Magnetic nanoparticles for gene and drug delivery

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Abstract: Investigations of magnetic micro- and nanoparticles for targeted drug delivery began over 30 years ago. Since that time, major progress has been made in particle design and synthesis techniques, however, very few clinical trials have taken place. Here we review advances in magnetic nanoparticle design, *in vitro* and animal experiments with magnetic nanoparticle-based drug and gene delivery, and clinical trials of drug targeting.

Keywords: magnetic nanoparticles, gene delivery, biotechnology

Introduction

The concept of using magnetic micro- and nanoparticles for drug delivery was proposed in the late 1970s by Widder, Senyi and colleagues (Senyi et al 1978; Widder et al 1978). The basic premise is that therapeutic agents are attached to, or encapsulated within, a magnetic micro- or nanoparticle. These particles may have magnetic cores with a polymer or metal coating which can be functionalized, or may consist of porous polymers that contain magnetic nanoparticles precipitated within the pores. By functionalizing the polymer or metal coating it is possible to attach, for example, cytotoxic drugs for targeted chemotherapy or therapeutic DNA to correct a genetic defect.

Once attached, the particle/therapeutic agent complex is injected into the bloodstream, often using a catheter to position the injection site near the target. Magnetic fields, generally from high-field, high-gradient, rare earth magnets are focused over the target site and the forces on the particles as they enter the field allow them to be captured and extravasated at the target. While this may be effective for targets close to the body's surface, as the magnetic field strength falls off rapidly with distance, sites deeper within the body become more difficult to target. Some groups have recently proposed a way around this problem by implanting magnets near the target site, within the body (Kubo et al 2000; Yellen et al 2005).

Magnetic nanoparticle techniques can also be used for *in vitro* gene transfection. In this case, a high-field, high-gradient is positioned under the multi-well plate, culture flask or petri dish in which the cells are growing. DNA is attached to magnetic nanoparticles (the details of this process follow later in this review) and the magnet increases sedimentation rates, particle internalization and gene expression. Internalization normally occurs through a process of endocytosis and can be dependent on particle coating and cell type (Huth et al 2004; Berry et al 2006).

In this review we will focus primarily on the nanoparticles which are used for drug and gene delivery. In addition, applications and results of *in vitro*, animal and clinical experiments will be discussed. The theory and physical principles of magnetic nanoparticle-based drug and gene targeting have been reviewed elsewhere (Pankhurst et al 2003; Grief and Richardson 2005; Dobson 2006a, 2006b, 2006c).

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Design and synthesis of magnetic nanoparticles

In biotechnology, the essential features of nanoparticles are their nano-scale dimensions, their magnetic properties and their capability of carrying active biomolecules for specific

tasks (Moghimi et al 2001). In order to be easily localized/targeted inside the human body, the nano-scale dimensions of particles allow them not only to pass through the narrowest blood vessels but also penetrate through cell membranes when necessary (Willard et al 2004). If these particles are ferromagnetic/superparamagnetic, they can be manipulated by an external magnetic field, which can drive them to the target organs for gene (Plank et al 2003) or drug delivery (Lazaro et al 2005). The active biomolecules bound to the surface of these nanoparticles can then be released. As a result, a functional magnetic nanoparticle consists of a number of components; the magnetic core, the protective coating, and the surface functionality. For biomedical applications, magnetic nanoparticles should also have active biomolecules according to the specific applications. Figure 1 shows a schematic design of a functional magnetic nanoparticle for biomedical applications. Other entities may also be included for multifunctional particles such as hybrid fluorescent/magnetic particles. The challenge in this area is to put all these components together in a small, nanometer-scale space.

Synthesis of magnetic nanoparticles

Many different synthetic routes of magnetic nanoparticle synthesis have been reported (Li et al 2006). Some of them are one-step, while others are multi-step procedures. They all have advantages and disadvantages, and none of them provides a universal solution for all types of magnetic nanoparticles. One has to consider whether the chosen route is suitable for preparing a specific magnetic nanoparticles in a given environment with available instruments and facilities. Most of these procedures involve simple, basic inorganic chemistry, particularly iron chemistry. The following are several commonly used methods.

Wet precipitation and co-precipitation

Wet precipitation is one of the oldest methods for preparation of magnetic nanoparticles. By carefully controlling the pH of a iron salt solution, iron oxide forms as a fine suspension with particle sizes as small as 5 nm (Liu et al 2004). This simple method for making magnetic nanoparticles does not required any specialized facilities. Indeed, precipitation of the iron oxides is a simple, classic chemical testing method (qualitative analysis) for identifying the existence of iron(II) or iron(III) ions in an aqueous solution (Vogel 2000).

Mixed oxide particles (eg, magnetite Fe_3O_4, ferrites including $CoFe_2O_4$ $NiFe_2O_4$) can also be prepared by co-precipitation with a stoichiometric solution of the two metal ions. For example, magnetite can be prepared by adding base to a mixture of Fe^{2+} and Fe^{3+} solution following the equation:

$$Fe^{2+}\,(aq.) + Fe^{3+}\,(aq.) + 8OH^{-}\,(aq.) \rightarrow Fe_3O_4\,(s) + 4H_2O\,(l) \quad (1)$$

However, preparation of mixed oxides via the co-precipitation method is less straightforward, as these metals precipitate at different pH values (Pourbaix 1974).

Unfortunately, there are also some drawbacks with this procedure. Controlling the pH is vital in order to control the particle size, which is governed by kinetic factors (LaMer and Dinegar 1950; Lagally 1993). Nanoparticles with broad particle size distributions and irregular morphologies are usually produced by wet precipitation. Oxidation of the iron(II) precursor also must be avoided for successful synthesis of magnetite. Since a large quantity of water is involved during synthesis, scaling up is possible but not easy. Finally, since controlling the pH is delicate, it is virtually impossible to simultaneously precipitate a protective coating. After

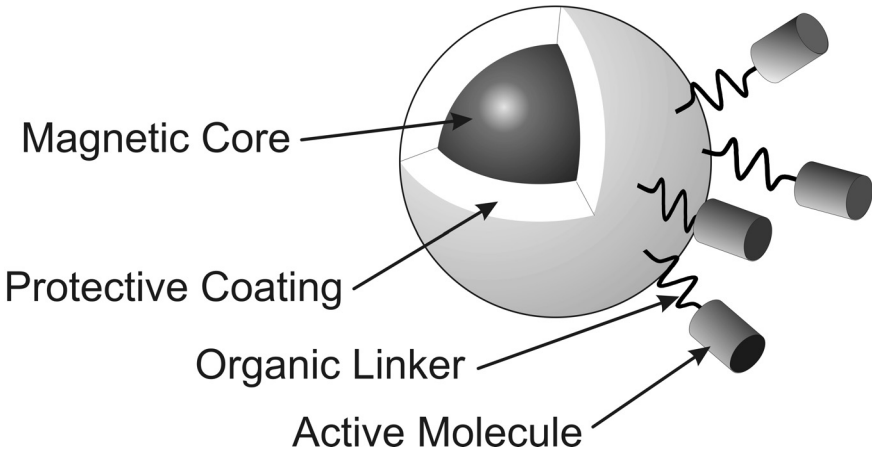

Figure 1 A typical design of a magnetic nanoparticle for biotechnology.

preparation, coating these nanoparticles individually, without aggregation can be difficult.

Reverse micelle mechanism

The formation of micelles is a classic phenomenon of surfactant chemistry (Meyers 2005). Surfactants are molecules with a hydrophilic head and a long, hydrophobic tail (Figure 2). The formation of micelles occurs when the concentration of surfactant molecules reaches a certain level, or critical micelle concentration 1 (CMC1, while CMC2 is the concentration triggering the self-assembly of liquid crystals which is not discussed here). Normal micelles form in an aqueous medium (such as using detergents in cleaning processes) but reverse micelles form in an oily medium (eg, hexane). The center of these reverse micelles is hydrophilic and stores the inorganic components of the reaction mixture. For the synthesis of iron oxide-based magnetic nanoparticles, inorganic precursors such as iron(III) chloride are dissolved in an aqueous medium and added to the oily reaction mixture with the surfactants. This is followed by the addition of pH regulators (eg, ammonia or NaOH) and inorganic coating materials (eg, silica or gold).

With the help of micelles, the size of the particles can be easily controlled and consequently nanoparticles prepared using reverse micelle routes tend to be very homogeneous in size. Also, the inorganic coating materials can be added to the micelles during synthesis, so nanoparticles produced by this method can be coated with an inorganic protective layer during the process. Magnetite nanoparticles with inorganic coatings such as silica have been prepared using this method (Santra et al 2001). The size of these nanoparticles is at the range of a few nanometers to tens, or hundreds, of nanometers with a narrow distribution.

One drawback of this technique, however, is that synthetic organic coatings are not possible as the monomers will remain in the organic phase of the micelle solution (ie, outside the micelles). The size of the nanoparticles is entirely dependent on the micelle size, which normally have a range of 20–500 nm. Synthesis of particles outside this range are not possible using the reverse micelle method. Finally, with such a large amount of organic solvent involved in making the micelles, the reverse micelle method is difficult to scale-up.

Chemical vapor condensation (CVC)

When some volatile metal compounds are heated in a inert gas atmosphere, these compounds decompose and form metal nanoparticles. This method is termed chemical vapor condensation (CVC). Metallic iron nanoparticles prepared using CVC mechanism have been reported (Choi et al 2001). In this work, iron carbonyl, $Fe(CO)_5$, was used as iron precursor and the particle size averaged to 5–13 nm. Further oxidation of these metallic iron nanoparticles is possible. Magnetite nanoparticles of 3 to 20 nm (Sun and Zeng 2002) and maghemite nanoparticles of 4 to 16 nm (Hyeon et al 2001) were prepared by oxidation of metallic iron nanoparticles.

Although this technique produces high-quality nanoparticles specialized facilities are required. More importantly,

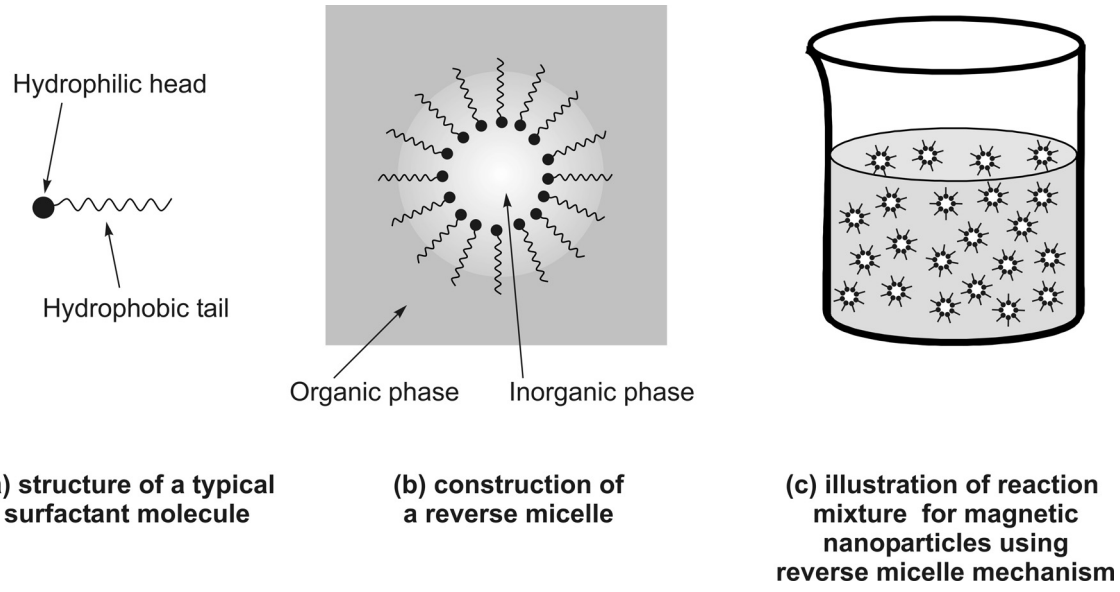

Figure 2 Illustration explaining the use of the reverse micelle mechanism in synthesizing magnetic nanoparticles.

some of the precursors such as $Fe(CO)_5$ are highly toxic (with CO as by-product) and difficult to handle.

Thermal decomposition and reduction

When metal oxy-salts (such as nitrates, carbonates and acetates) are heated to a certain temperature, they decompose to form metal oxides. For example, iron(III) nitrate decomposes to iron(III) oxide according to the following equation:

$$4Fe(NO_3)_3\ (s) \rightarrow 2Fe_2O_3\ (s) + 12NO_2\ (g) + 3O_2\ (g) \quad (2)$$

These metal oxide nanoparticles can be further reduced to metal by heating the oxides to a certain temperature under a reducing gas, usually hydrogen (H_2) or carbon monoxide (CO), following the equations:

$$MO + H_2 \rightarrow M + H_2O \quad (3)$$

$$MO + CO \rightarrow M + CO_2 \quad (4)$$

This reduction method applies to most metal oxides except those of alkaline and alkaline earth metals (Nurmi et al 2005).

With only very little solvent involved, this thermal method is popular with industry. However, control of the particle size is difficult and the same problems as wet precipitation for particle coating hampers its use in some laboratories.

Liquid phase reduction

Liquid phase reduction usually is applied to reduce magnetic or non-magnetic metal oxides to magnetic metal or metal alloy, with the use of powerful reducing agents, such as $NaBH_4$ and $LiAlH_4$. $NaBH_4$ is a particularly popular reducing agent in this area because it is soluble in both methanol and water. The reduction of metal oxides using $NaBH_4$ follows the equations in Table 1 (Morris et al 1985).

The mechanism of reduction using $NaBH_4$ can be complicated. Details of reduction for Fe, Co, Ni are available in the literature (Klabunde 1996). Magnetic alloy nanoparticles can also be formed using liquid phase reduction of mixed metal salts or oxides (Srivastava et al 2006).

Although most hydrides are moisture sensitive and difficult to handle, liquid phase reduction has some advantages over other synthetic methods. These hydrides are strong reactants so only mild conditions with standard laboratory facilities are required. Hydrides are also penetrative to some coatings, especially natural polymers so the particles can still be reduced even with "protective" coatings. However, some protective coatings may also be reduced by the hydride, including polyvinyl alcohol, polysaccharides and proteins.

Table 1 Standard redox potentials

Chemical reaction	E°/V vs NHE at 25 °C
$Fe^{2+} + 2e^- \rightarrow Fe$	−0.440
$BH_4^- + 4OH^- \rightarrow BO_2^- + 2H_2O + 2H_2 + 4e^-$	−1.73
$N_2H_4 + 4OH^- \rightarrow N_2 + 4H_2O + 4e^-$	−1.16

Magnetic core material

There are many magnetic materials available with a wide range of magnetic properties. However, many of these materials, such as cobalt and chromium, are highly toxic and unlikely to be used as biomedical agents *in vivo* without a non-toxic, protective coating with high mechanical strength. Iron oxide-based materials such as magnetite and maghemite, however, are relatively safe and are currently in use in the clinic as MRI contrast agents. The following are some magnetic materials suitable for use in biomedical applications. For a detailed review of their magnetic properties see Dobson (2007).

Magnetite Fe_3O_4

Magnetite is a common mineral which exhibits ferro (ferri)magnetic properties. Descriptions of the physical properties of magnetite are widely available (http://www.mindat.org/min-2538.html). The structure of magnetite belongs to the spinel group, which has a formula of AB_2O_4. Its ferromagnetic structures arise from alternating lattices of Fe(II) and Fe(III). This gives it a very strong magnetization compared to naturally occurring antiferromagnetic compounds such as the ferrihydrite core of the ferritin protein.

Maghemite γ-Fe_2O_3

Maghemite, a topotactic oxidation product of magnetite, has the same lattice structure as magnetite but all iron atoms are in Fe(III) oxidation state. It can be thermally transformed to other forms of iron(III) oxides such as heamitite, which is antiferromagnetic. The strong magnetization of maghemite (about 100 times stronger than hematite and ferrihydrite), which is on the order of magnetite, is due to lattice vacancies which give rise to uncompensated electron spins within the structure.

Maghemite is one of the most suitable materials for the core of magnetic nanoparticles because it is least likely to cause any health hazard. Iron (III) ions are widely found in human body so leaching of metal should not cause significant side-effects. As a result, maghemite is a popular choice for making magetic nanoparticles, especially for biomedical applications.

Iron-based metal oxides

There are many iron-based metal oxides which exhibit strong magnetic properties and can be used as magnetic cores for building the magnetic nanoparticles. Preparation procedures of mixed oxide nanoparticles such as $CoFe_2O_4$, $NiFe_2O_4$, $MnFe_2O_4$ are commonly found in the literature (Shafi et al 1998). It is worth noting that these materials have a remarkably similar spinel structure to magnetite Fe_3O_4. However, using these mixed oxide nanoparticles in biomedical research can be hampered by the high toxicity of these transition metals (Co, Ni, Mn). Non-permeable coatings are needed to prevent leaching of these metals. Other common examples of mixed oxides involve alkaline earth metals such as barium ($BaFe_{12}O_{19}$) and strontium ($SrFe_{12}O_{19}$), which belong to the magnetoplumbite-system (Pankov 2004). Again, leaching of these alkaline earth metals can cause problems in biomedical applications.

Iron alloys

Although iron metal itself is a good material for magnetic applications, it is seldom used as core material for the synthesis of magnetic nanoparticles unless they are coated with an inert, protective coating. Iron is exceptionally vulnerable to corrosion in presence of water, ie, rusting. Robust, non-porous coatings are essential for nanoparticles with iron metal cores. Also, functionalizing the iron surface is not straightforward. Therefore, iron alloys, such as FePt and FeAu, are more popular as core materials for magnetic nanoparticles.

Other materials

Other possible core materials for magnetic nanoparticles include rare earth metal alloys and transition metal clusters. The use of these materials for magnetic nanoparticle core synthesis is still rare due to their potential toxic effects on the human body.

Coating materials

Nanoparticles are more reactive than bulk materials due to their high surface to volume ratio (Klabunde 1996). As a result, these magnetic core nanomaterials need to be protected against corrosion. This coating also prevents the leaching of potentially toxic components into the body during *in vivo* applications. There are many choices of coating materials. One has to consider the nature of the coating and the ease of further functionalization to suit specific applications.

Natural polymers

Coating magnetic nanoparticles with natural polymers such as carbohydrates and proteins is common (Schroder et al 1986; Berry et al 2003; Nitin 2004; Ito et al 2005; de la Feuten and Penades 2006; Liang et al 2006; McDonald and Watkin 2006). Many natural polymers are biocompatible and therefore suitable for coating nanoparticles for biomedical applications. Table 2 shows some examples of magnetic nanoparticles with natural polymer coatings for biomedical applications.

Carbohydrates are particularly popular as coating materials for magnetic nanoparticles because of their biocompatibility. For example, dextran-coated magnetic nanoparticles have been

Table 2 Properties of natural and synthetic polymers for coating magnetic nanoparticles

Polymer	Hydrophobicity	Applications	Reference
Natural polymers			
Carbohydrates:			
Dextran	Hydrophilic	Drug delivery	Yuan et al 2006
		Radioimmunoassay	Li et al 1996
		MR imaging	Morales et al 2003
		Hyperthermia	Jordan et al 1999
Starch	Hydrophilic	Tumur targeting, MR imaging, x-ray imaging	Alexiou et al 2001
Proteins:			
Albumin	Hydrophilic	MR imaging	Roser et al 1998
RGD	Hydrophilic	Fluorescent imaging and MR imaging	Montet et al 2006
Lipids	Hydrophobic	Immunoassay	Matsunaga and Takeyama 1998
Synthetic polymers			
Poly(ethyleneglycol) (PEG)	Hydrophilic	MR imaging	Kohler 2005
			Veiseh et al 2005
			Kohler 2005
		Drug delivery	Gupta and Curtis 2004
Polyvinyl alcohol (PVA)	Hydrophilic	Drug delivery	Schulze et al 2005
			Schulze et al 2006

used in many biomedical applications such as cancer treatment (Subramani 2006) and MRI (Chouly et al 1996), and they are commercially available. Functionalization is also possible by making use of the hydroxyl groups on the carbohydrate skeletons (Heinze et al 2006). In order to diversify the surface properties, dextran has also been used as a blend with other polymers (including chitosan, poly-L-latic acid, and silica) to form blended coatings for magnetic nanoparticles (Grüttner et al 2001).

Unfortunately, some of these natural polymer coating materials are water soluble and lack mechanical strength. Cross-linking is needed to prevent them from breaking down in water but they are still mechanically weak. Also, these coatings tend to be porous and sometimes show non-selective adsorption (Markovic 2006).

Synthetic organic polymers

Since many natural polymers lack mechanical strength while others, such as cellulose, are too rigid to be manipulated to coat nanoparticles, synthetic polymers may provide a solution to this problem. Synthetic polymers such as poly(ethyleneglycol) (PEG) (Nitin et al 2004), polyvinyl alcohol (PVA) (Godovsky 1999; Qiu and Winnik 2000) and poly-L-lactic acid (PLA) (Mikhaylova 2004; Mertz et al 2005) are some examples or coatings for magnetic nanoparticles. The choice of synthetic polymer coating depends on the required surface properties for particular applications (Table 1).

One interesting example is PVA coating, which has the structure shown in Figure 3. The hydroxyl groups (-OH) on the polymer skeleton ensure the hydrophobic property of the coating, which resembles the surface chemistry of carbohydrates such as dextran. The use of PVA-coated magnetic nanoparticles in biomedical applications has been reported (Schulze et al 2006). This research group has demonstrated the internalization of PVA and PVA co-polymer-coated magnetic nanoparticles by synoviocytes and by cells of the synovial membrane in sheep (Schulze et al 2005).

Although synthetic polymers have better mechanical strength than many natural polymers, some coatings formed

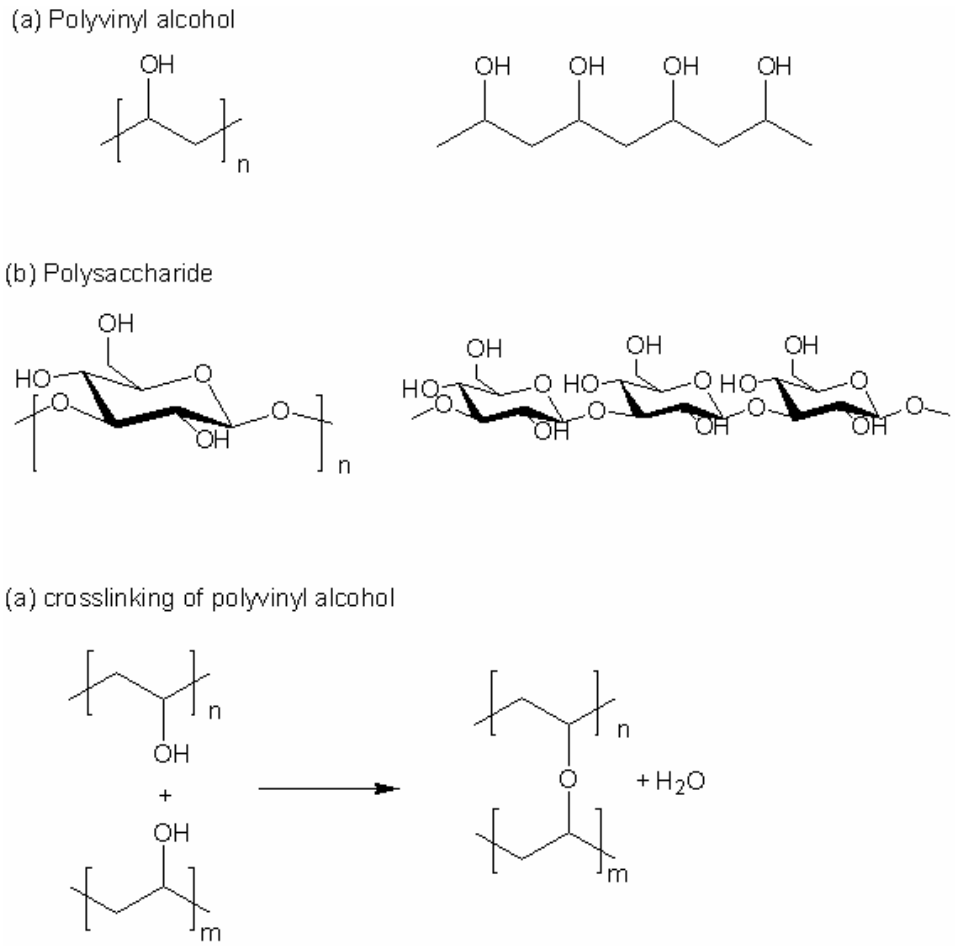

Figure 3 The structure of PVA (**a**) which is compared with a polysaccharide (**b**) as both materials have abundant hydroxyl groups on surface. The crosslinking reaction is shown in (**c**).

from synthetic polymers are still porous on molecular scales, which means that corrosion of the magnetic core is still possible. Also, some of these polymers are difficult to further functionalize. For example, PEG is a polyether and has no apparent site for simple organic functionalization, except the end groups. Linking active biomolecules with organic linkers becomes difficult.

Silica

Silica is an amorphous material with high mechanical strength. It carries negative charges at pH <3 because of the silanol groups (–Si–OH) on the surface. In order to alter the surface chemistry of silica, silylation can be carried out following scheme 1 with use of functional alkoxysilane, such as aminopropyltriethoxysilane, or ATPES (X = NH_2 in Figure 4). Techniques for functionalizing silica surfaces for various biomedical applications are widely available in literature (Yiu et al 2001; Yiu and Wright 2005).

Coating silica on iron oxide particles can be difficult as its amorphous structure prohibits silica from forming a homogeneous layer on the surface of the iron oxide. It normally results in the formation of silica spherical particles on the iron oxide surface with size comparable to the iron oxide nanoparticles. Hence, the overall particle size and shape are hard to control without structural directing agents such as surfactants (see section 2.1.2) In general, silica coating is carried out as the hydrolysis of tetraethyl orthosilicate (TEOS, also known as tetraethoxysilane) at a certain pH (8–10) or the neutralization of silic acid.

Gold

Gold is one of the most commonly used materials for bioscience interfaces (Hu et al 2006; You et al 2006). It is not only very stable but also easily functionalized via thiol linkers (–SH) (Bertilsson and Leidberg 1993). Figure 5 shows how gold-coated magnetic nanoparticles can be functionalized with thiol linkers. It is well-known that thiols, and many other sulphur compounds, have high affinity to the gold surface. Scientists have been exploiting this phenomenon in biotechnology, such as binding antigens for immunoassay (Ameur et al 2000; Susmel et al 2000).

Gold-coated magnetic nanoparticles were first reported in 2001 when Lin et al (2001) prepared so-called "Fe@Au" (gold-coated iron) nanoparticles (18–80 nm in diameter) via the reverse micelle mechanism. The iron metal nanoparticles were prepared inside the micelle followed by gold coating. To avoid aggregation, 1-dodecanethiol ($C_{12}H_{25}SH$) was bound to the gold surface of the nanoparticles through a self-assembly mechanism. These gold-coated nanoparticles can be functionalized for binding biomolecules by using thiol linkers with a functional group (such as amine) at the other end of the molecules.

Organic linkers

Without surface modification, biomolecules may not bind to the magnetic nanoparticles. Even if they do, the interaction between biomolecules and the surface of nanoparticles can be very weak, resulting in the instant release of these molecules during the delivery with little control. As a result, surface modification is necessary to create strong interactions to enhance the binding process of biomolecules and also to control the release mechanism.

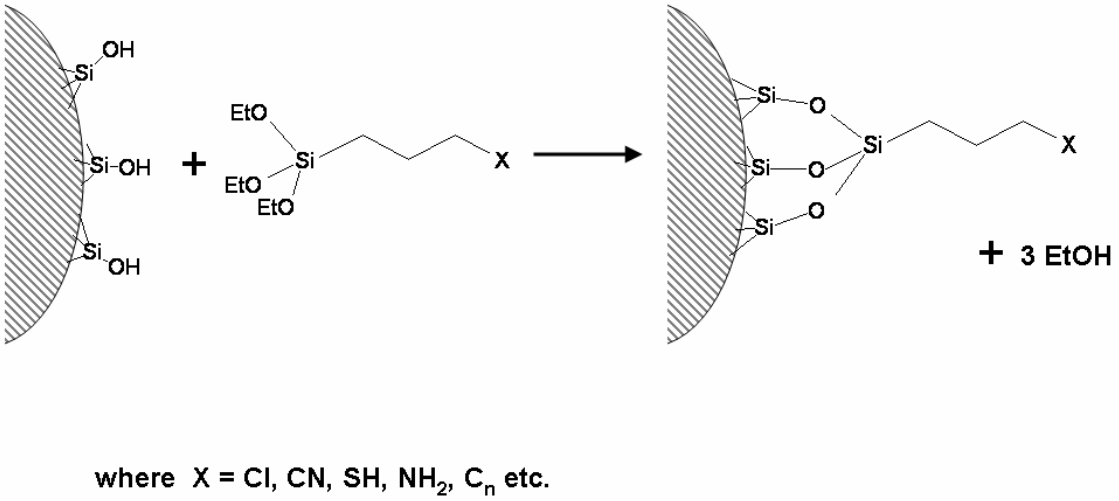

Figure 4 Silylation of a silica surface using triethoxysilane.

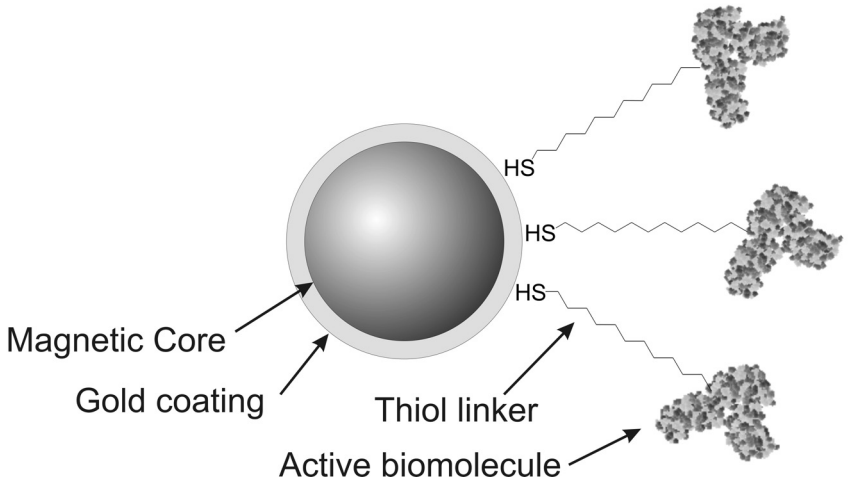

Figure 5 Illustration of the use of thiol linkers for binding biomolecules onto gold coated magnetic nanoparticles.

Modification through organic linkers is commonly used, as organic linkers provide a wide range of surface properties to suit various biomolecules in many conditions. Common organic linkers are listed in Table 3. Among these organic linkers, those creating electrostatic interactions are the most popular as the binding force is relatively easy to manipulate, usually by the addition of ions or altering the pH of the media. In other cases, catalytic or redox reactions may be involved for the releasing process.

For the application of magnetic nanoparticles in gene and drug delivery, the most suitable surface is that which is strongly positively charged. For gene delivery, the nanoparticles have to bind a large amount of negatively charged DNA molecules, ideally through electrostatic interactions, and release them after internalization of the nanoparticles into the cell (McBain et al 2007).

For drug delivery, although not all drugs are negatively charged, there are large numbers of common drugs that carry carboxylic acid groups in their molecular structure, such as ibuprofen and aspirin. These drugs are likely to bind strongly to nanoparticles with a positively charged surface. Once these drug-nanoparticle complexes reach the target organs, the drug molecules will be released in the presence of anions (such as chlorides and phosphates). In other cases, the binding strategy has to be considered individually.

It is worth noting that organic linkers can be built up on top of each other through organic reactions if necessary. For example, aldehyde is a commonly used functional surface for binding protein but it is usually built from reacting glutardialdehyde with a primary amine surface (Wong 1993). However, it is advisable to keep the surface simple. If a multiple linker system is built on the nanoparticles,

Table 3 Properties of organic linkers and their applications

Linkers	Formula	Property	Target biomolecules	Examples
Amine	-NH_2	Positively charged	Drugs	Ibuprofen Aspirin DNA
	-NHR	-NH_2 can form amide bond with – COOH	Proteins	
	-NR_2		DNA molecules	
Carboxylic acid	-COOH	Negatively charged	Proteins	Lysozyme
		Can form amide bond with –NH_2		Antibodies
		Can form ester bond with –OH		
Aldehyde	-CHO	Form imide bone with –NH_2	Proteins	Enzymes
Thiol	-SH	Form disulfide bridge	Proteins (with	Cytochrome c
		(-S-S-) with other thiol groups or	cystine in structure)	Trypsin
		cystine group		Lipases

their size will be increased dramatically and thus reduce the efficiency of delivery.

Magnetic nanoparticles for gene delivery

Magnetic nanoparticles have been in clinical use for a number of years, primarily as contrast enhancement agents for magnetic resonance imaging (Pankhurst et al 2003). However, in order for magnetic particles to act as effective carriers for DNA or pharmaceutical agents, the surface of the particles must first be modified to enable attachment of the target molecules, as discussed above. Molecules may be attached to the surface of the particles in a number of ways, for example by employing cleavable linkers or alternatively by utilizing electrostatic interactions between the particle surface and the therapeutic agent. Alternatively, the target molecule(s) may be incorporated into a degradable outer shell which releases the molecule as the shell is broken down.

In the first study to demonstrate targeted delivery of DNA using magnetic particles Cathryn Mah, Barry Byrne and colleagues (Mah et al 2000) at the University of Florida, coated adeno-associated virus (AAV) encoding Green Fluorescent Protein (GFP) to the surface of magnetic particles using a cleavable heparin sulfate linker. In this study, AAV2 conjugated to magnetic microspheres gave increased transduction efficiency in both C12s cells cultured *in vitro* and *in vivo* following intramuscular injection to 129/svJ mice (Mah et al 2002).

Although the use of target specific linkers undoubtedly provides an elegant approach to the attachment of target molecules it is not always possible. An alternative approach for attaching DNA to the surface of particles is to employ the electrostatic interactions between the negatively charged phosphate backbone of DNA and positively charged molecules linked to the particle surface. A popular choice for this approach is the cationic polymer Polyethyleneimine (PEI). This was among the first reported transfection agents and binds and condenses DNA due to the large number of secondary amine groups present along it's chain length (Abdallah et al 1996). In addition, PEI facilitates lysosomal release of the complex following internalisation by buffering the intralysosomal pH causing the lysosome to rupture and release it's contents (Akinc et al 2005). Since it is now understood that particle DNA complexes typically enter the cell by endocytosis through clatharin-dependent pits (Schillinger et al 2005), it is possible that this feature of PEI may remain beneficial for PEI-coated particles.

Polyethyleneimine-coated *magnetic* particles were first reported by Scherer et al in 2002 (Scherer et al 2002) and provided the first example of *in vitro* magnetic nanoparticle-mediated non-viral gene delivery. In addition to facilitating targeted gene delivery, the principle advantage of this approach is that the rapid sedimentation of the gene-particle complex onto the target area significantly reduces both the time and dose of vector to achieve efficient transfection. In their original study, Scherer et al demonstrated that association of DNA vectors with superparamagnetic nanoparticles increased the transfection efficiency of a number of commercial transfection reagents *in vitro* and enabled the duration of gene delivery to be reduced to as little as 10 minutes. Furthermore, conjugation of adenoviral vectors to the particles enabled transduction of a number of cells lines that expressed little or no Coxsackie and adenovirus receptor (CAR). This finding provided further evidence to support the idea that associating viral vectors with nano- or microparticles may extend the host tropism to non-permissive cells. Since this original study, *magnetofection* has been used to transfect a number of cell types including primary lung epithelial cells (Gersting et al 2004) and blood vessel endothelial cells (Krotz, Sohn et al 2003). These particles have also been used to successfully deliver antisense oligonucleotides (Krotz, de Wit et al 2003), and small interfering RNA (siRNA) to downregulate gene expression. In a recent study by Schilinger et al (2005) siRNA associated with magnetic particles significantly reduced retrovirally mediated expression of luciferase in Hela cells.

We have recently reported an alternative approach for synthesizing PEI coated magnetic particles based upon covalently coupling PEI to the surface of composite iron oxide, dextran silica particles using glutardialdehyde linkers. (McBain et al 2007)

To date, much of the work based upon linking DNA vectors to magnetic particles has centered upon the ability of this approach to reduce the time needed for transfection, or minimize the dose of vector. Recent work by our group has focused on improving the overall transfection efficiency of this technique by using dynamic magnetic fields produced from oscillating arrays of permanent rare earth magnets. Preliminary data from these studies suggest that this approach can improve the level of transfection >10 fold compared to static magnetic fields. We hypothesize that the oscillating fields introduce extra energy to the system which improves particle uptake. In addition, the non-linear motion of the particles as they move along the field gradient may aid tissue penetration for *in vivo* applications and help overcome the extracellular barriers (such as mucus layers) to gene delivery

that exist in some clinical targets for gene delivery such as the CF lung.

Another novel, and interesting approach to nanoparticle mediated gene delivery has recently been reported by Cai et al (2005). Termed nanotube spearing, this approach is based upon using nickel embedded carbon nanotubes coated in DNA. When the nanotubes are introduced to cells in the presence of a specifically orientated magnetic field, the nanotubes align with the magnetic flux lines as they are pulled towards the cells. This enables the nanotubes to spear the cells, pass through the membrane and deliver the target DNA, and has been successfully used to transfect a number of different cell types including Bal17 B-lymphoma, ex vivo B cells and primary neurons, whilst maintaining a high rate of cell viability after transduction.

Magnetic nanoparticles for use in drug delivery

In essence, the idea of using magnetic micro- or nanoscale particles to target delivery of therapeutic agents can be traced back to the late 1970s. Work by Widder et al (1978, 1979) employed magnetically responsive micropsheres to deliver anti tumor drugs. Since these early studies, a number of other groups have demonstrated the efficacy of this approach in numerous small animal studies (Alexiou 2000; Lubbe et al 2001).

Although magnetic targeting has been successful in a number of such studies, there remains only a small number of clinical trials to date. The first Phase I clinical trial of magnetically targeted drug delivery was performed by Lubbe and co-workers in 1996 (Lubbe, Bergemann, Huhnt et al 1996; Lubbe, Bergemann, Riess et al 1996). In this study, epirubicine was complexed to nanoparticles on the basis of electrostatic interactions between phosphate groups bound to the surface of the particle and amino sugars present within the drug. The clinical study built upon previous work in mice and rats in which two forms of treatment were studied; mechanical occlusion of the tumor with high concentrations of ferrofluid and magnetically targeted delivery of epirubicin using lower concentrations of particles. Interestingly, no LD50 could be found for the particle during these studies. In the clinical trial, of the 14 patients studied, epirubicin was effectively targeted to the tumor site in 6 patients. As with many similar *in vivo* studies the particles not attracted to the site of the tumor accumulated in the liver but appeared to produce no abberent effects.

A second clinical trial was performed in 2002 by Koda et al (2002) on 32 patients with hepatocellular carcinoma. In this study, doxorubicin hydrochloride was coupled to a magnetic particle carrier and delivered by sub selective hepatic artery catheterization. The particle-drug complex was targeted to the tumor site using an external magnetic field (500mT) and particle localization examined with MRI. Of the 32 patients studied, tumors were targeted effectively in 30. At the time the article was published, analysis of 20 of these tumors in 17 patients showed that 15 tumors had remained stable or reduced in size and only 5 had progressed.

In a similar study, a third trial performed in 2004 examined the efficacy of magnetic targeting for the treatment of four patients with hepatocellular carncinomas (Wilson et al 2004). In this study, doxorubicin linked to magnetic carriers was delivered via the hepatic artery using concurrent magnetic resonance imaging. The particles were targeted to the tumor sites by using rare earth magnets placed on the body surface. The results suggested that the particle/drug complex was well focused to the tumor sites with between 64 and 91% of the tumor volume affected by the drug.

Conclusions

Though progress in clinical applications of magnetically targeted carriers has been slow since first introduced in the 1970s, the potential for this technique remains great. Rapid developments in particle synthesis have enabled the use of new materials for more efficient capture and targeting and novel strategies are being developed for applying magnetic fields which could lead to treatments for diseases such as cystic fibrosis and localized cancerous tumors. Though clinical trials are few, the results have been promising. While magnetic targeting is not likely to be effective in all situations, with further development it should provide another tool for the effective treatment of a variety of diseases.

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