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TOPICAL REVIEW

Functionalisation of magnetic nanoparticles for applications in biomedicine

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Abstract

Magnetic nanoparticles have been proposed for use as biomedical purposes to a large extent for several years. In recent years, nanotechnology has developed to a stage that makes it possible to produce, characterize and specifically tailor the functional properties of nanoparticles for clinical applications. This has led to various opportunities such as improving the quality of magnetic resonance imaging, hyperthermic treatment for malignant cells, site-specific drug delivery and the manipulation of cell membranes. To this end a variety of iron oxide particles have been synthesized. A common failure in targeted systems is due to the opsonization of the particles on entry into the bloodstream, rendering the particles recognizable by the body's major defence system, the reticulo-endothelial system. This review discusses each of the above bio-applications of such magnetic nanoparticles and details some of the main recent advances in biological research.

1. Introduction

The application of small particles in *in vitro* diagnostics has been practised for nearly 40 years. This is due to a number of beneficial factors including a large surface area to volume ratio, and the possibility of ubiquitous tissue accessibility. In the last decade increased investigations and developments were observed in the field of nanosized magnetic particles, the term nanoparticle being used to cover particulate systems that are less than 1 μm in size, and normally below 500 nm. Nanoparticles that possess magnetic properties offer exciting new opportunities including improving the quality of magnetic resonance imaging (MRI), hyperthermic treatment for malignant cells, site-specific drug delivery and also the recent research interest of manipulating cell membranes, each of which will be addressed in this review.

Iron oxide magnetic nanoparticles tend to be either paramagnetic or superparamagnetic, with particles approximately 20 nm being classed as the latter. In most cases superparamagnetic particles (usually Fe_2O_3 and Fe_3O_4) are of interest for *in vivo* applications, as they do not retain any magnetism after removal of the magnetic field. This is important as large domain magnetic and paramagnetic materials aggregate after exposure to a magnetic field (Bonnemain *et al* 1998, Wang *et al* 2001).

One major hurdle that underlies the use of nanoparticle therapy is the problem of getting the particles to a particular site in the body. A potential benefit of using magnetic nanoparticles is the use of localized magnetic field gradients to attract the particles to a chosen site, to hold them there until the therapy is complete and then to remove them. This involved some fairly advanced design of systems for producing these fields. Additionally, such equipment should ideally contain other molecules to show that the particles have been actually located in the appropriate region of the body.

The particles may be injected intravenously, and then blood circulation would be used to transport the particles to

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the region of interest for treatment. Alternatively in many cases the particles suspension would be injected directly into the general area when treatment was desired. Either of these routes has the requirement that the particles do not aggregate and block their own spread. This leads to questions about the best way to produce a suspension that is stable.

Fortunately there is appreciable intracellular space in the body through which nanoparticles can diffuse out of flow. A large proportion of this space is between cells. Brightman found that 9 nm diameter ferritin particles would diffuse rapidly through intercellular spaces to achieve a near uniform distribution in a few minutes (Brightman 1965). Two tissues were not accessible by this route; those that are beyond the blood brain barrier, and those inside spaces of the kidney tubules. These regions have special types of cell to cell contacts called the zonulae occludentes, which have contacts less than 1 nm wide.

The diffusion to the general mass of tissues was presumably aided by pressure gradients from the blood vessels (chiefly microcapillaries) to the tissue spaces. Larger particles of 50–100 nm diameter did not transport in this way and remained in circulation or attached to the walls of the vascular system. Attaching particles to the vascular walls may be a method of therapy in some instances, but carries the risk of thromboses.

These considerations suggest that nanoparticles of about 5–10 nm diameter should form the ideal particles for most forms of therapy but that there will also be problems of formulating the particle concentrations and suspending media to obtain best distributions.

Magnetic nanoparticles are physiologically well tolerated, for example dextran-magnetite has no measurable toxicity index LD_{50} (Babincova *et al* 2000). However the fate of nanoparticles following intravenous administration, as indicated in figure 1, represents the diverse biological events that need to be considered. After particles are injected into the bloodstream they are rapidly coated by components of the circulation, such as plasma proteins. This process is known as opsonization, and is critical in dictating the circumstance of the injected particles (Davis 1997). Normally opsonization

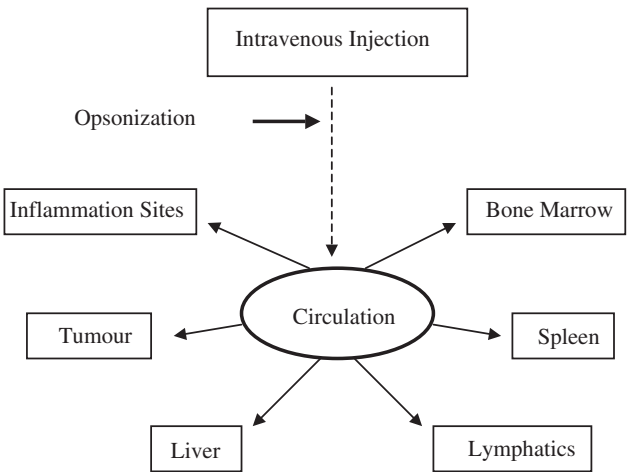

Figure 1. The fate of nanoparticles following intravenous injection. Particles are conditioned immediately on injection by plasma proteins (opsonization).

renders the particles recognizable by the body's major defence system, the reticulo-endothelial system (RES). The RES is a diffuse system of specialized cells that are phagocytic (i.e. engulf inert material) associated with the connective tissue framework of the liver, spleen and lymph nodes (Kreuter 1994, Araujo *et al* 1999). The macrophage (Kupffer) cells of the liver, and to a lesser extent the macrophages of the spleen and circulation, therefore play a critical role in the removal of opsonized particles. As a result, the application of nanoparticles *in vivo* or *ex vivo* would require surface modification that would ensure particles were non-toxic, biocompatible and stable to the RES.

Particles that have a largely hydrophobic surface are efficiently coated with plasma components and thus rapidly removed from the circulation, whereas particles that are more hydrophilic can resist this coating process and are cleared more slowly (Gaur *et al* 2000). This has been used to the advantage when attempting to synthesize RES evading particles by sterically stabilizing the particles with a layer of hydrophilic polymer chains (Allemann *et al* 1993). In the literature the most common coatings are derivatives of dextran, polyethylene glycol (PEG), polyethylene oxide (PEO), poloxamers and polyoxamines (Lacava *et al* 2001). The role of the dense brushes of polymers is to inhibit opsonization, thereby permitting longer circulation times (Shen *et al* 1996, Portlet *et al* 2001, Briggar *et al* 2002). A further strategy in avoiding the RES is by reducing the particle size (Gref *et al* 1994, Moghimi *et al* 2001). Despite all efforts, however, complete evasion of the RES by these coated nanoparticles has not yet been possible (Gaur *et al* 2000).

An interesting possibility in the attachment of particles to cells may be the presentation of topographical cues to other cells. It was proposed in 1964 that cells reacted to the topography of their environment (Curtis and Varde 1964), and recent studies have demonstrated that cells can respond to nanometric cues *in vitro* (Curtis and Riehle 2001, Curtis and Wilkinson 2001). It is further postulated that cells respond to topographies presented *in vivo* by proteins such as collagen (with the 64 nm repeat banding) and that cells may also respond to nanotopography present by neighbouring cells (Curtis and Wilkinson 1999). Particles attached to cells would most likely present nano-spheres or nano-bumps to neighbouring cells. Studies by Chen *et al* (1997) showed that cells reacted to micropatterned surfaces exhibiting differently sized extracellular matrix covered islands or bumps. The endothelial cells tested were encouraged to switch from growth to apoptosis with decreasing island size. This phenomenon was attributed to the patterned island influence on cell spreading, which in turn influenced cell shape, feeding back to govern cell viability status. More recent studies have also shown that cells alter their spreading on nano-sized island topographies (Dalby *et al* 2002). In this case three island heights were investigated (13, 35 and 95 nm), with the largest cell response to the 13 nm islands, resulting in increased cell spreading and cytoskeletal formation. This importance of topography supporting cell extension and subsequent spreading may be relevant when considering particle attachment to the cell surface, as this may in turn influence attachment of neighbouring cells.

Nanotechnology has reached a stage that makes it possible to produce, characterize and specifically tailor the functional

properties of nanoparticles for clinical applications. This short review will detail the main areas of biomedical applications and current research using such magnetic nanoparticles including as MRI contrast agents, for use as potential hyperthermia treatment of malignant cells, targeted drug delivery and also as laboratory tools to manipulate cell membranes.

2. MRI

Imaging of soft tissue structure of the musculoskeletal system has become the domain of MRI due to its superiority over other imaging techniques. The technique measures changes in the magnetization of hydrogen protons in water molecules sitting in a magnetic field after a pulse of radio frequencies has hit them. Protons from different tissues react differently, giving a picture of anatomical structures. These images can be enhanced adding 'contrast agents' which sharpen the contrast by affecting the behaviour of protons in their proximity (Lok 2001). In standard clinical MRI scans contrast agents travel through the bloodstream and tissues, increasing contrast wherever they go. Although the more commonly used MR contrast media are gadolinium (Gd) chelates, these tend to be non-specific with rapid accumulation in the liver, thus they only allow a short time imaging window (Kubaska *et al* 2001, Low 2001).

Colloidal iron oxides therefore play an important role as MRI contrast agents, as superparamagnetic iron oxide particles were the first liver-specific contrast agents used (Halavaara *et al* 2002). It has been known for many years that the inclusion of magnetic particles within tissue enables a very large signal to be obtained from a MRI scanner. To date a wide variety of particles have been produced, differing in size (hydrodynamic particle size varying from 10 to 500 nm) (Van Beers *et al* 1995) and type of coating material used (such as dextran, starch, albumin, silicones, poly(ethyleneglycol)) (Babes *et al* 1999). They tend to be classified in two main groups according to their size, as this affects plasma half-life and biodistribution. The first group are termed SPIOs (supreparamagnetic iron oxides) where nanoparticles have a size greater than 50 nm (coating included) and the second type termed USPIOs (ultrasmall superparamagnetic iron oxides) where nanoparticles are smaller than 50 nm (Brigger *et al* 2002). The particle size influences both their physicochemical and pharmacokinetic properties. The main present and future applications using the particles are imaging of gastrointestinal tract, liver and spleen, lymph nodes (Fahlvik *et al* 1990, Gellissen *et al* 1999). However, USPIO particles are also blood pool agents which could be used for perfusion imaging (i.e. brain or myocardial ischemic diseases) (Muhler *et al* 1995, Moore *et al* 2000, Moghimi *et al* 2001). Both types of particle can be now purchased commercially. Lumirem®, silicon-coated particles with 300 nm diameter, and Endorem®, magnetite particles with a 150 nm diameter, are two examples of SPIOs on the market (Bonnemain 1998). These contrast agents are used for gastro-intestinal tract imaging and for live and spleen disease detection, respectively. Sinerem®, magnetite particles with a 30 nm diameter, is an example of a USPIO available in the market, and is currently being used for tumour detection (Bonnemain 1998).

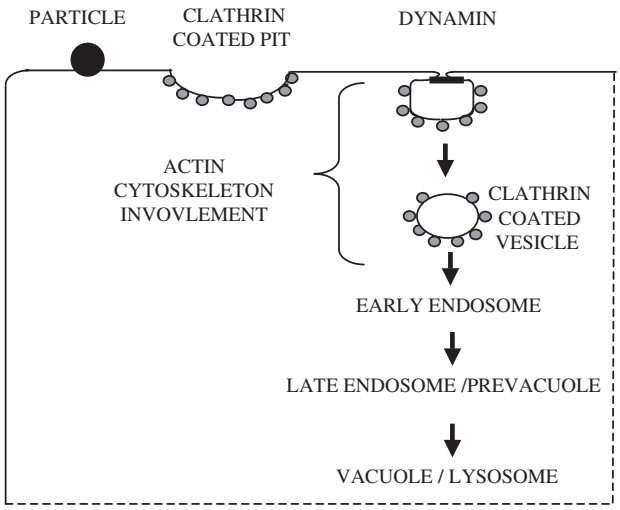

Figure 2. Schematic of the basic cell events occurring during clathrin mediated endocytosis. Once the coated nanoparticle has docked at its receptor, receptor-mediated endocytosis usually occurs. Clathrin coated pit invaginations are observed at the cell membrane, which with the combined action of the GTPase dynamin, pinch off from the membrane to form isolated vesicles leading to the formation of vacuoles.

Biologically speaking, the main requirement for MRI is that the cells efficiently capture the magnetic particles they are exposed to following the endocytosis pathway. The endocytic process is a process whereby nano-sized material is ingested by a cell. The proccess can be broken down into main steps as illustrated in figure 2. These steps include membrane invagination, clathrin coated pit formation, coated pit sequestration, detachment of the newly formed vesicle via action of the small GTPase dynamin and finally movement of this new endocytic compartment away from the plasma membrane into the cytosol (Schafer 2002). Vesicle formation during endocytosis involves a complex protein machinery and additional proteins to control it. Although general understanding has grown rapidly over the last few years, little is known about how it interconnects functionally with the cortical cytoskeleton underlying the plasma membrane (Qualmann *et al* 2000). There is therefore continuing debate in the field of cell biology as to the role of the actin component of the cell cytoskeleton in all or any of these aforementioned steps (Fujimoto *et al* 2000, Fujimoto *et al* 2000). Understanding the basics behind the endocytosis pathway will aid future optimization of cell loading with magnetic nanoparticles.

Previous studies have reported cellular uptake of dextran-coated nanoparticles varying from 0.011 to 0.118 pg of iron per cell (usually post 1 h incubation) in various tumour cells and a maximum load of 0.97 pg in primary isolated peritoneal mouse macrophages (Schulze *et al* 1995, Moore *et al* 1997, 2000). If a cell can be sufficiently loaded with magnetic material, then MRI can also be adopted for use in cell tracking, as it can have a resolution of 20–25 μm, approaching the size of single cells (Bulte *et al* 2002). The history of tracking cells with a MR-detectable tracer dates back about a decade, where attempts were made to label leukocytes, lymphocytes and monocytes with superparamagnetic iron oxides. The cells could then be used for the study of immune trafficking. Various strategies included the use of dextran coated particles, however

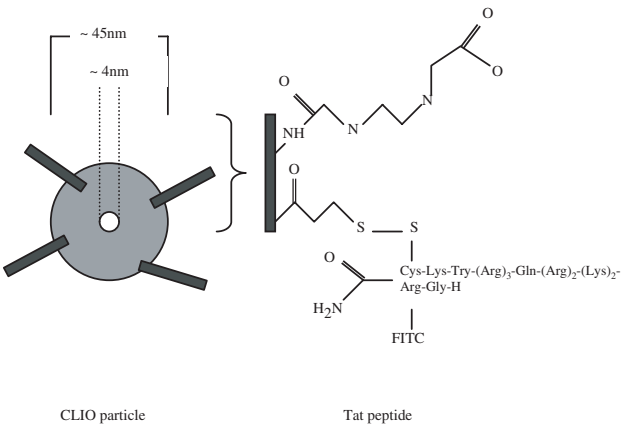

Figure 3. Schematic diagram of CLIO-Tat superparamagnetic nanoparticle. The particles consist of a central iron oxide core, surrounded by cross-linked dextran. The tat peptide attached to the dextran, and can feature FITC (yielding an average of four peptides per particle) (drawing adapted from Allport and Weissleder (2001)).

uptake was low and improvements were needed (Weissleder *et al* 1997, Dodd *et al* 1999). In particular there were several problems associated with tracking some cell types using MRI due to the fact that some cells, such as lymphocytes, do not possess high-efficiency internalizing receptors for endocytosis (Schoepf *et al* 1998). Recently, a new strategy for high-efficiency internal labelling of large numbers of cells has been developed. The technique involves the use of the HIV-1 tat peptide, which has been shown to move freely through cellular and nuclear membranes. It carries a transmembrane and a nuclear localization signal within its sequence (Vives *et al* 1997) and is therefore capable of translocating exogenous molecules into cells (Zhao *et al* 2002). The attachment of this peptide bearing the translocation sequence to dextran cross-linked iron oxide nanoparticles (figure 3) has been shown to increase their uptake over 100-fold into lymphocytes when compared to untagged particles (Josephson *et al* 1999, Allport and Weissleder 2001, Wunderbaldinger *et al* 2001). This huge increase in internalization resulted in substantially greater detectability of labelled cells and therefore increased ability to isolate and sort cells from tissues following *in vivo* experiments such as transplantations.

Another recent strategy to increase uptake was the use of a new class of iron oxide nanoparticles, anionic maghemite nanoparticles (AMNP). It has been shown that bare AMNPs, free form any dextran coating, exhibited a surprisingly high level of cell internalization that was comparable with nanoparticles modified with the tat peptide (Wilhelm *et al* 2002). In this case, the particles were found to interact strongly and non-specifically with the plasma membrane thanks to their surface negative charges. This adsorption step preceded the internalization step and therefore governed the overall cell uptake. The identification of such particles, that non-specifically adsorb onto virtually any mammalian cell with subsequent internalization, offers the opportunity to label a wide variety of cells with comparable efficiency , and opens up further possibilities for MRI tracking of cell transplants.

A further goal is to exploit the recent advantages in MRI techniques to image transgene expression. To date many other approaches have been used to image gene expression, including optical (e.g. green fluorescent protein expression) and nuclear imaging. However problems with resolution and depth penetration have hindered progress (Weissleder *et al* 2000). Although this work is still in the early stages, preliminary study results have demonstrated the feasibility of MRI to depict the activity of endocytotic receptors like the asialoglycoprotein receptor (Josephson *et al* 1990). In addition there have been more recent successes with the expression of an engineered transferrin receptor (ETR). This research was aimed at developing MRI for imaging transgene expression by using the ETR as a marker gene and conjugates of transferrin and monocrystalline iron oxide (Tf–MION) as an MR imaging probe for this receptor (Hogemann *et al* 2001, Moore *et al* 2001). The availability of a universal MR marker gene to image gene expression may permit monitoring of gene therapy in which exogenous genes are introduced into the body to eliminate a genetic defect or to add an additional gene function to tumour cells (see later). There is a growing number of cell biologists, molecular biologists and geneticists who are currently working with experts in MRI with the aim of developing techniques that can non-invasively visualize where, when, and in some cases, at what level a gene is being expressed in living animals (Lok 2001).

3. Hyperthermia treatment for malignant cells

The preferential killing of cancer cells without damaging normal cells has been a desired goal in cancer therapy for many years. However, the various procedures used to date, including chemotherapy, radiotherapy or surgery, can fall short of this aim. The potential of hyperthermia as a treatment for cancer was first predicted following observations that several types of cancer cells were more sensitive to temperatures in excess of 41°C than their normal counterparts (Jordan *et al* 1996, Nielsen *et al* 2001). In the past external means of heat delivery were used such as ultrasonic or microwave treatments, but more recently research has focused on the injection of magnetic fluids directly into the tumour body, or into an artery supplying the tumour. The method relies on the theory that any metallic objects when placed in an alternating magnetic field will have induced currents flowing within them. The amount of current is proportional to the size of the magnetic field and the size of the object. As these currents flow within the metal, the metal resists the flow of current and thereby heats, a process termed inductive heating. If the metal is magnetic, such as iron, the phenomenon is greatly enhanced. Therefore, when a magnetic fluid is exposed to an alternating magnetic field the particles become powerful heat sources, destroying the tumour cells (Babincova *et al* 2000). The magnetic fluids used are preferably suspensions of superparamagnetic particles, prepared much as described for MRI contrast agents, as these produce more heat per unit mass than larger particles (Mitsumuri *et al* 1996). The level heating is simply controlled by the materials Marie Curie temperature, that is, the temperature above which materials lose their magnetic properties and thus their ability to heat (Rehman *et al* 2002).

The use of iron oxides in tumour heating was first proposed by Gilchrist *et al* (1957) and there are now two different

approaches. The first is called magnetic hyperthermia and involves the generation of temperatures up to 45–47°C by the particles. This treatment is currently adopted in conjunction with chemotherapy or radiotherapy, as it also renders the cells more sensitive (Hilger *et al* 2002). The second technique is called magnetic thermoblation, and uses temperatures of 43–55°C that have strong cytotoxic effects on both tumour and normal cells (Jordan *et al* 1996, Hilger *et al* 2001). The reason for this use of increased temperatures is due to the fact that about 50% of tumours regress temporarily after hyperthermic treatment with temperatures of up to 44°C, therefore authors prefer to use temperatures up to 55°C (Hilger *et al* 2001). The problem of deleterious effects on normal cells is reduced by intratumoural injection of the particles.

The heating power of the particles is quantified as the specific absorption rate (SAR) and describes the energy amount converted into heat per time and mass (Moroz *et al* 2002). Apart from the particle size and shape influencing their magnetic properties, thus consequently their heating power, there is also a dependency between temperature elevation and magnetic field amplitude which must be considered when comparing experiments with different tissue parameters. On the basis of recent studies, tumours with volumes of approximately 300 mm^3 can be heated and no potential problems were expected with larger tissue volumes (e.g. $>1000\,mm^3$) if there is proper regulation of the magnetic mass used and the intra-tumoural particle distribution (Hilgar *et al* 2001). The frequency should be greater than that sufficient to cause any neuromuscular response, and less than that capable of causing any detrimental heating of healthy tissue, ideally in the range of 100–1000 kHz (Babincova *et al* 2000). If suitable frequencies and field strength combinations are used, no interaction is observed between the human body and the field.

4. Targeted drug delivery

The main problems currently associated with systemic drug administration include even biodistribution of pharmaceuticals throughout the body, the lack of drug specificity towards a pathological site, the necessity of a large dose to achieve high local concentration, non-specific toxicity and other adverse side effects due to high drug doses. Drug targeting aims to resolve many of these problems (Torchilin 2000). Amongst the current principle schemes of drug targeting is magnetic targeting, i.e. the targeting of a drug immobilized on magnetic materials under the action of an external magnetic field.

To enhance target specificity the drug is associated with another molecule capable of specific recognition and binding to the target site. Target recognition can occur at different levels: on the level of the whole organ, the level of certain specific cell types for a given organ, or on the level of individual components characteristic for these cells such as cell surface antigens. The most common type of associated molecules are antibodies (and their fragments), lectins, proteins, hormones, charged molecules and some low molecular weight ligands such as folate (Sudimack *et al* 2000).

A highly publicized example of magnetic drug delivery is as a replacement or to augment chemotherapy/radiotherapy treatments. The development of techniques that could selectively deliver drug molecules to the diseased site, without a concurrent increase in its level in healthy tissues, is currently one of the most active areas of cancer research. The first clinical trials in humans with a magnetic drug targeting worldwide were reported by Lübbe *et al* (1997), who used a ferrofluid (particle size 100 nm) to which the drug epirubicin was chemically bound. Epirubicin is a well-known antibiotic antracylin that has a wide range of application for the treatment of solid tumours (Bonadonna *et al* 1993). In brief, special starch polymers coat the magnetic particles together with anionic phosphate groups so that a cationic binding to the positively charged amino sugars of epirubicin was possible. Preliminary successful animal trials lead to these human trials, where for the first time documented tolerance and efficacy was observed in mice and rats, in which no LD_{50} could be found for the ferrofluid. The treatment protocol consisted of the intravenous infusion of the chemically bound drug and one course of conventional chemotherapy. During infusion, and for 45 min after, a magnetic field was built up as close to the advanced and unsuccessfully pretreated tumour as possible (distance assured to be less than 0.5 cm). It was shown that the ferrofluid could be successfully directed to the tumours in about half of the patients. However it was also concluded, based on MRI techniques, pharmacokinetics and clinical detection that although the treatment seemed safe, improvements were needed to make it more effective (Lübbe *et al* 1997).

To understand this new form of pharmacological application as well as the mechanism of action, there are many considerations, which may be subdivided into several categories. In the first instance there is the ferrofluid's parameters, which include particle size, surface characteristics, concentration, volume and strength of drug–particle binding. Secondly there is access to the organism, where considerations involve the infusion route, such as the duration and rate of the injection. Finally there are the physiological parameters to consider, ranging from the organism's weight, blood volume, cardiac output, circulation time through to tumour volume/location/blood flow (Lubbe *et al* 2001). Therefore the step from animal trials to human trials is not straightforward (Lübbe *et al* 1999).

There are, however, fundamental problems associated with the use of magnetic directed drug targeting. Targeting, for example, to a specific cell type, may be possible with directed coatings, but retaining the particles localized at the cell membrane for any length of time is difficult as the cell tends to automatically instigate receptor-mediated endocytosis, as was described in figure 3. In addition, the ability of magnetic particles to concentrate will depend on both the blood flow rate and the intensity of the magnetic field. The success therefore depends to a large extent on the construction of strong magnets, able to produce high magnetic field gradients at the target sites. It has been shown that most of the available fields are only strong enough for the manipulation of particles against the diffusion and bloodstream velocities found in living systems over a distance of a few centimetres from the sharp end of a magnet pole (Lübbe *et al* 2001). This means that it is difficult to build up and sustain a field strength sufficient to counteract the linear blood flow rates in tissues so as to effectively retain the drug carrier at a specific location.

A further research interest in the use of targeted magnetic nanoparticles is in the field of gene therapy. Gene therapy

represents an exciting development in medical treatment, however many experts in the field have the opinion that the true benefits of gene therapy cannot be realized until current gene delivery systems are perfected or new vectors are developed (Nichol and Kim 2001). The theory is that by insertion of plasmid DNA into target cells, it may be possible to rectify genetic disorders, and to produce therapeutic agents in the form of peptides and proteins to stimulate the immune system. Attractive targets for gene therapy include the epithelial surfaces of the lungs and gastrointestinal tract, endothelial cells lining the blood vessels, muscle myoblasts and skin fibroblasts (Svensson *et al* 1996, Caplen 2001).

One of the impediments to successful gene therapy is the inefficient delivery of genes because of short *in vivo* half-life, lack of cell-specific targeting and particularly low transfection efficiencies (Pap *et al* 2002). The latter, that DNA transfection efficiency is limited, was attributed to the fact that there may be too low a concentration at the cell surface (Luo and Saltzman 2000). This concept has recently been adopted, alongside the principle of magnetic drug targeting, by Scherer *et al* (2002) to produce gene vectors associated with iron oxide particles. The application of targeted delivery using superparamagnetic particles (∼400 nm) and gene vectors has been termed magnetofection. The associated particles were initially tested *in vitro* using in-house designed culture plates encompassing permanent magnets beneath cell monolayers, strong enough to sediment the applied magnetic vectors onto the cells. Peak transfection levels were achieved with as little as 10 min incubation of cells with the vectors. These results were in agreement with parallel work by Hughes *et al* (2001) who also recently attached retroviral vectors via biological linkage to commercially available magnetic particles. In this case a similarly encouraging improvement in vector targeting and efficacy by magnetic force was discovered. Combined with the existing concepts of magnetic drug delivery, magnetofection may provide additional specificity and efficacy that is required in many gene therapy approaches today.

5. The future of cell membrane manipulation?

Although it is known that mechanical forces are important in regulating many cell functions, including cell growth, proliferation, protein synthesis and gene expression, it is not well understood how mechanical signals are transduced into biological responses (Eastwood *et al* 1998, Zhu *et al* 2000). Many methods have been used to apply mechanical stresses and/or strains to cell surfaces of a single cell or to a population of cells. Popular methods for single cell manipulation have included micropipette aspiration, cell poking and surface probe microscopes while seeding cells on flexible culture substrates or fluid shear stresses have been used for cell populations (Ives *et al* 1986, Gilbert *et al* 1994, Berry *et al* 2002).

The advantage in probing single cells is that regional differences in mechanical properties can be studied. In addition, cell deformability can also be assessed, which is important in a variety of cell functions such as cell contraction, spreading and crawling. Recently developed systems are attempting to use magnetic particles in what is described as magnetic twisting cytometry (MTC) (Mijailovich *et al* 2002). In theory, controlled mechanical stresses can be applied

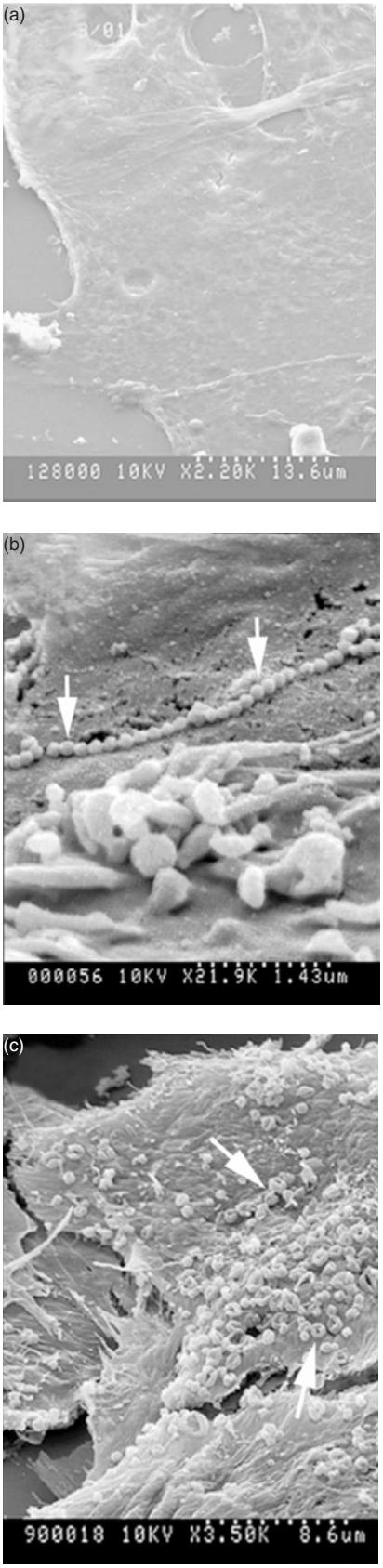

Figure 4. Scanning electron micrographs demonstrating both control cell membrane with no particles present (*a*), transferrin coated particles attached to the cell membrane ((*b*)—see arrows) and endocytosis of albumin coated iron oxide nanoparticles ((*c*)—see arrows) (personal work).

directly to a specific cell surface receptors via ligand-coated particles, and cell responses could be recorded simultaneously (Chen *et al* 2001). MTC probes the mechanical properties of a cell by applying a torque to the magnetic particle that should be tightly bound to the cell surface. Much of such work is based on the criteria that external mechanical forces are transmitted across the cell membrane to the cytoskeleton by transmembrane receptors (e.g. integrins) (Ingber 1997, Chen and Ingber 1999). Therefore coating particles with proteins recognized by integrins, such fibronectin or the tripeptide RGD (Arg-Gly-Asp), should localize the particles to the receptors (Bauch *et al* 2001). By using MTC techniques research groups are starting to make baseline measurements of cell deformability, for example in terms of stiffness, creep response, elastic modulus (Bausch *et al* 1998, 1999). Studies are also underway to test the effect of drugs/hormones on the membrane mechanical properties and the cell response (e.g. ion channel opening, gene upregulation) (St Pierre and Dobson 2000, Chen *et al* 2001).

However there are problems with using MTC that require improvement. The main problem associated with most studies appears to be that although the particles may localize to a cell membrane receptor, most are still internalized into the cell via receptor-mediated endocytosis, resulting in cell morphology as shown in figure 4 (Chen *et al* 2001, Stearns *et al* 2001). This inhomogeneity in particle attachment introduces complications. Completely internalized particles distribute their torque over a larger area than attached particles and attached/partially internalized particles may produce a shear stress. In addition, cell responses may be affected depending on particle location during force application, thereby confusing the results.

6. Conclusion

The aim of this review was to describe the current use of magnetic nanoparticles in bio-research and to a limited extent biomedicine. As highlighted, this requires the production of nanoparticles as stable, highly magnetized (preferably superparamagnetic) suspensions, capable of being functionalized with biocompatible properties that will enable evasion of the RES system where needed, and targeting to specific cell types. Due to the overriding problem with particle specificity, where particles are not reaching the cell of interest, or on doing so are rapidly endocytosed, research is concentrating on synthesis of particles with specific functional properties. The success of particle development and the application in clinical and biological laboratories is a factor of the interdisciplinary work that is involved. The collaboration in the fields of materials science, chemistry, magnetic characterization, cell engineering and testing, as well as clinical tests, is one of the great challenges of such research.
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