsorbed onto the surface of a nanoparticle. The drug may be released via degradation of the carrier particle or may be triggered by heat or pH. Many drug delivery systems use polylactic-co-glycolic acid (PLGA), polycaprolactone (PCL) and their copolymers, as these are biodegradeable, FDA-approved materials and the degradation rate can be controlled by the particle formulation [126]. Careful choice of surfactants can allow hydrophobic drugs to be transported throughout the body [127].

Ionically bound pharmaceuticals have the advantage that the active-low-molecular weight substances can desorb from the carriers after a defined time span and diffuse from the vascular wall into the tissue. The diffusion through the vascular wall can significantly change the desorption kinetics of the pharmaceutical [128]. Epirubicin chemoadsorptively bound to a polymer-coated particle can desorb according to physiological environment (pH, osmolarity, and temperature) [129]. The half-life of the drug desorption can be fixed to be approximately the same as the desired time for magnetic field targeting.

6.1. Chemotherapy

Cancer is a leading cause of death worldwide. Chemotherapy, a common treatment, is non-selective and causes significant negative side effects. Chemotherapy dosages are calculated primarily by the individual tolerance levels of a particular patient, which means that physically weaker patients are not able to receive adequate doses for successful treatment [122]. Targeting the drugs and preventing release until they reach the tumor decreases the damage to normal cells and increases the dosage at the tumor.

The first evidence for the utility of nanospheres in cancer treatment was demonstrated in the early 1980s. Widder, *et al.* showed significant remission of Yoishida sarcoma without drug toxicity in rats using magnetically targeted albumin microspheres with doxorubicin [69, 130, 131]. The majority of magnetic nanoparticles used for drug delivery are based on iron-oxide or iron-carbon combinations. Mitoxantrone, doxorubicin, mitomycin C, etoposide, paclitaxel, oxaliplatin and epirubicin have been bound to iron oxide or Fe-C fluids for magnetically targeted cancer treatment [16–18, 39, 67, 69, 131, 132]. MTC™ is a micron-sized Fe-C particle with 3–10 times greater susceptibility than the corresponding-sized Fe_3O_4 particles [133]. The high susceptibility is necessary for the goal of magnetically targeting hepatocellular (liver) carci-

noma, which requires the particles to be captured at depths up to 14 cm. Despite the recent suspension of one U.S. Phase II/III liver-cancer trial that was using magnetically targeted Fe-C particles, other studies suggest high potential for treatment of cancer in humans.

6.2. Radionuclide Therapy

Radiotherapeutics attack cancer by causing radiation damage to DNA in cells. The requirements differ from those for drug delivery because the radiotherapeutic can act at a distance and does not have to separate from the delivery particle. Radiotherapeutics can be selected to provide action over a range of distances, from tens of nanometers to hundreds of microns. Three radiotherapy modalities can be identified. Brachytherapy, most often used with beta-emitters, uses tightly enclosed radioactive material that is brought in close proximity to the tumor. A second modality is intravenous injection so that the radiopharmaceutical binds to the outside of the tumor cells or is taken up by the cell and irradiates from within. The third approach uses a carrier loaded with the radiotherapeutic that is transported to the vicinity of the target cells, and then released.

Y-90 is a stable beta emitter, releasing less than 5% of the bound radioactivity within 3 weeks [134, 135]. The polymer decay rate can be matched to the 64.1-hour half-life, so that once the particles loose a significant amount of their radioactivity, they decay into lactic acid. This treatment could deliver up to 100 times greater dose of radiation to the tumor than conventional external beam radiation therapy and minimize the damage to healthy tissue [135, 136]. Squamous cell carcinoma in rabbits has been treated using 100-nm multidomain magnetic iron oxide/hydroxide particles onto which I^{123} has been ionically bonded [137, 138]. A permanent magnet is used for magnetic targeting and the biodistribution of the ferrofluid is detected using a gamma camera [138].

6.3. Magnetic Switches

Magnetic fields can be used to activate the release of a drug remotely. For example, liposomes can be made to encapsulate magnetic nanoparticles and drugs. An applied ac field causes the magnetic particles to heat, which in turn opens the lipid layer and releases the encapsulated drug [139, 140]. A similar effect can be used with thermoresponsive gels, which are chemically cross-linked polymer network characterized by pores, elasticity, and the ability to change volume when stimulated by temperature. Entrapping magnetic nanoparticles in the gel allows control of the pore size via the external ac magnetic field. Drug entrapped in the pores is released when the gel swells and the pores expand [141, 142].

Magnetically induced stress has been used to control drug release. Polymer spheres filled with magnetic nanoparticles and drug subjected to an oscillating magnetic field produced small stress-induced cracks in the polymer. The cracks allowed liquid to enter the spheres and carry out drug. Using magnetism as a "release-on-demand" mechanism could be useful for insulin-dependent diabetics [143, 144].

Innovent, Inc. has developed a magnetic capsule made of two or more parts that are held together magnetically. Demagnetizing the magnetic capsule by applying alternate pulses of opposite magnetic polarity allows the capsule to open. The resulting capsule parts are small enough for the patient to eliminate. The capsule is smaller than ones now used for endoscopy and could be used for delivery of drugs to the gastrointestinal tract [145].

6.4. Gene Delivery

Transfection refers to the incorporation of exogenous DNA into a cell; however, the incorporation process is diffusion limited and thus slow. "Magnetofection" uses magnetic targeting of polyethylenimine-coated magnetic nanoparticles to which gene vectors are electrostatically attached. The magnetic targeting overcomes the limitations of diffusion, allowing increases in uptake in the target tissue [146–148]. Research remains to be done on the influence of the externally applied magnetic field beyond merely attracting the nanoparticles to the tissue. Magnetic targeting also has been used to deliver stem cells [149].

7. MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) aids in diagnosis, research, and treatment of a wide variety of pathologies. The first attempts to use nuclear magnetic resonance for medical purposes were made by Odeblad and Lindstrom in 1955 [150], but it was not until 1973 that Lauterbur developed magnetic-field-gradient methods capable of generating images that could be used clinically [151]. Although hydrogen atoms are the usual targets, magnetic nuclei such as ^{13}C, ^{19}F, ^{23}Na and ^{31}P can be detected in biological tissue. MRI measures change in the magnetization of the protons in water molecules in a magnetic field after being subjected to a radio-frequency magnetic field pulse. Protons in different types of tissues relax differently, thus providing the contrast necessary to distinguish between different types of tissues.

One mm^3 of water contains 6.7×10^{19} H nuclei. The nuclear magnetic moment of the sample is proportional to the magnetic field strength and inversely proportional to temperature and an external magnetic field creates a surplus

of nuclear spins aligned parallel to the field. The surplus in room-temperature water at a magnetic field of 1 T corresponds to a fraction of 3.2×10^{-6} of the protons. A radio-frequency field at a frequency near the Larmor frequency stimulates precession of the nuclear magnetization about the static field. The amplitude of the processing nuclear magnetization decays with time until the original equilibrium state is recovered.

Two time constants describe the relaxation process: T_1 is the longitudinal relaxation time (a spin-lattice relaxation involving energy transmission to the surroundings) and T_2 is the transverse relaxation time (a spin-spin relaxation). An individual moment sees many other moments in thermal (Brownian) motion and thus experiences a continuously changing magnetic perturbation field. Spectral components of the field that correspond to the Larmor frequency induce longitudinal relaxation, while transverse relaxation is due to the frequency of collisions between the molecules [152]. The slope of the inverse relaxation time vs. concentration plot is called the relaxivity, with r_1 being the relaxivity corresponding to T_1 relaxation and r_2 being the relaxivity corresponding to the T_2 relaxation. Fat has a very efficient energy exchange and thus a short T_2, while water is less efficient and has a longer T_2. The white matter of the brain has a T_2 around 60–100 ms and a T_1 an order of magnitude larger.

Magnetic contrast agents work by changing the relaxivity of the water in the area near the contrast agent, thus improving differentiation between otherwise similar types of tissue. Individual atoms such as Gd and Fe decrease T_1 by orders of magnitude; however, they must be chelated for *in vivo* use to prevent toxicity. A disadvantage of these types of contrast agents is that they equilibrate rapidly throughout the interstitial space, making it difficult to identify specific regions with high precision [153]. Superparamagnetic nanoparticles, either coated metal particles or polymeric nanoparticles loaded with metal atoms, preferentially affect the *transverse* relaxation time T_2 as protons diffuse within the inhomogeneous magnetic field created by the magnetic clusters.

Most clinically used nanoparticle contrast agents are iron-oxide based and are grouped into two categories: Superparamagnetic iron oxides (SPIOs) are between 500 nm and 50 nm, and ultra-small SPIOs (USPIOs), which are smaller than 50 nm.

SPIOs are used for gastrointestinal tract, liver, and spleen imaging and take advantage of the natural clearance of the RES, the transit time through the gut or preferential uptake by specific cells. The most promising uses of SPIOs are to improve the sensitivity of detection and localization of primary and metastatic brain tumors, inflammation and ischemia (insufficient supply of blood to an organ) [154–156].

USPIOs can be used as "blood pool agents" because their smaller size allows them to remain in the bloodstream for longer times. They can be used to assess perfusion (the passage of blood into an organ or tissue) in areas of ischemia and provide information about capillary permeability. They also can be used to study the extent of tumor neovascularity and associated permeability changes [153].

Nanoparticle T_1 contrast agents are being studied as well. Gd-chelates can be surrounded by a polymer with a high affinity for the metal and again by a porous hydrophobic polymer shell that modulates access to the core [157]. The coatings increase the circulation time, which has been the primary limitation of Gd chelates. When administered intravenously, the nanoparticles remain in the intravascular space, and thus provide excellent visualization of the vasculature.

Natural targeting can be used by incorporating magnetic nanoparticles within the lipid phase of liposomes. The relaxivities of magnetoliposomes vary enormously, depending on size and surface characteristics, and on whether the magnetoliposomes are free or bound [139]. Changes in the relaxivity can indicate bound vs. unbound magnetoliposomes and even changes in the ratio r_1/r_2 also can be used for contrast.

Magnetic contrast agents can be targeted; however, targeting is usually chemical due to the magnetic nature of the resonance measurement. Restenosis, a complication of coronary angioplasty that involves the proliferation and migration of vascular smooth muscle cells, and arterial plaques can be detected by targeted Gd contrast agents [158]. The acoustic reflectivity increases when the nanoparticles are bound, thus allowing high contrast between the targeted tissue and the background. MRI also can be used to confirm the targeting of drug-delivery nanoparticles [159].

MRI resolution can be 20–25 pm, which means that real-time tracking of single cells is possible if a cell can be loaded with sufficient magnetic material [160]. These techniques are useful in understanding how cells migrate in response to diseases. Biocompatible magnetic nanosensors have been designed to detect molecular interactions in biological media via MRI. Changes in relaxation times due to the nanoparticles binding with the target molecule are detected by MRI contrast, thus allowing simultaneous study of the location and chemical functionality of specific types of cells. These magnetic nanosensors can detect specific mRNA, proteins, enzymatic activity, and pathogens with sensitivity in the low femtomole range (0.5–30 fmol) [161–163]. Other applications include imaging gene expression [164, 165].

Work is in progress on chip-scale integration for MRI of very small samples. Micromechanical cantilever oscillators allow for high sensitivity magnetic measurements [166]. Integrating DC and RF magnetic field sources into such a chip would allow for magnetic resonance measurements with significantly improve sensitivity.

8. HYPERTHERMIA

Cancer growth is slowed or stopped at temperatures in the range of 42–48 °C, while normal cells can tolerate even higher temperatures [167, 168]. Heat treatments can be characterized as hyperthermia, in which the temperature is limited to less than about 50 °C, and thermal ablation, which involves higher temperatures.

Hyperthermia induces almost reversible damage to cells and tissues; however, it can enhance radiation and chemotherapy injury of tumor cells [169]. The enhancement is attributed to heat-induced malfunction of the processes that ordinarily repair DNA. Hyperthermia also affects the activity of regulatory proteins, kinases and cyclins, which in turn alters cell growth and differentiation, and can induce apoptosis. Thermoablation produces necrosis, coagulation or carbonization that could be sufficient to eliminate the need for radio- or chemo-therapy [68, 170]. Thermoablation has been studied primarily for difficult-to-treat cancers (such as liver cancer) with limited treatment options, and for areas of the body far away from vital organs (e.g. breast cancer). Targeting of the magnetic nanoparticles to specific sites eliminates the systemic side effects that result from oncologic treatments (i.e. nausea or radiation pneumonitis); however, whole body hyperthermia produces non-selective damage and thermal ablation has a higher risk for collateral damage.

Hyperthermia and thermoablation have been accomplished using capacitive or inductive coupling of rf fields (10–100 MHz), microwaves (> 300 MHz), ultrasound, lasers or external heat [171–177]. Macroscopic metal implants of Cu and other high-conductivity metals have been used to induce eddy-current heating. The absorbed power per mass is called the specific absorption rate (SAR), which can be expressed as

$$SAR = \frac{\Delta Q}{\Delta t}\left(\frac{1}{m_f}\right) \tag{6}$$

where ΔQ is the energy converted into heat. Δt is the time over which the conversion occurs, and m_f is the magnetic material mass [170]. The SAR is determined by the "rate-of-temperature-rise" method [178]

$$SAR = c\frac{dT}{dt} \tag{7}$$

where c is the specific heat and $dTldt$ is the rate of temperature change. The density of absorbed power is related to the SAR by

$$P = SAR\frac{m_f}{V} \tag{8}$$

The coupling of an external RF magnetic field to magnetic particles in the body results transfer of energy to the tissue by: 1) eddy current heating, 2) hysteretic heating: heat generated when a magnetic material is forced around part or all of the hysteresis loop, 3) viscous heating: heat generated by the kinetic motion of a particle within a viscous fluid, and 4) magnetic resonance. The loss power of the magnetic particles should be as high as possible so as to allow the lowest possible dose [179].

Avoiding neuromuscular electrostimulation requires frequencies greater than 50 kHz, and the penetration depth limits the frequency to less than 5–10 MHz, effectively eliminating effects from magnetic resonance [180]. Typical values used with iron-oxide nanoparticles are magnetic fields of frequency in the range 50–500 kHz and amplitude 1–15 kA/m [170, 181]. Jordan suggests that materials should be investigated for use at frequencies near 100 kHz so as to optimize the SAR in the magnetic material in comparison to the potential SAR in tissues due to eddy currents [182]. For thermal ablation, a frequency of 400 KHz and 6.5 kA/m should be tolerable for the exposure of parts of the body with diameters of up to 15 cm, if short exposure times are used [170, 183]. In many nanoparticle systems, the SAR is best be described by

$$SAR = kf^n H^2 \tag{9}$$

where n ranges from 1.1 to 1.5, suggesting that there must be a frequency-dependent process of magnetic relaxation that accounts for the changes in the power n [180]. Theoretical predications for Rayleigh loops suggest a H^3 dependence.

Modeling magnetic hyperthermia is difficult due to the complex magnetization reversal mechanisms found in nanoparticles. Optimization of nanoparticle properties is important to limit the amount of material that must be introduced. The SAR achievable for a given combination of field, frequency, and type of particle usually must be determined experimentally. SAR values range from a few W/g to a few hundred W/g for optimized values (i.e. using the field and frequency that provides the best results for the particular system being studied) [167]. The SAR depends on many factors, including the effect of coating on surface spin dynamics, the effect of surface properties on Brownian relaxation, size, and crystallinity among other factors.

The SAR due to losses along a hysteresis loop is:

$$SAR_{hyst} = \frac{v\mu_0}{m_f} f \oint H \mathrm{d}M \qquad (10)$$

Magnets with large-area loops are thus preferred for hysteresis hyperthermia; however, the size of the magnetic field at the location of the nanoparticles may be limited, making only minor loops accessible.

In macroscopic implants, shape anisotropy can be used to maximize the area of the loop; however, local heating ("hot spots") is a concern. In nanoparticles, overcoming the energy barrier to rotation of the magnetization (with the particle fixed) determines the properties of the loop. Ferromagnetic particles much larger than the superparamagnetic limit have no implicit frequency dependence to the hysteresis in the frequency range considered. The physical basis of the heating due to superparamagnetic particles shows that the frequency dependence is more important than for their ferromagnetic counterparts [184].

The use of iron oxides for hyperthermia of tumors was first proposed by Gilchrist *et al.* [185]. Magnetic nanoparticle hyperthermia has a potential advantage over radio- and chemo- therapies because there is no systemic buildup in organs, so larger doses are possible. The nanoparticles can be introduced into the body once, and then used for multiple treatments. Nanoparticles can be magnetically targeted, injected directly into the tumor in some cases, or injected into the vasculature supplying the tumor. Chemical and/or magnetic targeting help limit side effects [186]. Tumors derive their nourishment from the supplying vasculature, so blocking the blood supply via magnetic nanoparticle thermoablation has been attempted [187–189].

The first clinical human trials using magnetic hyperthermia were reported by Lubbe, *et al.* [70, 129, 137, 190] who used 100-nm starch-coated iron-oxide particles bound with epirubicin for treatment of advanced solid cancers. Jordan recently reported positive results from ongoing trials of advanced cancer patients who received magnetic nanoparticle hyperthermia in conjunction with conformal external beam radiation therapy [191]. The therapy was well tolerated by the patients and significant increases in the length and quality of life were observed.

The greatest challenge to the efficacy of hyperthermia using magnetic nanoparticle fluids is balancing the rate of thermal energy deposition with the mechanisms responsible for thermal dissipation. Modeling heating is complex due to the multiple factors affecting temperature change, including tissue density, amount of fat, and blood flow. Tumors in highly perfused organs, such as the kidney, lung and liver, are harder to heat due to the high blood flow [68, 170]. The dependence of temperature rise on distance is steep, and there are delays in heating and cooling after the field is applied or removed. Larger bones, such as the pelvis and skull shield tissues and produce inhomogeneous heating. Non-uniformity of tumors also poses a complication, as large tumors heat at a greater rate than small tumors due to the poorer tissue cooling and differences in heat conduction in the necrotic regions of large tumors [167, 192, 193].

One approach to controlling temperature is to use materials with a Curie temperature between 42 and 50 °C, as these materials automatically "turn off" when the temperature becomes too high. Substituted ferrites such as $(Co_{1-x}Zn_x)Fe_2O_4$, manganates such as $La_{1-x}Me_xMnO_3$ [Me=Sr, Ba, Pb, Ag, Na] and substituted yttrium-iron garnet $Y_3Fe_{5-x}Al_xO_{12}$ are ideal candidates due to their stability against oxidation (relative to metals) and the ability to tune the Curie temperature by composition [56, 194, 195]. Ni-Cu and Ni-Pd alloys have been investigated, but biocompatibility issues must be addressed [196–200].

9. OTHER APPLICATIONS

Magnetic nanoparticles can be used simultaneously for more than one of the applications discussed; for example, MRI can be used to confirm magnetic nanoparticle distribution prior to using the same particles to administer hyperthermia [201]. A combination of fluorescence and magnetic-nanoparticle-enhanced MRI was used for preoperative magnetic resonance imaging and as an intraoperative optical probe during surgery, allowing clearer delineation of brain tumors. These "multimodal nanoparticles" may allow radiologists and neurosurgeons to see the same probe in the same cells and thus improve identification of tumor margins [202, 203]. A nanoparticle combining near-infrared fluorescent dye with magnetism allows the particles to be located by MRI while the fluorescent dye provides simultaneous information about the molecular environment about the nanoparticle [204].

Although most applications try to avoid agglomeration, the intentional formation of a blockage of magnetic nanoparticles to block blood supply to a tumor has been investigated [67, 70, 137]. Aggregation and selective uptake has been used to destroy cells via the application of pulsed magnetic fields and subsequent rupture of cells [205].

Retinal detachment is a major cause of vision loss in adults. The usual treatment is the scleral buckle, which is a silicone band sewn to the outside of the eye that compresses the wall of the eye inward to close the holes in the retina. Sterically stabilized 4–10 nm magnetic particles in a poly(dimethylsiloxane) biocompatible fluid can be held in place with an external magnetized scleral buckle, thus providing a stable internal blockage that encircles the entire eye

periphery with a ring of silicone oil. No fluid contacts the central vitreous cavity or the lens, thus decreasing the chances of undesired contact and/or damage [56, 206].

Many neurodegenerative diseases indicate the disruption of normal iron homeostasis in the brain. Recent experimental work indicates that nanoscale magnetic biominerals (primarily magnetite and maghemite) may be associated with senile plaques and fan filaments found in brain tissue affected by these diseases. Understanding the role of iron in neurodegenerative disease could help understand the origin, diagnosis and treatment of these diseases [175].

10. CONCLUSION

The field of nanobiomagnetics is exceptionally broad, involving researchers from medicine, pharmaceutical science, chemistry, physics, biology, engineering, and materials science. The literature is similarly decentralized, making it impossible not to omit some contributions in this article. Recent reviews mentioned in this chapter provide additional information for the reader interested in more detail on a specific topic. Nanomagnetics researchers have much to contribute to this new and exciting field.

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