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TOPICAL REVIEW

Progress in applications of magnetic nanoparticles in biomedicine

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Abstract
A progress report is presented on a selection of scientific, technological and commercial advances in the biomedical applications of magnetic nanoparticles since 2003. Particular attention is paid to (i) magnetic actuation for *in vitro* non-viral transfection and tissue engineering and *in vivo* drug delivery and gene therapy, (ii) recent clinical results for magnetic hyperthermia treatments of brain and prostate cancer via direct injection, and continuing efforts to develop new agents suitable for targeted hyperthermia following intravenous injection and (iii) developments in medical sensing technologies involving a new generation of magnetic resonance imaging contrast agents, and the invention of magnetic particle imaging as a new modality. Ongoing prospects are also discussed.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

In 2003, when we wrote our original review on the applications of magnetic nanoparticles in biomedicine [1], the field, and those working in the field, were on the brink of a major expansion in both activity and scope. After many years of painstaking research and development, it seemed that suddenly it had all come together, and there was a sharp increase in both the number of groups working in the area, and in their ambitions and objectives. Consequently, the last six years have seen myriad new prospects and ideas come forward, and, perhaps most exhilarating, many new companies and ventures formed to take those ideas on the long road to commercial success and the ultimate goal of delivering real clinical and biomedical solutions to real people.

At the same time it has been noticeable that more and more large, cross-disciplinary teams are being formed to work in specific areas towards chosen targets of known clinical need. It has always been the case that biomagnetics is a field that relies on close collaborations between medics, clinicians, life scientists, pharmacologists, physical scientists and engineers, but now more than ever it appears to be imperative that the relationship is both close and free-flowing. The benefit is focus, momentum and the ability to set achievable, feasible and pragmatic goals. There are downsides of course, such as the management overhead, and the potential for both 'mission creep' and for disillusionment when, as often happens, the expectation for quick and early results comes up against the harsh realities of the uncertainties of fundamental research, the vagaries of ethics committee proposals, and the very major obstacle of satisfying regulatory authorities. Nevertheless, progress is being made, and at a much better rate than we could have hoped for in 2003. For that reason, it is timely now to assess current progress in the field.

In our 2003 paper we covered a good deal of the underlying physics involved. We reviewed some of the relevant basic

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concepts of magnetism, including the classification of different magnetic materials. We described how a magnetic field can exert a force at a distance, and described the physics of magnetic actuation. We considered the way that energy can be transferred from an exciting field into a magnetic dipole, and how this can be harnessed into the protocol of magnetic field hyperthermia. We also attempted to demystify the physics of magnetic resonance imaging (MRI), and the role of magnetic nanoparticles as MRI contrast enhancement. We will not repeat those discussions here, for which the reader is directed to the original paper [1]. Instead, in this review we will concentrate on progress since 2003 in the realms of magnetic actuation, magnetic heating or hyperthermia, and magnetic sensing, the latter covering not just MRI, but also an intriguing new modality in the stable, magnetic particle imaging (MPI). We will conclude with a discussion of lessons we can learn from our past and current experiences, and of the prospects that lie ahead in the application of magnetic nanoparticles in biomedicine.

2. Magnetic targeting for drug and gene delivery

2.1. Progress in magnetically mediated cancer and gene therapies

As discussed in our previous review of this subject [1], physical constraints placed upon magnetic targeting, such as the rapid diminishing of field strength with target depth in the body and the difficulties of bypassing intervening vasculature and tissue structures [2, 3], have hampered the clinical realization of this technology. Much of the recent work in this area has focused on the development of high-moment magnetic nanoparticle carriers with novel, multifunctional coatings and novel techniques for enhancing the body's own 'targeting' systems.

The development of novel magnetic nanoparticle carrier formulations continues apace. The progress in this area has been reviewed elsewhere (including a companion paper in this issue). In general, advances are focusing on novel, multifunctional coatings, the use of high-moment materials for the particle cores and the development of thermoresponsive hydrogels and particles [4–6]. Mathematical modelling is also beginning to inform some of the experimental studies [7] and our understanding of *in vivo* magnetic targeting is beginning to move forward based on this work.

Although there have been numerous small animal studies reported since our last review, due to the technical barriers mentioned above, the goal of clinical applications remains largely unfulfilled. However, in 2004 Wilson *et al* published encouraging results of a clinical study combining magnetic targeting and MRI, in which they were able to monitor the trans-catheter delivery of magnetically targeted doxorubicin to the hepatic artery using intra-procedural MRI [8]. The study demonstrated selective targeting to the tumour with a final fraction of treated tumour volume of 0.64 to 0.91 compared with only 0.07 to 0.30 in the normal liver tissue [8].

In addition, the last few years have seen some innovations in magnetic targeting aimed at overcoming some of these hurdles to clinical application. One example of this is the use of magnetic needles and meshes inserted at the target site to create a high-gradient magnetic field. As seen in our last review, the force on the magnetic carriers is proportional to the gradient of the field, and by implanting a needle or mesh, it is possible to create a field and gradient of sufficient magnitude to facilitate capture at the target. The theory of this variation of magnetic targeting was demonstrated by Iacob, Hayden, Hafeli and others [9–11]. They also modelled and evaluated the potential advantages of planar, periodic magnetic bandages and Halbach arrays for enhanced targeting [10, 11].

An alternative approach to tumour targeting, which harnesses an innate cell targeting mechanism, was recently revealed by Muthana *et al* [12, 13]. As solid tumours grow, they can outgrow their blood supply, resulting in the formation of a hypoxic, semi-necrotic tumour core. The well vascularized regions of the tumour are accessible to intravenously administered chemotherapy drugs that may destroy this part of the tumour. However, the lack of a blood supply to the core means that it is largely unaffected. Within the core reside dormant tumour cells, which then send out chemical signals to recruit macrophages into the core. These macrophages then begin to rebuild the blood supply, allowing the tumour to begin growing again.

The group essentially hijacked this process by loading human macrophages with magnetic nanoparticles and placing magnets near the site of a human prostate tumour grown in mice. The 'therapeutically armed' macrophages, carrying a reporter gene, invaded the tumour at a rate more than three times that of the non-loaded cells (figure 1). This demonstration of magnetic targeting overcomes some of the clinical limitations by virtue of the fact that the cells do not need to be pulled out of the bloodstream at the target by brute force. Rather, they need only be slowed down enough so that a higher proportion of the loaded cells respond to the chemical signals from the tumour core. As the macrophages are loaded with magnetic nanoparticles, they can then be destroyed by hyperthermia after delivering the therapeutic drug or gene.

Work on magnetic nanoparticle-based gene transfection has also significantly progressed over the past five years. Since 2000, when Mah *et al* [14, 15] first described magnetic micro- and nanoparticle-based gene transfection (*in vitro*) by linking viral vectors to magnetic carriers, there has been a dramatic expansion of work aimed at adapting this technique for non-viral transfection of DNA, siRNA and other biomolecules [16, 17]. Magnetic transfection, or 'magnetofection', works on similar physical principles to magnetic targeting. A high-field, high-gradient magnet is generally placed underneath a cell culture dish or multi-well plate. The particle–gene complex is introduced into the cell growth medium and the magnetic field rapidly pulls the particles into contact with the cells growing on the bottom of the dish. This has been shown to promote endocytosis of the particles, resulting in rapid and efficient transfection [18].

Several groups have also successfully employed non-viral nanomagnetic transfection to introduce siRNA into cells for gene knockout studies [19]. This involves attaching strands of short-interfering RNA to the particles. As the particles are

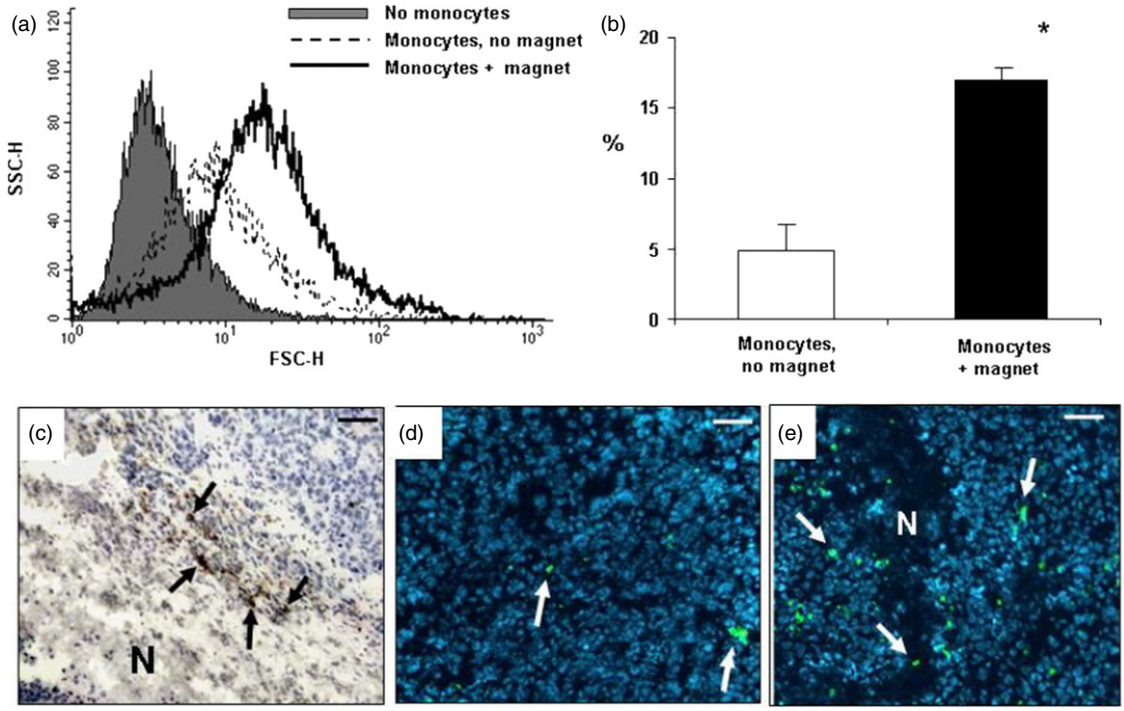

Figure 1. Magnetic targeting of GFP-transfected monocytes to prostate tumours *in vivo*, reproduced from Muthana *et al* [13]. GFP-transfected human monocytes loaded with magnetic nanoparticles were injected intravenously into male nude mice bearing PC3 tumour xenografts. Flow cytometric analysis of enzymatically dispersed tumours showed (*a*) shift in the FACS profile and (*b*) increased proportion of CD14+/GFP+ human monocytes in tumours exposed to an external magnet compared with tumours with no magnet present or tumours from uninjected mice. (*c*) The presence of human monocytes in tumours was confirmed by immunostaining using an antibody to human CD68 (which does not detect murine CD68 as seen by the absence of staining in tumours from mice not injected with human monocytes). Fluorescence microscopy of tumour sections revealed green GFP expression by human transfected monocytes and blue DAPI staining of nuclei in live cells in tumours in the absence (*d*) or the presence (*e*) of an external magnet. N denotes an area of necrosis. Panels (*a*), (*c*), (*d*) and (*e*) are from representative tumours. In panels (*c*)–(*e*), bars are 50 μm. Panel (*b*) is pooled data from four identical experiments (means $\pm$ SEMs). $^{*}P < 0.002$ with respect to tumours injected with GFP+ magnetic monocytes but in the absence of an external magnet.

taken into the cells, the siRNA blocks the activity of the target gene, knocking out its function. These studies are particularly important for examining specific genes involved in disease pathways.

More recent advances have been made using oscillating magnet arrays placed beneath the culture dish as well as pulsed electromagnets oriented perpendicular to the magnetization vector of the magnet below the culture dish [20–22]. The oscillations introduce a lateral component of motion to the particle–gene complex, which is superimposed on the *z*-axis motion due to the permanent magnet beneath the culture plate. This mechanical stimulation promotes more efficient endocytosis of the particle–gene complex, significantly increasing transfection efficiency compared with other non-viral methods.

A novel approach examined by Stride *et al* combines two physical transfection techniques, magnetofection and ultrasound [23]. The transfection efficiency of magnetic microbubble/nanoparticle complexes was found to be greater in Chinese hamster ovary cells when both magnetic fields and ultrasound were applied simultaneously. This interesting combination of methods may point to future directions for enhancing non-viral gene transfection both *in vitro* and *in vivo*.

As with magnetic drug targeting, though, the development of magnetic targeting for *in vivo* gene delivery remains elusive. In a 2006 study, Xenariou *et al* were not able to demonstrate gene transfection in a mouse model of cystic fibrosis. However, in 2008 Hüttinger *et al* published the results of a phase I trial of a veterinary application. The group showed that magnetofection was well tolerated as a potential gene therapy for feline fibrosarcomas [24]. Although the study was aimed at evaluating toxicity, 10 of the 20 cats were recurrence-free after one year, pointing towards a potential novel *in vivo* application for this technology.

2.2. *Magnetic actuation for control of cells and cellular function*

The manipulation and control of cells and sub-cellular structures through magnetic nanoparticle-based actuation is a relatively new technique that has led to novel and exciting biomedical applications. From its genesis as a theoretical model developed to predict the response of magnetic iron compounds in the brain to environmental electromagnetic fields, it has evolved into an elegant technique for examining cellular mechanics, ion channel activation kinetics and tissue engineering (TE) and regenerative medicine (RM) applications. The ability to manipulate and remotely control specific cellular components has the potential to provide clinicians and scientists with a powerful tool for investigating cell function and molecular signalling pathways, as well as to

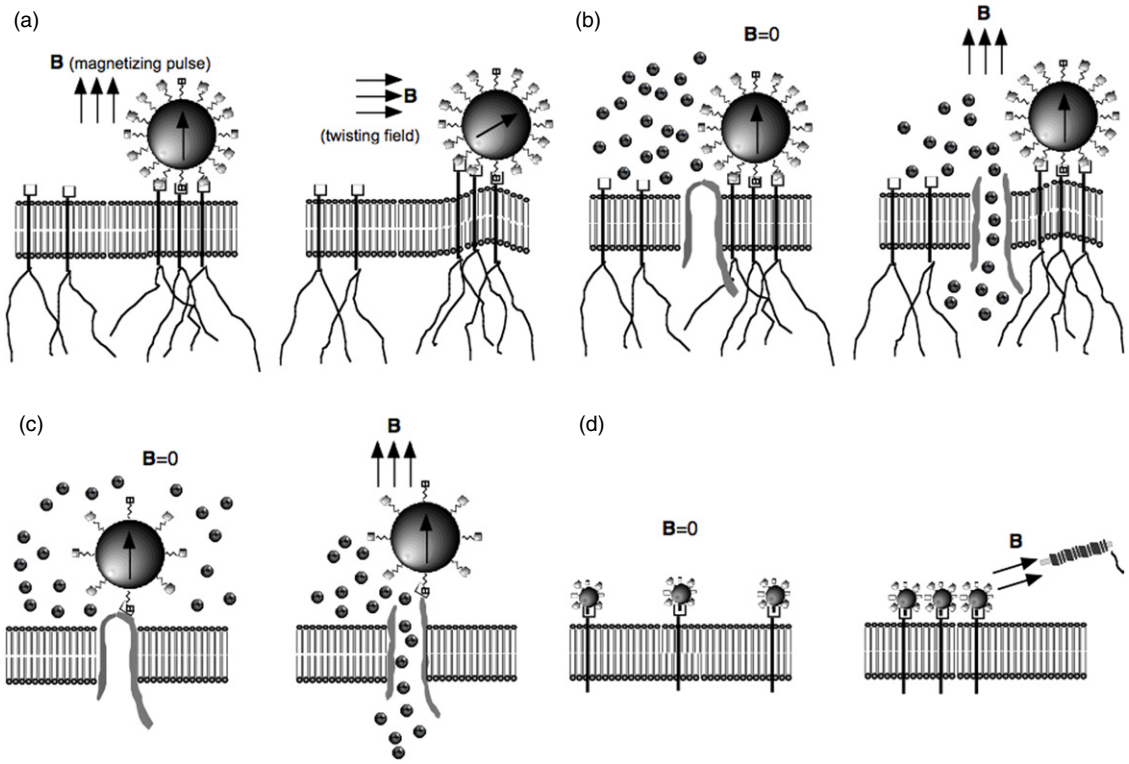

Figure 2. Schematic representation of nanomagnetic actuation for biomedical applications, adapted from Dobson [45]: (*a*) *Magnetic twisting cytometry*: micrometre-sized magnetic particles are linked to actin filaments via integrin receptors bound to RGD molecules coated onto the particle surface. A magnetizing pulse is applied (left) which gives the particle a remanent magnetization ($\boldsymbol{B}$ = magnetic field vector). A torque is then applied (right) to the particle via a 'twisting field' and the force required to twist the particle is related to the mechanical properties of the actin filaments. (*b*) *Mechanosensitive ion channel activation:* magnetic particles, again generally larger than 1 μm in size, are bound to the integrin receptors (left) and, upon the application of a high-gradient magnetic field (left) the particles are pulled towards the field, deforming the cell membrane and activating adjacent mechanosensitive ion channels. (*c*) *Targeted ion channel activation*: magnetic nanoparticles are attached to an ion channel via an antibody (left). Upon activation of a high-gradient magnetic field source (right), the ion channel is forced open. (*d*) *Receptor clustering*: magnetic nanoparticles are bound to IgE–FcεRI receptor complexes. In the absence of a magnetic field (left) the receptors are spaced along the membrane surface. When a field is applied via a high-gradient magnetic needle, the receptors are pulled towards the field source, initiating receptor clustering.

provide a platform for the development of new treatments for a myriad of medical conditions.

The use of magnetic micro- and nanoparticles to probe the mechanical/rheological properties of cells via magnetically generated stresses dates back to studies by Heilbronn and Seifriz in the 1920s and Crick and Hughes in the 1950s [25–27]. In the 1980s Valberg and others used magnetic microparticles to investigate the rheological properties of the cytoplasm by twisting and measuring their magnetic fields [28–30]. However, the use of the technique to control specific cellular functions, such as ion channel activation, appears to originate in a theoretical model developed to explain the interaction of magnetic iron compounds in the brain with environmental electromagnetic fields. In 1992, Joseph Kirschvink at the California Institute of Technology proposed a mechanism by which relatively weak magnetic fields from mains-powered electrical devices could activate mechanosensitive ion channels via actuation of nanoparticles of biogenic magnetite which had recently been discovered in the human brain [31]. The model demonstrated how a particle of magnetite with a stable magnetization (magnetically blocked) would 'twist' in response to a magnetic field applied at an angle to the magnetization vector of the particle. If such a particle was coupled, in some way, to a cellular ion channel, the torque on the particle would be strong enough to force open the channel, activating and deactivating the channel in response to a sinusoidal magnetic field. The model was expanded to examine pulsed fields a few years later [32].

In addition to twisting magnetically blocked nanoparticles, it is also possible to 'pull' the particles towards a magnetic field source, provided there is a gradient to the field, as described previously [1]. When applied to magnetic micro- or nanoparticles that are attached in some way to cell membrane receptors or cellular components this attractive force, sometimes in combination with torque, can be used to actuate and control specific cellular processes.

One of the earliest applications of magnetic actuation for examining cell function was the development of magnetic twisting cytometry. Originally conceived in the 1990s by Wang, Bulter and Ingber at MIT and Harvard, the technique exploits the model proposed by Kirschvink by coating magnetically blocked microparticles with molecules which bind to integrin receptors on a cell's surface [33, 34]. These receptors are extracellular protrusions of the cell's cytoskeleton and, by attaching particles to these receptors and manipulating them in a controlled fashion, it is possible to investigate the mechanical properties of the cell (figure 2(*a*)).

Around the same time as Ingber and Wang were developing magnetic twisting cytometry, other researchers began to investigate whether the technique could be used to activate mechanosensitive ion channels. These channels respond to membrane deformation by changing conformation from 'closed' to 'open' (or vice versa) and are particularly ubiquitous in cells which rely on mechanical stress for the production of specific proteins such as bone, cartilage and muscle cells (figure 2(*b*)). By using micro- and nanomagnetic actuation to apply precisely controlled forces to the cell membrane, combined with a variety of particle binding motifs, it proved possible to elucidate mechanical activation pathways and evaluate ion channel kinetics [35–40].

Recent work has focused on targeting specific ion channels to initiate controlled responses by the cell. The objective is to attach magnetic nanoparticles directly to mechanosensitive regions on one type of ion channel in order to control it without interfering with the normal functioning of the other channels in the cell's membrane. Proof of principle was initially demonstrated on the TREK-1 potassium channel by inserting a histidine tag into the external loop of the channel and inserting the clone into the membrane of COS-7 cells [41]. Magnetic nanoparticles were attached to the tag via a Ni–NTA linker that facilitated selective activation of the channel (figure 2(*c*)). More recently, it has been shown to be possible to activate the channel by using anti-TREK antibodies that bind directly to the native channel, eliminating the need to insert the histidine linker.

A similar technique has been used by Ingber and colleagues in an elegant experiment which used magnetic actuation to promote membrane receptor grouping in RBL-2H3 mast cells [42]. In order to achieve this, a magnetic needle was used which can focus magnetic forces to small areas for highly targeted nanomagnetic actuation [43]. By promoting clustering of the IgE–FcεRI receptor complexes, it was possible to activate intracellular calcium signalling in those cells (figure 2(*d*)).

The Ingber group has also recently developed magnetically actuated cellular microchips. These microchips are patterned magnetic arrays which, when activated, promote adhesion of cells (in this case human umbilical vein endothelial cells, HUVECs) bound to magnetic nanoparticles [44]. HUVECs depend on substrate adhesion for survival and upon deactivation of the magnetic array, the cells rapidly detach and undergo apoptosis. The chip can be configured to investigate multiple cells as well as multiple substrate ligands simultaneously [44]. These applications are reviewed in more detail elsewhere [45].

2.3. Magnetic nanoparticles in TE and RM

Over the past decade another novel application of magnetic nanoparticles has emerged: nanomagnetic actuation for TE and RM. One aspect of this is the magnetic targeting of stem cells to sites of injury in the body, an approach that was first reported *in vitro* by Sura *et al* in 2008 [46] and *in vivo* by Kyrtatos *et al* in 2009 [47]. In the latter a six-fold increase in the localization, to the carotid artery, of magnetically labelled endothelial progenitor cells was achieved in a rat model of vascular injury [47]. However, magnetic actuation can also be used to influence the growth and differentiation characteristics of stem cells.

The primary goal of TE is to grow functional tissue from a patient's own cells outside the body, in a bioreactor (a type of sophisticated tissue culture environment). TE involves the manipulation of the patient's cells within his or her own body to promote tissue regeneration or healing. For many TE/RM applications, mechanical cues provide vitally important stimuli to the cells that promote the production of functional tissue matrix, especially bone, cartilage, muscle and connective tissue. However, applying the correct stress profiles to cells growing in a 3D scaffold within a bioreactor or within a patient's body has proven difficult. To overcome this problem, nanomagnetic actuation has been developed to apply targeted, controlled stress to cells growing in bioreactors and *in vivo*.

In 2002, Cartmell *et al* presented results of a magnetic force bioreactor in which magnetic nanoparticles were coupled to human osteoblasts and magnetically activated mechanical conditioning was shown to promote the generation of bone matrix [48]. Subsequent work has shown that magnetic actuation can be used to promote the upregulation of genes related to both bone and cartilage matrix [49].

Following on from this work, other groups have used magnetic nanoparticles to control the formation of sheet and tubular structures. Superparamagnetic iron oxides can be loaded onto and into cells, which are then seeded onto culture plates with magnets underneath. The magnets promote adherence and sheet formation and, once the field is removed, the sheets can be harvested to create, for example, sheets of skin [50]. This technique, pioneered by Ito, Honda and others, has also been used to roll, using a magnetic rod, the harvested sheets into tubular tissue structures for use as blood vessels and urothelial tissue [51–53].

Interestingly, it is now apparent that mechanical cues are as important as, or potentially more important than, biochemical cues for directing the differentiation of human mesenchymal stem cells, particularly for bone and connective tissue. By utilizing nanomagnetic actuation of specific ion channels and surface receptors on the stem cell membrane, Sura *et al*, have been able to direct their differentiation completely without the use of chemical agonists [54]. By activating the TREK-1 potassium ion channel on these cells, expression of cartilage-related genes was induced, indicating that the cells are moving down a chondrocyte lineage [54]. By activating other surface receptors, it should be possible to control the differentiation of these stem cells into bone, muscle, cartilage and tendon.

Although the use of magnetic nanoparticles for TE/RM and stem cell research and therapy is at an early stage, the potential for this technology to make a major contribution to this field is great.

3. Nanomagnetism in therapeutic hyperthermia

3.1. First clinical trials of magnetic hyperthermia

In 2003 we reviewed the biomedical applications of nanomagnet technology that included a summary of some of

the principles underlying its implementation in therapeutic hyperthermia for the treatment of cancer [1]. Since that time a number of excellent reviews have been published describing the state of the art and outlining the challenges that still exist [55–58].

The most important advance in the last six years has been the commencement of the first-ever clinical studies of therapeutic hyperthermia induced by heating from implanted magnetic nanoparticles. The group at Berlin's Charité Hospital, headed by Andreas Jordan, has been publishing in this field since 1993 [59]. In 2007 this group reported the results of the first study into the feasibility of thermotherapy (hyperthermia) using magnetic nanoparticles in human patients [60]. The study involved 14 patients receiving treatment for recurrent glioblastoma multiforme, a particularly severe type of brain cancer, via a combination of fractionated external beam radiotherapy and several sessions of thermotherapy. Thermotherapy was effected by heat generated from aminosilane coated iron oxide nanoparticles that had been injected into multiple sites throughout each tumour. The choice of injection sites was based on data from a comprehensive series of MRI scans of the cranium coupled with a specially developed software planning system which they have trade-marked as NanoPlan®. The superparamagnetic iron oxide nanoparticles (core size 15 nm) were dispersed in water at a concentration of 112 mg_{Fe} ml^{-1}. Each tumour was injected with from 0.1 to 0.7 ml of the magnetic fluid per ml of tumour and then exposed to a magnetic field of 3.8 to 13.5 kA m^{-1} alternating at 100 kHz.

The study successfully demonstrated that this form of thermotherapy using magnetic nanoparticles could be safely applied to the treatment of brain tumours and that hyperthermic temperatures could be achieved. Very small deposits (0.1 ml) of the magnetic fluid could be precisely deposited within the targeted area. Follow-up CT scans and reproducible temperature measurements confirmed that these deposits were stable over several weeks. Patient survival and local tumour control were not considered primary endpoints of this study, however, clinical outcomes were observed to be promising with the therapy being well tolerated by all patients. More complete evaluation of clinical outcomes is to be assessed in a phase II study on 65 patients with recurrent glioblastoma multiforme.

The Jordan group have also begun clinical studies of their technology applied to the treatment of prostate cancer [61, 62], and Jordan and several of his collaborators have formed a company, MagForce Nanotechnologies AG, to commercialize the technology. To the best of our knowledge, this remains the only group to be undertaking clinical investigations of thermotherapy based on heating from magnetic nanoparticles.

The rather long period of gestation from first *in vitro* studies to eventual clinical application reflects the considerable technological and regulatory difficulties to be overcome in any attempt to develop a clinically acceptable and useful therapy of this type. It is not merely enough to develop magnetic particles that heat upon exposure to an alternating magnetic field (AMF), although that is clearly an important prerequisite. It is also important to know how to appropriately administer enough of the particles to the intended target tissue and to be able to generate enough heat from them, by exposure to a tolerable level of AMF that does not in itself cause any undesirable side effects. The methodology developed by the MagForce group successfully addresses each of these issues. In this context it is particularly interesting to note the significance of the NanoPlan® software platform, and its important role in ensuring that the right treatment is given to each subject.

3.2. Interstitial heating from multiple sources

If it is the aim to generate enough of a temperature rise throughout the target tissue volume for the induced hyperthermia to be therapeutic in its own right, then the method used to get the nanoparticles into the target becomes critical [63]. MagForce have pursued the concept of interstitial heating via multiple-site direct injection of their nanoparticles and have developed sophisticated measures to ensure that the specific absorption rate (SAR) throughout the entire target volume will be enough to result in a therapeutic thermal dose, expressed as cumulative equivalent minutes at 43 °C for 90% of the tumour volume (CEM 43 T_{90}) [64]. This is an extremely demanding requirement since it only requires a very small part of the target volume to remain cool for the entire treatment to be compromised. The two most obvious reasons why a small section of a tumour may not be heated are either because of a locally increased level of blood flow, say because of a nearby blood vessel, or an inadequate concentration of implanted magnetic nanoparticles.

Earlier attempts to develop interstitial heating technology based on implantable 'thermoseeds', such as ceramic ferrite cores encased in metal sheaths [65], have suffered from the difficulty of implanting a clinically tolerable number of thermoseeds in an array that does not leave regions of under-dosed tissue between the implants [65]. The MagForce approach improves on this earlier concept by exploiting the increased flexibility available by using directly injected magnetic fluids to tailor the implant configuration to closely match tumour specific, theoretically modelled deposition patterns generated by their NanoPlan® platform. The group's early reports of thermal dose calculated for individual tumours treated in this way showed quite a wide variation in CEM 43 T_{90} (from 2.3 to 502, median value 7.7) [60], which is a reflection of the difficulty in obtaining optimum distributions of the deposited nanoparticles on a consistent basis.

A study published by one of us in 2003 [66] highlights the difficulty in obtaining a uniformly effective thermal dose throughout the tumour volume. Here an animal model was used to examine the effect on tumour growth of nanoparticle-mediated hyperthermia by comparing two methods of nanoparticle administration. In one group, small deposits of a viscous emulsion consisting of magnetic nanoparticles mixed with histoacryl (a tissue adhesive used to prevent migration of the nanoparticles) and lipiodol were injected directly into the centre of the tumours (the DIH group), while in the second group microspheres (ca 30 μm in diameter) containing the same type of magnetic nanoparticles were administered via the arterial blood supply to the tumours

(the AEH group) before exposure to the AMF. In both cases the thermal response, as measured by discrete temperature probes located in and around the tumour, appeared to be adequate although the DIH group heated much more rapidly than the AEH group. The somewhat unexpected result, however, was that the therapeutic outcomes were very different and revealed a distinct advantage of the AEH approach, despite an apparently inferior thermal response initially. The authors concluded that this result could be explained by the differences in the distribution of the magnetic particles throughout the tumour that are not reflected in the measured thermal response.

3.3. Progress towards targeted hyperthermia

Another approach that has garnered substantial attention in recent years is that of conjugation of magnetic nanoparticles with monoclonal antibodies to enable the targeted delivery of the therapeutic agent, i.e. the nanoparticles for hyperthermia, directly to the cells of interest via systemic administration. This clearly worthwhile aspiration has an advantage over other methods involving the immuno-targeting of more toxic agents, such as radioisotopes or drugs, in that the nanoparticles are relatively harmless until exposed to the AMF. Hence, the problem of non-specific binding to healthy tissue can potentially be overcome by the use of a magnetic field system that only exposes the target area to the high frequency AMF. In addition, it may be possible to use MRI to obtain confirmation that the desired distribution of the immuno-targeted nanoparticles has been achieved prior to the application of the AMF. The main challenge of the method, as with all the methods described here, is to be able to obtain sufficiently high concentrations of the nanoparticles in the local environment of the cancer to result in useful heating at clinically tolerable levels of AMF.

In recent years DeNardo *et al* [67] have published the results of experimental studies of their monoclonal–antibody-linked iron oxide nanoparticle 'bioprobes' in athymic mice bearing human breast cancer HBT 3477 xenografts. Their bioprobes each consisted of one or two ^{111}In-chimeric L6 (ChL6) monoclonal antibodies linked to commercially sourced 20 nm superparamagnetic iron oxide beads with a pegylated dextran coating. The ^{111}In radiotracer was a useful way to confirm adequate uptake of the bioprobes to the target prior to exposure to the AMF. The prescribed dose of bioprobes was injected into a lateral tail vein in tumour-bearing mice. Three days later these mice were exposed to either a 1300 Oe, 1000 Oe or 700 Oe AMF alternating at a frequency of 153 kHz. The tumours in all the treated groups showed a statistically significant decrease in growth rate compared with controls [67]. Some toxic side effects in the form of acute death and observed acute erythematic skin changes were apparent for mice in the 1300 Oe group, but none was observed in the 1000 Oe and 700 Oe groups.

Following these encouraging results, the DeNardo group went on to publish the results of further studies along similar lines that included more information about the pharmacokinetics of the bioprobes, the SAR of the particles used, measurements of bioprobe concentrations in tumour and calculations of thermal dosimetry [68]. They reported a mean concentration of bioprobes per gram of tumour of about 14% of the injected dose, equivalent to around 0.315 mg of bioprobe per gram of tumour or about 315 μg per ml of tumour. This is an exceedingly small amount of magnetic material being used to heat the tumour mass compared, for example, with the intratumoural concentrations obtained by the direct injection method of Jordan *et al*, which were greater than 10 mg ml^{-1} of tumour. In the DeNardo experiments the low nanoparticle concentration in tumour was compensated for by application of the very high magnetic field strengths. In an earlier study [69] the same group examined the tissue heating effects of the AMF alone including the idea of reduced duty cycle to limit the non-specific heating of tissue via eddy currents.

3.4. Intrinsic frequency limits for the AMF

The low nanomagnet concentration that can be achieved *in vivo* is likely to remain one of the key challenges of the immuno-targeting approach. There is limited scope to increase the SAR by increasing the strength and frequency of the AMF, despite what is suggested by the equations describing the rate of heat generation from superparamagnetic nanoparticles, such as equation (11) in [1]. This is due to the eventual onset of indiscriminate eddy current heating of tissue or peripheral neural stimulation or even, in some operational regimes, cardiac muscle stimulation, all of which are unavoidable consequences of Faradays law of induction.

Interestingly, the exact same issues are becoming more and more important in the design of new MRI machinery where these effects impose a limit on the strength and modulation rate of gradient fields [70]. A number of authors in the last decade have published analyses of the biological effects of time-varying magnetic fields. The stimulation thresholds shown in figure 3 are derived from the information provided in Reilly [71] for the frequency dependent thresholds for magneto-stimulation in a typical human exposed to a spatially uniform longitudinal field. The eddy current heating threshold also shown in figure 3 is derived from equation (3) in [59] and assumes a tolerated maximum rate of eddy current heating of 25 mW ml^{-1}.

There are several interesting features displayed in these graphs: (1) cardiac tissue and peripheral nerves show a different frequency dependent responsiveness to the AMF, (2) the threshold for cardiac muscle stimulation, which would be a potentially fatal situation, is always at a higher field amplitude than the threshold for peripheral nerve stimulation, hence there exists an inbuilt safety warning mechanism, (3) beyond a certain corner frequency, f_e, around 120 Hz for the heart and anywhere between 500 and 5400 Hz for peripheral nerves (depending on whether the nerve fibre is myelinated or unmyelinated and how thick the fibre is; see [71] for a detailed treatise), the stimulation thresholds become almost independent of frequency, (4) the eddy current threshold becomes the limiting threshold at frequencies beyond several hundred kilohertz. Of course, all these threshold calculations only apply to whole body exposure. In cases where it is possible to restrict exposure to a smaller region, e.g. the head or

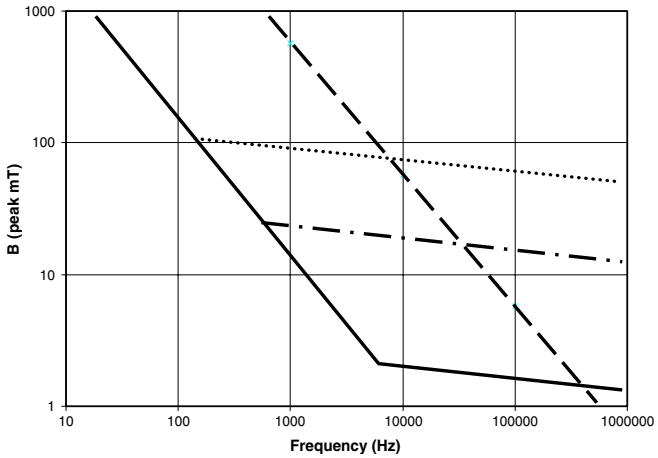

Figure 3. Thresholds for stimulation of peripheral nerves or cardiac tissue by a sinusoidal magnetic field applied along the longitudinal axis (i.e. parallel with the long axis) of an average adult human, calculated from data given in Reilly [71]. Curves are shown for cardiac tissue (dotted line), for which there is a 'corner' frequency, $f_e = 120$ Hz, beyond which the stimulation thresholds become almost independent of frequency, and for a variety of peripheral nerves which, depending on their physiology and size, have corner frequencies ranging from $f_e = 500$ Hz (dot–dashed line) to $f_e = 5.4$ kHz (solid line). Also shown (dashed line) is the applied magnetic field limit that would result in eddy current heating of peripheral tissues at a rate of ca 25 mW cm^{-3} for a torso of radius 15 cm and tissue conductivity 0.4 S m^{-1}. Note that the power deposition per cubic centimetre of tissue due to eddy currents scales with the square of the radius, and thus the limits imposed by eddy current heating of tissue increase as the radius decreases.

using a focused beam of magnetic field, the thresholds would be increased since the induced electric field that gives rise to these phenomena is proportional to the radius of the exposed region.

The machine developed by the Jordan group for use in combination with their magnetic fluid, the MFH®300F [72], operates at 100 kHz and produces up to 18 kA m^{-1} (226 Oe) in a cylindrical treatment volume of 20 cm diameter. Johannsen *et al* [62] reported that patients receiving thermotherapy treatment for their prostate cancer using this machine were able to tolerate up to 5 kA m^{-1} for an hour or so but any increase in field strength beyond this level resulted in some discomfort. For intracranial thermotherapy, field strengths from 3.8 to 13.5 kA m^{-1} (median 8.5 kA m^{-1}, 107 Oe) appeared to be quite well tolerated [60]. Interestingly, the $H = 5$ kA m^{-1} limit reported by Johannsen *et al* (which corresponds, since the relative magnetic permeability of tissue is approximately one, to $B = \mu_o H = 6.3$ mT) is in good agreement with Harvey and Katznelson [73] who claim that 5.9 mT is the B field value below which stimulation is not possible, irrespective of rise time, frequency or slew rate.

3.5. Prospects

So what are the implications for magnetic nanoparticle hyperthermia? An excellent analysis of the various opportunities and limitations was published in 2007 by Hergt and Dutz [74], who have expanded on the work of Rabin [75] to highlight the difficulties of using currently available magnetic nanoparticles to heat anything smaller than a 10 mm diameter tumour. The issue is essentially one of heat loss into the surrounding tissue. If one wishes to generate and sustain a large temperature imbalance within a tumour, the heat flow into that tumour has to be so large as to overcome the heat flow out. Roughly speaking, the bigger the tumour, the smaller the surface area to volume ratio, the less important is the outward heat flow, and the easier it is to heat.

Hergt and Dutz have followed this argument through and concluded that the specific loss power (SLP)[6] of the magnetic nanoparticles must be unrealistically high, certainly several orders of magnitude greater than the best currently reported, to heat a 3 mm cluster of cells, even with concentrations of iron in the cellular mass of 10 to 50 mg ml^{-1}. These figures are relevant given that ca 3 mm is the size of a subclinical metastasis that is undetectable by normal imaging techniques, and 10 mg ml^{-1} is substantially more than was used *in vivo* by DeNardo *et al*, but in the realm of that used by Jordan *et al*. The situation becomes even worse if the aim is to heat individual cells.

So the quest to develop magnetic nanoparticles with improved SLP characteristics is well justified if this form of therapy is to flourish. Several excellent reviews of the state of the art are now available [55, 57, 76]. Whilst other types of oxides have been investigated by some (e.g. [77]), the overwhelming majority of research is focused on magnetite and maghemite. The key appears to be to develop or select nanoparticles of just the right size to maximize heat transfer, and to reduce the polydispersity of the nanoparticles as much as possible, to increase the resultant SLP. Jordan *et al* [78] have found that magnetic fractionation can be used to select a sub-population of particles with approximately twice the SLP of the bulk sample. Fortin *et al* [79] examined the effects of crystal size, carrier fluid viscosity and anisotropy constant using samples of maghemite and cobalt ferrite. They found a best SLP result of 1650 W g^{-1} for their largest maghemite particles (diameter 16.5 nm) dispersed in water and exposed to an AMF of amplitude 24.8 kA m^{-1} and frequency of 700 kHz.

In an interesting counterpoint from the natural world, in 2005 Hergt *et al* [80] reported on the development of bacterial magnetosomes that yielded an impressive 960 W g^{-1} at 410 kHz and 10 kA m^{-1}. This SLP could be further increased to 1400 W g^{-1} in the presence of a large static magnetic field applied along the same axis as the AMF (magnetic texturing). Interestingly, the size of the magnetosomes was reported to be around 38 to 39 nm with a narrow size distribution. The authors suggest that these particles are not strictly superparamagnetic but that they are best described as being in the transitional region between superparamagnetic and stably ferromagnetic. Presumably this would make it difficult to understand their heating characteristics in terms of the currently popular theoretical description based on relaxation in superparamagnetic particles.

In the foregoing discussion it is important to recognize that the SLP/SAR parameter is an extrinsic parameter which

[6] In practical terms the SAR and SLP refer to the same fundamental concept: heat dissipation in a target material. As such they are currently used interchangeably in the literature. Both are measured in watts per unit mass.

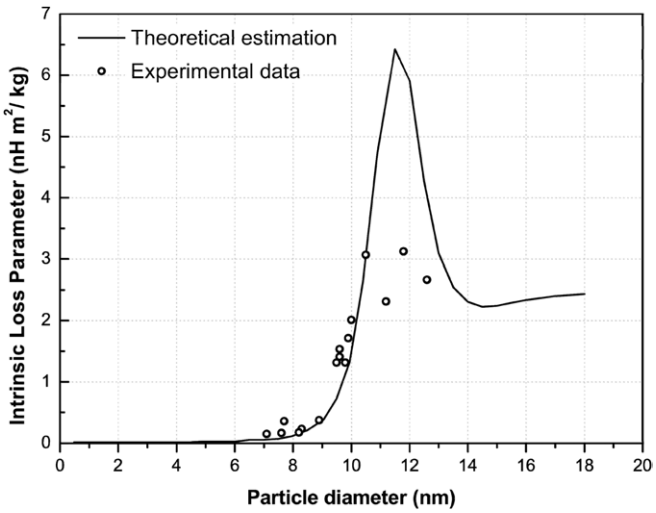

Figure 4. Comparison between theoretical predictions and experimental data on the size-dependent intrinsic heating characteristics of magnetic fluids, expressed in terms of the intrinsic loss parameter, ILP. The experimental data are reproduced from Kallumadil *et al* [76], and refer to a selection of commercially prepared fluids. The particle sizes were determined by magnetic means, and refer to the mean crystallite diameters of the constituent nanoparticles. The theoretical curve is adapted from Suto *et al* [81], and is the superposition of a peak in ILP due to Neel relaxation, and a tail at larger sizes due to Brownian relaxation.

depends not only on the magnetic heating properties of the particles themselves, but also on external factors such as the AMF magnitude and frequency. In an attempt to allow better comparisons between measurements, Kallumadil *et al* [76] have introduced the concept of intrinsic loss power (ILP), and used it to compare several commercially available magnetic nanoparticle candidates. The ILP parameter is simply the SLP/SAR parameter normalized to $H^2 f$. It is easily derived from measured heat loss data, namely the ILP measured in $\mathrm{nH\,m^2\,kg^{-1}}$ equals the SLP/SAR parameter measured in $\mathrm{W\,kg^{-1}}$ divided by the square of the field strength H measured in $\mathrm{kA\,m^{-1}}$ and the frequency f measured in kHz.

The ILP concept is a useful way to compare results from different groups who often obtain their results using different AMF conditions from one another. It is also useful in making direct comparisons with theoretical models. Figure 4 illustrates the use of the ILP parameter, with data on the ILP of commercial magnetic fluids [76] being plotted as a function of the mean particle size, and being compared with a theoretical model of the size-dependence [81]. It is notable that the comparison is quite respectable, implying that the commercial samples are approaching the best achievable results. In particular, ILPs of order $3.1\,\mathrm{nH\,m^2\,kg^{-1}}$ were obtained for samples from Micromod, Bayer Schering and Chemicell. It is interesting to note that the ILP of the best-heating synthetic particles reported to date, Fortin's maghemite particles [79], have an ILP of $3.8\,\mathrm{nH\,m^2\,kg^{-1}}$, which is not yet at the theoretical limit. Furthermore, it is intriguing to note that Hergt's bacterial magnetosomes mentioned above [80], which have an ILP of $23.4\,\mathrm{nH\,m^2\,kg^{-1}}$, are presumably operating via a different heating mechanism than that which applies to the synthetic materials.

Eggeman *et al* [82] have also looked at the effect of particle aggregation and interactions using particles synthesized in their own labs as well as a sample from Chemicell. They conclude that it is probably crucial to understand the influence of local clustering of particles in order to fully optimize the heating from real samples of magnetic nanoparticles.

Lastly, we should note that an underlying assumption that seems to be universal is the requirement for hyperthermia therapy to be able elevate target tissue temperatures to at least 43 °C and to maintain this temperature for anything up to an hour in order to be a successful treatment, i.e. deliver a thermal dose of some substantial CEM 43 throughout the tumour. Whilst this is undoubtedly true, and should remain the ultimate aim, there is increasing evidence from the clinic that even quite modest temperature rises to only 39 or 40 °C, or a low CEM 43 T_{90} figure, can still provide substantial therapeutic benefits when chemotherapy or radiotherapy is combined with hyperthermia; see, for example, [83]. In this context, the prospect of magnetic nanoparticle-mediated hyperthermia still appears to hold significant promise, and warrants the attention it receives.

4. Imaging using magnetic nanoparticles

4.1. New MRI contrast agents—metals and alloys

Iron oxide nanoparticles were the first, and are the most commonly used magnetic nanoparticle-based contrast agents for MRI. They have been so used because of their chemical stability, lack of toxicity and biodegradability. Importantly, they also have been taken through regulatory approval and may be safely, and legally, used in humans. The reader is directed to several recent reviews of magnetic iron oxide nanoparticles that include discussion of their application as MRI contrast agents [84–88]. Here we focus on rather more complex or novel contrast agents, and consider their potential application as the next generation of MRI contrast agents.

Cobalt nanoparticles have an intrinsic advantage over iron oxide nanoparticles in their much higher room temperature saturation magnetization, $1422\,\mathrm{emu\,cm^{-3}}$ [89] compared with $395\,\mathrm{emu\,cm^{-3}}$ for iron oxide [90]. This means that cobalt nanoparticles may have a larger effect on proton relaxation, giving improved MR contrast and allowing smaller particle cores to be used without compromising sensitivity. However, it is rather difficult to fabricate water-soluble Co nanoparticles since they are prone to oxidation. Through a recent development in chemical synthesis, the Thanh group has been able to produce water-stable Co nanoparticle [91], and as a result, for the first time MRI responses can be evaluated using Co nanoparticles [92].

In their work the effects of particle size, magnetic field and temperature were studied for two samples with core diameters of 3.9 and 3.3 nm [92]. In a 1.5 T field, the larger particles had a larger r_1 relaxivity ($7.4 \pm 1.1\,\mathrm{mM^{-1}\,s^{-1}}$) than did the smaller ones ($3.9 \pm 0.8\,\mathrm{mM^{-1}\,s^{-1}}$). This difference was less marked in a 3 T field. For r_2 relaxivity, magnetic field or particle size had no significant effect, while the rather high value of $r_2 = 99 \pm 36\,\mathrm{mM^{-1}\,s^{-1}}$ make Co nanoparticles

suitable as a negative contrast agent. This is an encouraging result, especially since it is seen in 3–4 nm particles, and it is known that below ca 8 nm, inorganic nanoparticles can be readily excreted from the body by renal clearance [93].

The toxicity effects of cobalt in man are difficult to evaluate, as they are also dependent on nutritional factors [94]. Many patients have taken up to 50 mg of cobalt per day in the drug Roncovite®, which is routinely prescribed for the treatment of refractory anaemia, for long periods, with little or no toxicity being found. However, a daily dose of Roncovite® also contains 100 mg of ferrous sulfate, which may affect the amount of cobalt absorbed, since cobalt and iron share a common absorption pathway. In contrast, it has been suggested that the 10 mg cobalt ingested per day by heavy beer drinkers in a study in the 1960s may have resulted in cardiomyopathy [94]. Here the disease was thought that the combination of inadequate protein and thiamine intake, zinc depletion and alcohol may have rendered the heart more sensitive to Co^{2+} toxicity.

It is also important to note that there are currently no data available on the toxicity of cobalt nanoparticles *per se* By keeping the cobalt stable from chemical oxidation through the design of appropriate ligand shells, it should be possible to prevent the formation of Co^{2+}. In such a case, there may well be the potential for cobalt to be used in humans, after suitable toxicity and pharmacokinetic studies in animals.

Other metals and also alloy nanoparticles are of interest as MRI contrast agents. The r_2 and r_2* relaxivity of Fe nanoparticles is significantly higher than that of iron oxide at a comparable particle size [95]. FePt alloy nanoparticles, as reported by Maenosono *et al* in 2008 [96], are better still. They synthesised chemically disordered, face-centred cubic FePt nanoparticles with a mean diameter of 9 nm via pyrolysis of iron(III) ethoxide and platinum(II) acetylacetonate. The r_2/r_1 relaxivity ratio of the FePt nanoparticles was found to be 3–14 times larger than that of conventional iron-oxide-based contrast agents [96]. However, to administer the FePt particles into a rat, the surface ligands were exchanged from oleic acid to tetramethylammonium hydroxide (TMAOH), a protocol that does not have long-term stability. It appears that further improvement in biostabilization and fuctionalization of these alloys is needed.

4.2. New MRI contrast agents—oxides and core-shell particles

The efficacy, as MRI contrast agents, of iron oxide nanoparticles depends to a large extent on their physicochemical properties, particularly their size and surface chemistry, the latter being modified through conjugation with biologically active substances such as antibodies, receptor ligands, polysaccharides and proteins [97]. For example, water-dispersible Fe_3O_4 nanocrystals stabilized with phosphine-oxide–PEGs show size-dependent MR contrast [98]. Nanocrystals with core diameters of 18, 11 and 5 nm at the same iron concentration of 300 mM showed spin–spin relaxation times (T_2) of 23 ms, 38 ms and 99 ms, respectively, demonstrating that the larger particles exhibited the larger T_2 effect [98].

The intrinsic magnetization of the particles is also important. Enhanced MRI sensitivity was reported in 2007 by Lee *et al* in spinel ferrite nanoparticles with exceptionally high and tunable magnetisations [99]. Spinel MFe_2O_4 ferrites, where M is a +2 cation of Mn, Fe, Co or Ni, were synthesised using divalent metal chloride in a high-temperature, nonhydrolytic reaction between divalent metal chloride (MCl_2) and iron tris-2,4-pentadionate, in the presence of oleic acid and oleylamine as surfactants. These particles were made water-soluble by exchanging the hydrophobic ligands with 2,3-dimer-captosuccinic acid. $MnFe_2O_4$ nanoparticles showed the highest mass magnetization value of 110 emu g^{-1} of magnetic atoms. The $MnFe_2O_4$ particles also had the highest magnetic susceptibility, and the strongest r_2 relaxivity value of 358 $mM^{-1}\,s^{-1}$. The r_2 values systematically decreased to 218 $mM^{-1}\,s^{-1}$, 172 $mM^{-1}\,s^{-1}$ and 152 $mM^{-1}\,s^{-1}$ for nanoparticles of Fe_3O_4, $CoFe_2O_4$ and $NiFe_2O_4$, respectively. Lee *et al* commented that given its high sensitivity, $MnFe_2O_4$–Herceptin conjugates would enable the MR detection of tumours as small as 50 mg, a size of $2 \times 5 \times 5\,mm^3$ [99].

In 2008 Barcena *et al* [100] presented a mixed spinel $Zn_{0.34}Fe_{0.66}Fe_2O_3$ with a comparable MRI detection sensitivity. Their T_2-weighted images of $Zn_{0.34}Fe_{0.66}Fe_2O_3$ coated with poly(ethylene glycol)-block-poly(D,L-lactide) yielded a detection limit of 0.8 $\mu g\,ml^{-1}$, which corresponds to an r_2 value of 294 $mM^{-1}\,s^{-1}$. In comparison, the sensitivity of one of the gold-standard commercial contrast agents (sold as Feridex® in the United States and as Endorem® in Europe, and made by Guerbet LLC in Paris) is 2.1 $\mu g\,ml^{-1}$, which corresponds to a much smaller r_2 of 110 $mM^{-1}\,s^{-1}$. With comparable FDA reference daily intake values to those of Fe, the toxicity of Zn would not be a major biocompatibility concern [100].

Core-shell nanoparticles are also of great interest as new, and flexible, contrast agents. In 2008 Kim *et al* showed that superparamagnetic Fe_3O_4@$mSiO_2$ particles, comprising a magnetite core and a mesoporous silica shell, have multiple functionalities applicable to simultaneous multimodal imaging and therapy [101]. The r_1 and r_2 relaxivity values of Fe_3O_4@$mSiO_2$ particles with a 15 nm core were 3.40 $mM^{-1}\,s^{-1}$ and 245 $mM^{-1}\,s^{-1}$, respectively. The fluorescent and T_2-weighted MR images of phantoms showed that as the concentration of the nanoparticles was increased, a brighter fluorescence and a darker T_2 signal was observed 2 h after injection, and that the accumulation of nanoparticles in tumours could be detected in the T_2-weighted MR images. Even at 24 h after injection the nanoparticles still remained in the tumour sites. The latter was attributed to an appreciable accumulation of nanoparticles in tumours through the enhanced permeability and retention (EPR) effect [101].

4.3. Magnetic particle imaging

In what may prove to be a significant development for the future of magnetic imaging in the human body, in 2005 Gleich and Weizenecker from Philips Research in Hamburg published the first report [102] on a new imaging modality, magnetic particle

imaging (MPI). The technique takes advantage of the nonlinear magnetization curve of small magnetic particles to generate harmonic responses to time-varying fields that can be detected using standard lock-in methods to a high degree of precision, and with very little background signal to contend with. Gleich and Weizenecker used a drive field of $H = 8\,\mathrm{kA\,m^{-1}}$ (100 Oe) at 25.25 kHz, and commented that fields twice as large, and frequencies up to 100 kHz, could be used in future. The imaging capability is the result of an elegant and simple concept: that in the presence of a large enough dc magnetic field, the magnetization curve is flat, and as such the harmonic signals disappear. The corollary of this is that if one applies a dc field to all but a small 'field-free point' on the sample, the only harmonic signal received comes from that field-free point, and all other signals are damped out.

Using this approach, Gleich and Weizenecker demonstrated a 2D spatial resolution of better than 1 mm, and a detection limit for Fe of ca $100\,\mu\mathrm{mol\,l^{-1}}$ [102]. The latter is within the range of the allowed dosage for medical use. In a subsequent paper in 2007, the Philips group described a further step towards the goal of video-rate imaging, showing MPI data taken at an encoding speed of 3.88 ms for a field-of-view of $1 \times 1\,\mathrm{cm^2}$ [103]. Small phantoms composed of several dots, each filled with 200 nl of undiluted Resovist® (a commercial MRI contrast agent comprising $500\,\mathrm{mmol(Fe)\,l^{-1}}$, made by Bayer-Schering Pharma in Berlin) were scanned. A resolution of better than 1 mm was achieved at a frame rate of $25\,\mathrm{frames\,s^{-1}}$ [103].

As well as the Philips Research team, other groups have taken up the technical challenge of developing the MPI technique. Amongst these, in 2008 Weaver *et al* demonstrated experimentally that the addition of an offset magnetic field introduces even harmonics in the nanoparticle signal that are significantly larger than the odd harmonics, so the total signal produced is increased significantly [104].

MPI has great potential for medical applications such as vascular or small intestine imaging, where fast dynamic information is required, and the targets are located relatively deep below the skin, the latter because the MPI signal is virtually unattenuated by intervening tissue. Its sensitivity is improving, with a report in 2009 showing that it is already capable of imaging Resovist® at concentrations as low as $40\,\mu\mathrm{mol(Fe)\,l^{-1}}$, and with temporal and spatial resolutions comparable to established modalities: namely 21.5 ms at sub-millimetre resolution for a 3D field-of-view of ca $20 \times 12 \times 17\,\mathrm{mm^3}$ [105]. Another major development, reported by Sattel *et al* in 2009 [106], is that MPI can overcome the problem of the specimen needing to be placed in a total-surround scanner (such as an MRI scanner) through use of a single-sided scanner, which is applied to the object of interest from one side only. The first single-sided results show a resolution of about 1 mm, and are promising [106].

5. Discussion

The sheer diversity and scope of the innovations briefly described above is a clear indication of the burgeoning state of the field of biomagnetics. It is quite remarkable how much has been accomplished in just a few years, and the prospects for even more breakthroughs to come look very good.

In vitro applications based on magnetic actuation are becoming significant players in the non-viral transfection market, rivalling existing virus-based methods for transporting genes and proteins across cell membranes. Novel practices involving oscillating magnets or combinations of magnetically loaded microbubbles and ultrasound are achieving much higher transfection rates than otherwise possible, indicating that there may be more to come in this area. On the other hand, the 'holy grail' of efficient *in vivo* actuation for drug delivery and gene therapies is still elusive, with the fundamental problem of the drop-off in magnetic force with distance in the body, and with smaller targets such as individual nanoparticles, as well as the body's own physiological defence mechanisms against 'foreign agents', all working against us. Nevertheless, progress is being made, with improvements in the delivery of magnetic forces via magnetic needles, meshes and bandages, as well as new methods for creating 'stealth' delivery vehicles using magnetic particles incorporated into macrophages or stem cells. There have also been some promising *in vivo* results reported on a pre-clinical trial of gene transfection in cats for the treatment of feline fibrosarcomas, which may point to a way forward in this work: namely, to refine our approaches to drug delivery and gene therapy in the veterinary market first, as a stepping-stone towards human treatments.

At the same time a good deal of work is being done to understand and control, at the level of cells and cell membranes, the influence between localized forces and cellular function. This is now showing promise in applications including RM, where magnetic actuation is being used to promote differentiation of progenitor cells into pre-specified cell types, and TE, where entire tubular tissue structures destined to become implantable blood vessels and the like are now being grown. This is an area where continued progress is likely in the coming years.

In magnetic heating or hyperthermia, the big news in 2007 was the commencement of the first human clinical trials on brain cancer, which was later expanded to prostate cancer, both being conducted by Jordan and colleagues at the Charité Hospital in Berlin. Although undoubtedly a major achievement, it is interesting to note that Jordan's approach is one of the utmost simplicity: direct injection at multiple sites in the tumour rather than the often-repeated aspiration of targeted delivery via intravenous injection of a suitably modified vector. It is also clear that a great deal of attention has been paid to the question of dose-response characteristics, and the need to have a clear and unequivocal answer to the regulator's question of 'how can you assure me that your treatment will do no harm?' This pragmatic approach has allowed the trial to be set up, and initial results are promising.

Nevertheless, targeted hyperthermia remains a major goal that many groups around the world are working towards, with steady, if not yet spectacular, success. Conjugated monoclonal antibodies and magnetic nanoparticles have been the subject of many studies, and loadings in mouse tumours of up to $0.3\,\mathrm{mg\,ml^{-1}}$ have been reported, which is approximately 30 times less than the loading that Jordan achieves by direct

injection, but even so a respectable amount, and promising for future work. Much chemical synthesis work is being done towards improving the intrinsic heating properties of the magnetic particles, although the issue of comparability has continued to dog the field. We recommend the adoption of the ILP as a step towards normalizing results between different laboratories. Other ways to improve the heating efficiency are also being pursued, such as increasing the frequency and field strength of the applied alternating field. In this context there is something of a sea-change in progress, with challenges to our preconceptions on the allowable limits for field and frequency in therapeutic applications. The limits illustrated in figure 3 are a case in point, and increasingly groups are using MHz frequencies and field amplitudes of 10 kA m^{-1} and more, in an attempt to achieve therapeutically viable heating.

MRI continues to be the most important medical sensing technology that uses magnetic nanoparticles, and progress continues to be made in the development of new contrast agents, albeit that there appears to be little commercial interest at present in gaining regulatory approval for new diagnostic indications. Even so, a number of metal, alloy, complex oxide and core-shell nanoparticles are currently in development with substantially better relaxivities than those of existing iron oxide contrast agents. It is likely that there will need to be a specific target identified before such new agents will find their way into clinical trials; one possibility is the early diagnosis of breast cancer, where manganese ferrite nanoparticles conjugated with Herceptin are showing promise.

The medical imaging field is constantly evolving, and multi-modality probes and techniques are very popular. The newest modality to appear was announced by Philips Research in 2005, namely MPI. MPI has the potential to become a significant player in the development of magnetic particles for therapeutic use, especially if its resolution can be improved to rival that of MRI, without the need for whole-body scanners. The fact that to date the preferred MPI contrast agent is the commercial agent Resovist® has implications for its route to market. The fact that Resovist® is also one of the best hyperthermia agents yet produced, is perhaps a hint of things to come, where, for example, MPI provides an answer to the dose-response characterisation of a magnetic heating therapeutic.

6. Conclusions

In this review and progress report on the state of play in biomagnetics we have focused on the three main application pathways that are linked to the fundamental characteristics of magnetic particles: namely magnetic actuation, magnetic heating and magnetic sensing.

However, there are applications that do not fit neatly in these categories, but are instead defined by the clinical need that they are designed to meet. Although we will not discuss them in detail here, it is worth noting that there is substantial work being done in areas such as novel MRI techniques for monitoring iron levels in the liver, and the diagnosis of iron overload diseases [107]; methods for probing the life-cycle of the malaria parasite, which produces a magnetic mineral called hemazoin in inflected red blood cells [108]; proposals for using conjugated magnetic particles and anti-HER2 targets to enable a quantitative, magnetic form of immunohistochemistry on breast cancer biopsies [109]; the use of magnetically actuated viscous ferrofluids in the eye for the treatment of detached retinas [110]; magnetic stents and magnetically tagged endothelial cells for treating cardiovascular disease [111] and the development of novel hand-held probes based on magnetoresistive sensors [112] or ultra-sensitive susceptometers [113] for tracing lymphatic drainage from breast and lung cancer tumours.

It is also worth remembering that not all work in the field appears in the scientific literature, but rather it resides in company patents or is kept as know-how to enable commercialization. Thus another way to gauge advances in applications of magnetic nanoparticles in biomedicine is to look at the growth of new companies in the field, or the R&D involvement of larger or more established companies. We have already mentioned the work of MagForce Nanotechnologies AG and Philips Research NV with respect to magnetic hyperthermia and MPI, respectively, but there are many more companies of note. These include established magnetic particle synthesis companies such as Liquids Research Ltd, Chemicell GmbH, Micromod GmbH and Bayer-Schering Pharma, as well a new companies setting out to make bespoke materials, such as MidaTech Ltd, NanoPET GmbH, Promethean Particles Ltd and Pepric NV. There are many application-focused companies, including Endomagnetics Ltd for sentinel lymph node detection, NanoTherics Ltd for gene transfection, Aduro Biotech Inc and Sirtex Medical Ltd for magnetic hyperthermia, Resonance Health Ltd for non-invasive iron overload measurement and MagnaBioSciences LLC for magnetic immunoassays.

In conclusion, there is a lot of activity in this field, and the future is bright, so long as we pay attention to the primary criteria for success, making sure that there is a clearly identified clinical need that can be addressed, and addressed in a way that can be quantified or assessed to the satisfaction of the relevant licensing bodies. It may also be prudent to carefully assess the potential applications for any new approach to see whether there is a simple, straightforward target that may be addressed in the short term. Success begets success, and even if the need is small or the market tiny, it can be very useful as a way of gaining traction towards a more holistic application. The MagForce approach is a case in point here, where magnetic hyperthermia following direct injection is achievable now, whereas targeted hyperthermia following intravenous injection is still undergoing strenuous development. We look forward to more of these 'low hanging fruit' style of application in the coming years, alongside continued focused work on the fundamentals. If we get these first applications out into the marketplace and establish the profitability of biomedical applications of magnetic nanoparticles to investors and the world at large, the prospects for further scientific, technological and commercial advances are indeed bright.

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