



## Review

# Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications

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## Abstract

Superparamagnetic iron oxide nanoparticles (SPION) with appropriate surface chemistry have been widely used experimentally for numerous *in vivo* applications such as magnetic resonance imaging contrast enhancement, tissue repair, immunoassay, detoxification of biological fluids, hyperthermia, drug delivery and in cell separation, etc. All these biomedical and bioengineering applications require that these nanoparticles have high magnetization values and size smaller than 100 nm with overall narrow particle size distribution, so that the particles have uniform physical and chemical properties. In addition, these applications need special surface coating of the magnetic particles, which has to be not only non-toxic and biocompatible but also allow a targetable delivery with particle localization in a specific area. To this end, most work in this field has been done in improving the biocompatibility of the materials, but only a few scientific investigations and developments have been carried out in improving the quality of magnetic particles, their size distribution, their shape and surface in addition to characterizing them to get a protocol for the quality control of these particles. Nature of surface coatings and their subsequent geometric arrangement on the nanoparticles determine not only the overall size of the colloid but also play a significant role in biokinetics and biodistribution of nanoparticles in the body. The types of specific coating, or derivatization, for these nanoparticles depend on the end application and should be chosen by keeping a particular application in mind, whether it be aimed at inflammation response or anti-cancer agents. Magnetic nanoparticles can bind to drugs, proteins, enzymes, antibodies, or nucleotides and can be directed to an organ, tissue, or tumour using an external magnetic field or can be heated in alternating magnetic fields for use in hyperthermia. This review discusses the synthetic chemistry, fluid stabilization and surface modification of superparamagnetic iron oxide nanoparticles, as well as their use for above biomedical applications.

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**Keywords:** Magnetic nanoparticles; MRI; Drug delivery; Surface modification; Hyperthermia; Cell labelling; Iron oxide

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## 1. Introduction

In the last decade, nanotechnology has developed to such an extent that it has become possible to fabricate, characterize and specially tailor the functional properties of nanoparticles for biomedical applications and diagnostics [1–4]. As intermediates between the molecular and the solid states, inorganic nanoparticles combine chemical accessibility in solution with physical properties of the bulk phase [5]. They are thus ideal elements for the construction of nanostructured materials and devices with adjustable physical and chemical properties [1,6]. The application of small iron oxide particles in *in vitro* diagnostics has been practised for nearly 40 years [7]. In the last decade, increased investigations with several types of iron oxides have been carried out in the field of nanosized magnetic particles (mostly maghemite,  $\gamma$ - $\text{Fe}_2\text{O}_3$ , or magnetite,  $\text{Fe}_3\text{O}_4$ , single domains of about 5–20 nm in diameter), among which magnetite is a very promising candidate since its biocompatibility has already proven [8]. Magnetite,  $\text{Fe}_3\text{O}_4$ , is a common magnetic iron oxide that has a cubic inverse spinel structure with oxygen forming an fcc closed packing and Fe cations occupying interstitial tetrahedral sites and octahedral sites [9]. The electrons can hop between  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  ions in the octahedral sites at room temperature, rendering magnetite an important class of half-metallic materials [10]. With proper surface coating, these magnetic nanoparticles can be dispersed into suitable solvents, forming homogeneous suspensions, called ferrofluids [11,12]. Such a suspension can interact with an external magnetic field and be positioned to a specific area, facilitating magnetic resonance imaging for medical diagnosis and AC magnetic field-assisted cancer therapy [13].

Nanosized particles have physical and chemical properties that are characteristic of neither the atom nor the bulk counterparts [14]. Quantum size effects and

the large surface area of magnetic nanoparticles dramatically change some of the magnetic properties and exhibit superparamagnetic phenomena and quantum tunnelling of magnetization, because each particle can be considered as a single magnetic domain [15]. Based on their unique mesoscopic physical, chemical, thermal, and mechanical properties, superparamagnetic nanoparticles offer a high potential for several biomedical applications, such as [16–19]:

- cellular therapy such as cell labelling, targeting and as a tool for cell-biology research to separate and purify cell populations;
- tissue repair;
- drug delivery;
- magnetic resonance imaging (MRI);
- hyperthermia;
- magnetofection; etc.

For these applications, the particles must have combined properties of high magnetic saturation, biocompatibility and interactive functions at the surface. The surfaces of these particles could be modified through the creation of few atomic layers of organic polymer or inorganic metallic (e.g. gold) or oxide surfaces (e.g. silica or alumina), suitable for further functionalization by the attachment of various bioactive molecules [20]. They are either dispersed through a large volume of a polymeric bead or occur as core in colloidal reagent with a biodegradable shell. As the magnetic particles accumulate, e.g., in tumour tissue, they can play an important role in detection through MRI or electron microscopic imaging to locate and measure binding or as drug carrier for certain anti-cancer drugs. The magnetic nanoparticles having suitable surface characteristics have a high potential for the use in a lot of *in vitro* and *in vivo* applications. In all cases, superparamagnetic particles are of interest because they do not retain any magnetism after removal of magnetic

field [21]. The effectiveness of the particles depends upon:

- high magnetic susceptibility for an effective magnetic enrichment [22];
- size of particles which should be monosized in the range of 6–15 nm (Particles below a critical particle size (<15 nm) would consist of a single magnetic domain, i.e. a particle that is a state of uniform magnetization at any field with superparamagnetism and high saturation magnetization values [23]. The particles in this size range are rapidly removed through extravasations and renal clearance [24].);
- superparamagnetic behaviour [21];
- tailored surface chemistry for specific biomedical applications [1]; etc.

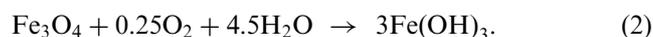
## 2. Synthesis of magnetic iron oxide nanoparticles

It has long been of scientific and technological challenge to synthesize the magnetic nanoparticles of customized size and shape. Physical methods such as gas phase deposition and electron beam lithography are elaborate procedures that suffer from the inability to control the size of particles [25–28] in the nanometer size range. The wet chemical routes to magnetic nanoparticles are simpler, more tractable and more efficient with appreciable control over size, composition and sometimes even the shape of the nanoparticles [29–31]. Iron oxides (either  $\text{Fe}_3\text{O}_4$  or  $\gamma\text{-Fe}_2\text{O}_3$ ) can be synthesized through the co-precipitation of  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  aqueous salt solutions by addition of a base [32]. The control of size, shape and composition of nanoparticles depends on the type of salts used (e.g. chlorides, sulphates, nitrates, perchlorates, etc.),  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  ratio, pH and ionic strength of the media [33,34].

Conventionally, magnetite is prepared by adding a base to an aqueous mixture of  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$  chloride at a 1:2 molar ratio. The precipitated magnetite is black in colour. The chemical reaction of  $\text{Fe}_3\text{O}_4$  precipitation is given in Fig. 1. The overall reaction may be written as follows [35,36]:



According to the thermodynamics of this reaction, a complete precipitation of  $\text{Fe}_3\text{O}_4$  should be expected between pH 9 and 14, while maintaining a molar ratio of  $\text{Fe}^{3+}:\text{Fe}^{2+}$  is 2:1 under a non-oxidizing oxygen-free environment. Otherwise,  $\text{Fe}_3\text{O}_4$  might also be oxidized as



This would critically affect the physical and chemical properties of the nanosized magnetic particles. In order to prevent them from possible oxidation in air as well as

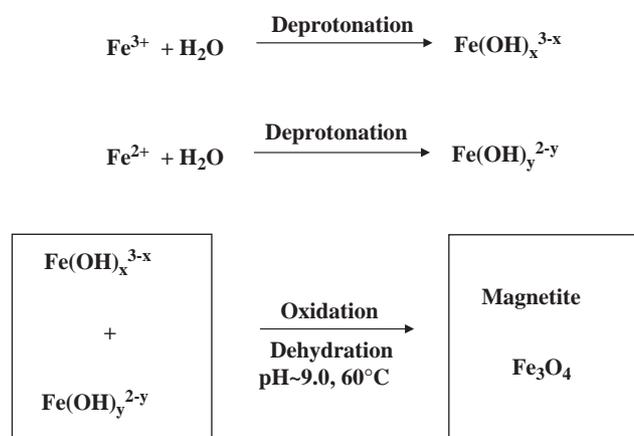


Fig. 1. Scheme showing the reaction mechanism of magnetite particle formation from an aqueous mixture of ferrous and ferric chloride by addition of a base. The precipitated magnetite is black in colour.

from agglomeration,  $\text{Fe}_3\text{O}_4$  nanoparticles produced by reaction (1) are usually coated with organic or inorganic molecules during the precipitation process. To control the reaction kinetics, which is strongly related with the oxidation speed of iron species, the synthesis of particles must be done in an oxygen-free environment by passing  $\text{N}_2$  gas. Bubbling nitrogen gas through the solution not only protects critical oxidation of the magnetite but also reduces the particle size when compared with methods without removing the oxygen [31,37].

Genesis of the particles in the solution under optimum synthetic conditions takes place by the formation of tiny crystalline nuclei in a supersaturated medium, followed by crystal growth [8]. The latter process is controlled by mass transport and by the surface equilibrium of addition and removal of individual monomers, i.e., atoms, ions, or molecules. Hereby, the driving force for monomer removal (dissolution) increases with decreasing particle size. Thus, within an ensemble of particles with slightly different sizes, the large particles will grow at the cost of the small ones. This mechanism is called Ostwald ripening and is generally believed to be the main path of crystal growth [38].

A different view of crystal growth is emerging from recent experiments by Penn and Banfield [39–41]. They observed that anatase and iron oxide nanoparticles with size of a few nanometers can coalesce under hydrothermal conditions in a way they call oriented attachment. In the so-formed aggregates, the crystalline lattice planes may be almost perfectly aligned or dislocated at the contact areas between the adjacent particles, leading to defects in the finally formed bulk crystals. They presented strong evidence that this type of crystal growth plays an important role in earth history during mineral formation. Other authors also proposed oriented attachment during crystal growth of  $\text{TiO}_2$  [42]

and for micrometer-sized ZnO particles during the formation of rod-like ZnO microcrystals [43].

Magnetite particles obtained under different synthetic conditions may display large differences regarding their magnetic properties. These differences are attributed to changes in structural disorder [44], creation of antiphase boundaries [45], or the existence of a magnetically dead layer at the particle surface [46]. The saturation magnetization ( $M_s$ ) values found in nanostructured materials are usually smaller than the corresponding bulk phases, provided that no change in ionic configurations occurs. Accordingly, experimental values for  $M_s$  (i.e. magnetic saturation) in magnetite nanoparticles have been reported to span the 30–50 emu/g range, lower than the bulk magnetite value; 90 emu/g [47]. Many studies have been reported on the origin of the observed reduction in magnetization in fine magnetic particles. The first studies on the decrease in magnetization performed in  $\gamma\text{-Fe}_2\text{O}_3$  by Coey [48] showed that this reduction is due to the existence of noncollinear spins at the surface, making the same mechanism appealing for  $\text{Fe}_3\text{O}_4$ . Also, in magnetite fine particles, Varanda et al. [49] have reported a linear correlation between saturation magnetization and particle size, suggesting that defects at the particle surface can influence the magnetic properties. The surface curvature of the nanoparticle was much larger for smaller particle size, which encouraged disordered crystal orientation on the surface and thus resulted in significantly decreased  $M_s$  in smaller nanoparticles.

The disadvantage of these bulk solution synthesis is that the pH value of the reaction mixture has to be adjusted in both the synthesis and purification steps. As a result, the production of significant quantities of narrowly dispersed, nanometer sized magnetic particles remains a significant challenge through these methods. The critical difficulty is that these particles form aggregates and grow to minimize the overall surface free energy, so that free precipitation is not a viable technique [50].

Advancement in the use of magnetic particles for biomedical applications depends on the new synthetic methods with better control of the size distribution, magnetic properties and the particle surface characteristics (see Table 3). Organized assemblies [51] or complex structures have been used as reactors [52] to obtain ultrafine magnetic iron oxide particles. Stable aqueous magnetic suspensions can also be fabricated using various saturated and unsaturated fatty acids as primary and secondary surfactants [53]. In practice, however, little control can actually be exercised over the size and size distribution of the nanostructures and, moreover, only small quantities of iron oxide can be obtained, owing to the constraints of low reagent concentrations necessitated by this synthetic procedure. A variety of other methods based on the principle of

precipitation in highly constrained domains have been developed; these include sol–gel preparation [54], polymer matrix-mediated synthesis [55], precipitation using microemulsions [56,57] and vesicles [58]. Small quantities of these materials have been produced in apoferritin cages and laboratory-grown bacteria [59].

The principle behind controlling the size of particles formed by precipitation of cations within constrained cavities can be understood as follows: If the aqueous core and particle are assumed spherical, the expected fully dense single-particle diameter upon cation exhaustion is  $d = 0.1D(M/M_w/\rho)^{1/3}$ , where  $d$  and  $D$  are particle and vesicle internal diameters,  $M$  is the internal cation molarity.  $M_w$  is the molecular weight of the product and  $\rho$  is the product density (in  $\text{g}/\text{cm}^3$ ). Particle size can therefore be controlled by varying the solution concentration and cavity size [60].

A microemulsion is defined as a thermodynamically stable isotropic dispersion of two immiscible liquids, since the microdomain of either or both liquids has been stabilized by an interfacial film of surface-active molecules [61]. In water-in-oil microemulsions, the aqueous phase is dispersed as microdroplets (typically 1–50 nm in size) surrounded by a monolayer of surfactant molecules in the continuous hydrocarbon phase [62]. When a soluble metal salt is incorporated in the aqueous phase of the microemulsion, it will reside in the aqueous microdroplets surrounded by oil. These microdroplets will continuously collide, coalesce, and break again [63]. Conceptually, when reactants A and B are dissolved in two identical water-in-oil microemulsions, they will form an AB precipitate on mixing. The growth of these particles in microemulsions can be conceptualized as a progress of interdroplet exchange and nuclei aggregation [64–66]. The finely dispersed precipitate so produced can be extracted from the surfactants.

Recently, we have utilized water-in-oil microemulsions to synthesize superparamagnetic iron oxide nanoparticles in narrow size range with uniform chemical and physical properties [29]. Highly mono-dispersed iron oxide nanoparticles were synthesized by

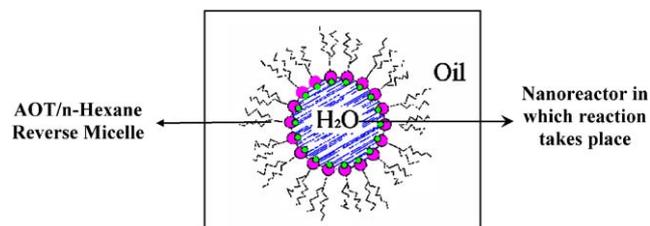


Fig. 2. Structure of reverse micelles formed by dissolving AOT, a surfactant, in *n*-hexane. The inner core of the reverse micelle is hydrophilic and can dissolve water-soluble compounds. The size of these inner aqueous droplets can be modulated by controlling the parameter  $W_o$  ( $W_o = [\text{water}]/[\text{surfactant}]$ ).

using the aqueous core of aerosol-OT (AOT)/*n*-hexane reverse micelles (w/o microemulsions) (Fig. 2). The reverse micelles have aqueous inner core, which can dissolve hydrophilic compounds, salts, etc. A deoxygenated aqueous solution of the  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  salts (molar ratio 2:1) was dissolved in the aqueous core of the reverse micelles formed by AOT in *n*-hexane. Chemical precipitation was achieved by using a deoxygenated solution of sodium hydroxide. Smaller and more uniform particles were prepared by precipitation of magnetite at low temperature in the presence of

nitrogen gas (Fig. 3) [67]. The size of the inner aqueous core of reverse micelles is in nanometer range [68], so the magnetic nanoparticles prepared inside these nanoreactors were found to be very small in size (less than 15 nm) with narrow size distribution (Fig. 4). The colloidal nanoparticles exhibit superparamagnetic behaviour with high magnetization values. The principle advantage of utilizing this type of microemulsion system for nanoparticle formation is that the size of nanoparticles can be controlled by modulating the size of aqueous micellar core [69].

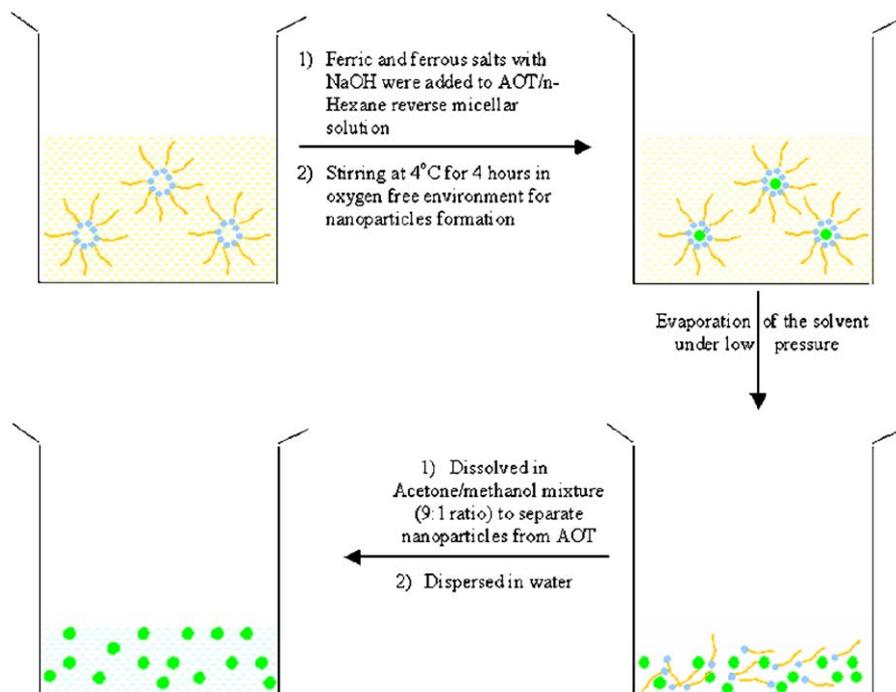


Fig. 3. Strategy of preparing highly monodispersed iron oxide nanoparticles inside the w/o microemulsion droplets. Iron salts were dissolved inside the aqueous cores of reverse micelles and precipitated using alkali solutions to get the particles of desired size.

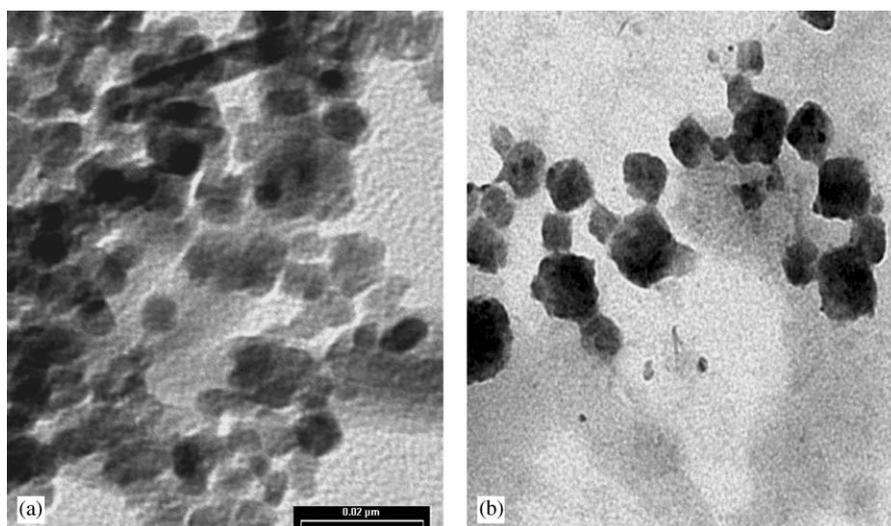


Fig. 4. Transmission electron microscopy pictures of magnetic particles prepared in (a) bulk solutions and (b) in w/o microemulsions.

Igartua et al. [70] have described the preparation of colloidal lipid particles containing magnetite from warm emulsions. A two-step method was used to obtain the spherical nanoparticles of 62 nm: (i) formulation of a transparent phase by heating an o/w emulsion formed by aqueous surfactant solution melted with a lipid phase, containing the ethyl oleate and soybean lecithin in which modified lipophilic magnetite was incorporated, and (ii) preparation of the nanoparticles by dispersing the warm transparent phase in cold water under mechanical stirring.

Yaacob et al. [60] have reported the production of magnetic nanoparticles by precipitation within spontaneous generated vesicles from mixtures of single-tailed cationic (cetyltrimethylammonium bromide (CTAB)) and anionic (dodecylbenzenesulphonic acid (DBSA)) surfactants. The CTAB/DBSA molar ratio was 7:3 and the magnetic particles were produced by gently heating the  $\text{Fe}^{2+}$  hydroxide precipitate that formed under the room temperature conditions used in the experiments. Since solution pH is an important factor controlling the stability of metal hydroxides precipitated in aqueous media [71,72], use of the acid form of the anionic surfactants affords an opportunity to tune to the pH. In another study, the same authors have shown that by using appropriate ratios of cationic to anionic surfactant in the suspension, magnetic nanoparticles can be formed directly at room temperature as a result of obtaining the “right” range of intravesicular pH [73].

### 3. Surface modification of magnetic nanoparticles for biomedical applications and their effect on stability and magnetization

In the preparation and storage of nanoparticles in colloidal form, the stability of the colloid is of utmost

importance. Ferrofluids are colloidal suspensions of magnetic particles ( $\text{Fe}_3\text{O}_4$  or  $\text{Fe}_2\text{O}_3$ ), forming magnetizable fluids that remain liquid in the most intense magnetic fields and find widespread applications. As a result of their composition, magnetic fluids possess a unique combination of fluidity and the capability to interact with a magnetic field [74–76]. In the absence of any surface coating, magnetic iron oxide particles have hydrophobic surfaces with a large surface area to volume ratio. Due to hydrophobic interactions between the particles, these particles agglomerate and form large clusters, resulting in increased particle size. These clusters, then, exhibit strong magnetic dipole–dipole attractions between them and show ferromagnetic behaviour [77]. When two large-particle clusters approach one another, each of them comes into the magnetic field of the neighbour. Besides the arousal of attractive forces between the particles, each particle is in the magnetic field of the neighbour and gets further magnetized [78]. The adherence of remnant magnetic particles causes a mutual magnetization, resulting in increased aggregation properties.

Since particles are attracted magnetically, in addition to the usual flocculation due to Vander Waals force, surface modification is often indispensable. For effective stabilization of iron oxide nanoparticles, often a very high requirement of density for coating is desirable. Some stabilizer such as a surfactant or a polymer is usually added at the time of preparation to prevent aggregation of the nanoscale particulate. Most of these polymers adhere to surfaces in a substrate-specific manner [79]. Scheme showing different strategies for fabrication and surface modification of magnetic iron oxide nanoparticles is shown in Fig. 5.

However, one should be careful about choosing the coating materials for the nanoparticles. Nanoparticle coatings may be comprised of several materials

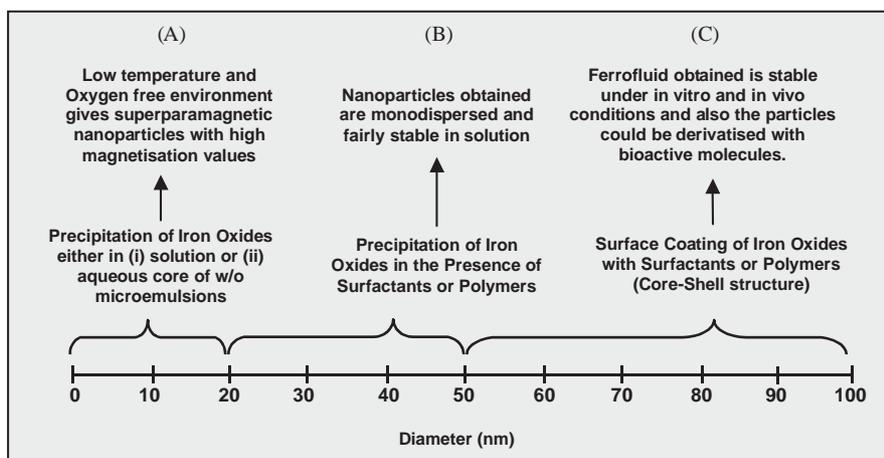


Fig. 5. Scheme showing different strategies for fabrication and surface modification of magnetic iron oxide nanoparticles. Smaller and more uniform nanoparticles can be prepared inside the aqueous droplets of reverse micelles. Also, the particles below 100 nm can evade RES and have long blood circulation times.

Table 1

Different polymers/molecules which can be used for nanoparticle coating to stabilize the ferrofluids and also for other biological applications

Polymers/molecules	Advantages	References
Polyethylene glycol (PEG)	Non-covalent immobilization of PEG on the surface improves the biocompatibility, blood circulation time and internalization efficiency of the nanoparticles	[29,198,215]
Dextran	Enhances the blood circulation time, stabilizes the colloidal solution	[20,102]
Polyvinylpyrrolidone (PVP)	Enhances the blood circulation time and stabilizes the colloidal solution	[249]
Fatty acids	Colloidal stability, terminal functional carboxyl groups	[95]
Polyvinyl alcohol (PVA)	Prevents coagulation of particles, giving rise to monodisperse particles	[250]
Polyacrylic acid	Increase the stability and biocompatibility of the particles and also helps in bioadhesion	[251]
Polypeptides	Good for cell biology, e.g. targeting to cell	[222,247]
Phosphorylcholine	Poorly complement and coagulation activating, colloidal solution stabilizer	[91]
Poly (D, L- lactide)	Biocompatible, low cytotoxicity	[85]
Poly( <i>N</i> -isopropylacrylamide) (PolyNIPAAAM)	Thermosensitive drug delivery and cell separation	[252]
Chitosan	A natural cationic linear polymer that is widely used as non-viral gene delivery system, biocompatible, hydrophilic, used in agriculture, food, medicine, biotechnology, textiles, polymers, and water treatment	[253]
Gelatin	Used as a gelling agent, emulsifier hydrophilic, biocompatible, natural polymer	[254]

including both inorganic and polymeric materials [80–82]. Polymeric coating materials can be classified into synthetic and natural. Polymers based on poly(ethylene-*co*-vinyl acetate), poly(vinylpyrrolidone) (PVP), poly(lactic-*co*-glycolic acid) (PLGA), poly(ethyleneglycol) (PEG), poly(vinyl alcohol) (PVA), etc. are typical examples of synthetic polymeric systems [83–85]. Natural polymer systems include use of gelatin, dextran, chitosan, pullulan, etc. [86–90]. Various surfactants, e.g. sodium oleate, dodecylamine, sodium carboxymethylcellulose, are also usually used to enhance dispersibility in an aqueous medium [91–93]. Table 1 provides a list of materials that could be used to stabilize the nanoparticles along with their biomedical applications.

### 3.1. Surface modification with non-polymeric organic stabilizers

In order to stabilize the colloidal dispersion, Gedanken et al. studied the adsorption of alkanesulphonic and alkanephosphonic acids on the surfaces of amorphous Fe<sub>2</sub>O<sub>3</sub> nanoparticles and proposed two possible bonding schemes for the phosphonate ions on Fe<sup>3+</sup>, i.e., one O or two O atoms of the phosphonate groups binding onto the surface [94]. Sahoo et al. [95] have reported the surface derivatization of magnetite by oleic acid, lauric acid, dodecylphosphonic acid, hexadecylphosphonic acid, dihexadecylphosphonic acid etc. to stabilize the nanoparticles in organic solvents. They found that alkyl phosphonates and phosphates could be used for obtaining thermodynamically stable dispersions of magnetic nanoparticles. The authors suggested on the basis of the results obtained from the temperature and enthalpy desorption studies that these ligands form a

quasi-bilayer structure with the primary layer strongly bonded to the surface of nanoparticles.

The ferrofluids, frequently dispersed in hexadecane (HD: C<sub>16</sub>H<sub>34</sub>) as the carrier medium, may be stabilized by various long-chain surfactants, the classic example being oleic acid, (CH<sub>3</sub>(CH<sub>2</sub>)<sub>7</sub>CH=CH(CH<sub>2</sub>)<sub>7</sub>CO<sub>2</sub>H), which has a C18 (oleic) tail with a *cis*-double-bond in the middle, forming a kink. Such kinks have been postulated as necessary for effective stabilization, and indeed stearic acid, (CH<sub>3</sub>(CH<sub>2</sub>)<sub>16</sub>CO<sub>2</sub>H), with no double-bond in its C18 (stearic) tail, cannot stabilize ferrofluid suspensions [96–98]. This is a puzzle in so far as molecules such as C<sub>9</sub>H<sub>18</sub>=C<sub>9</sub>H<sub>18</sub> and *n*-octadecane (C<sub>18</sub>H<sub>38</sub>), which are identical in structure to the tails of the respective surfactants, are both readily soluble in HD and so would be expected to provide similar stabilizing properties.

Oleic acid and stearic acid are similar surfactants which, however, lead respectively to stability and to precipitation of ferrofluid suspensions: to understand this, the forces between layers of oleic-like surfactants and between layers of stearic-like surfactants across a hexadecane (HD) medium were measured using a surface force balance (SFB), the force versus distance profiles between layers of oleic-tailed and of stearic-tailed surfactants, and the wettability of these layers by HD [98]. Using SFB, the surface separations can be measured to an absolute accuracy of 1–2 Å in the range from contact to several thousand angstroms by multiple beam interferometry. The two mica sheets are mounted on cylindrical quartz lenses in a crossed-cylinder configuration (inset). The distance *D* between them is measured to ±1–2 Å via optical fringes arising from interference of white light passing through the sheets. SFB can measure normal and shear stresses that are a

factor of some 5000–10,000 smaller than can be achieved with conventional scanning probe methods. The surfaces are separated by four molecular layers of cyclohexane, and when the shear force exceeds the frictional force the surfaces slide by a stick-slip mechanism as the thin films undergo consecutive melting and freezing [99,100]. Separate measurements revealed that only the oleic layers are solvated by HD, while the SFB results revealed that for both surfactants, a marked net attraction is present between the surfaces. Simple considerations based on these observations explain why, despite this attraction, ferrofluid dispersions are stabilized by oleic but not by stearic surfactants [98]. Because use of polymers leads to thick surface layers, Portet et al. have [101] developed monomeric organic molecules as coating materials. The main property of these small molecules is to produce a homogeneous coating of the entire iron oxide core that is able to inhibit the protein absorption. Phosphorylcholine (PC)-derived polymers are known to protect prosthesis against protein contamination, but pure PC coatings do not allow colloidal stability at physiological pH [91].

### 3.2. Surface modification with polymeric stabilizers

Polymeric coatings on magnetic nanoparticles offer a high potential in several areas of applications. Precipitation of inorganic particles in a cross-linked polymer matrix or network of gel often prevents coagulation of particles, giving rise to monodisperse particles [102,103]. The pioneering work of Ugelstad et al. [104], based on the preparation of hydrophobic monosized polystyrene magnetic particles, has stimulated the research in this domain. The methodology used is basically based on direct precipitation of iron salts inside in the pores of the porous polystyrene seed. The particles obtained exhibit large particle sizes (i.e. 2.8 and 4.5  $\mu\text{m}$ ) with a good magnetic separation. The hydrophilic magnetic latexes have been first reported by Kawaguchi et al. [105] using acrylamide as the initial monomer. The second type of hydrophilic magnetic nanoparticles has been reported by Sauzedde et al. [106,107] using Furusawa et al.'s methodology [108]. The hydrophilic temperature-sensitive latexes have been obtained by encapsulating adsorbed iron oxide nanoparticles onto oppositely charged polystyrene-core/poly(*N*-isopropylacrylamide) shell. The encapsulation has been performed using water-soluble monomers such as *N*-isopropylacrylamide, *N*-*N'* methylene bis acrylamide, itaconic acid, etc. The final particles exhibited thermal-sensitive property. In addition, various original methods (via non-conventional polymerization) have been investigated using natural polymers or proteins. In this domain, an interesting paper has been reported by Chatterjee et al. [109], such as cross-linked albumin

magnetic microspheres and their uses in the separation of red blood cells from whole blood.

Lee et al. [110] have modified nanoparticle's surface with PVA by precipitation of iron salts in PVA aqueous solution to form stable dispersion. They found that the crystallinity of the particles decreased with increasing PVA concentration, while the morphology and particle size remained almost unchanged. Recently, self-assembly of ligand-stabilized nanoparticles with size of a few nanometer into two- and three-dimensionally ordered arrays has been reported [111]. In these experiments, however, self-assembly is mainly driven by the interactions of the organic ligands rather than by the interaction of the particle cores. Contrarily, self-assembled oriented attachment was only observed for ligand-free nanoparticles.

For better dispersion, magnetite particles are often modified after precipitation [112]. In a recent publication, we have shown that the synthesis of hydrophilic magnetic polymeric nanoparticles with magnetite core and polymeric shell is possible using an inverse microemulsion polymerization process [29]. The strategy of utilizing inverse microemulsion approach to modulating the surface of magnetic nanoparticles with PEG is based on the following prior observations: (i) Preparation of hydrophilic nanoparticles is possible in the aqueous cores of reverse micellar droplets. (ii) The size of the particles can be modulated down to 10 nm diameter by regulating the size of the aqueous core of reverse micelles. (iii) Since the cross-linking and polymerization reactions take place in the aqueous core of reverse micelles, it is possible to coat the magnetic particles inside these nanoreactors [113]. The results obtained have demonstrated that the inverse microemulsion is a superior method over other bulk precipitation methods to synthesize magnetic polymeric nanoparticles with a good control over iron oxide amount and magnetic properties.

Saturation magnetization values of magnetite depend on the temperature and surface characteristics of the nanoparticles. It is well known that above blocking temperature ( $T_B$ ), superparamagnetic nanoparticles become thermally unstable and the magnetization values decrease exponentially as  $MV/kT$  become larger than 1 [114], where  $M$  is the magnitude of magnetization,  $V$  is the particle volume,  $k$  is the Boltzmann constant and  $T$  is absolute temperature. The superparamagnetic particles deflect uniquely to the strong field side of the gradient magnet, but this behaviour decreases as a function of increasing temperature. When the particles are chemically coated with sodium oleate, the blocking temperature is suppressed to lower temperature. In the absence of any coating, due to the increase in the large ratio of surface area to volume, the attractive force between the nanoparticles increases and agglomeration of the nanoparticles takes place. These agglomerated

nanoparticles act as a cluster, resulting in an increase in the blocking temperature [115]. In contrast, surface-coated particles are more freely aligned with the external field than the uncoated nanoparticles. The repulsive force between hydrophobic surfactant molecules coated on single particles can prevent them from agglomeration [31]. The total effective magnetic moment of such coated particles is found to decrease, which is most likely due to a non-collinear spin structure originated from the pinning of the surface spins and coated surfactant at the interface of the nanoparticles.

When considering the coating materials for drug delivery applications, it is usually required that particles should have sufficient hydrophilic surfaces and size less than 100 nm so that they can evade the reticuloendothelial system. However, nanoparticles have large surface area/volume ratios and tend to agglomerate and adsorb plasma proteins. When the nanoparticles agglomerate, or are covered with adsorbed plasma proteins, they are quickly cleared by macrophages in the reticuloendothelial system before they can reach target cells [116]. One possible approach, therefore, to increasing the circulation time of nanoparticles in the blood stream is to coat the particles with hydrophilic polymers such as PEG to disperse them and minimize or eliminate the protein adsorption [117].

### 3.3. Surface modification with inorganic molecules

Metallic core shell types of iron oxide nanoparticles have been investigated by several researchers. These nanoparticles have inner iron oxide core with an outer metallic shell of inorganic materials. The iron oxide nanoparticles have been coated with silica, gold or gadolinium, etc. These coatings provide not only the stability to the nanoparticles in solution but also helps in binding the various biological ligands at the nanoparticle surface for various biomedical applications. Chen et al. [118] synthesized two kinds of gold-coated iron-based particles: acicular and spherical, and studied the effect of heat treatment in acid on the coercivity and saturation magnetization of the particles. The gold-coated acicular particles had initial coercivities very close to those of the uncoated sample, and the changes after heating in acid were small. The relative saturation magnetization after treatment was higher than for the uncoated particles, indicating at least partial passivation of the Fe. The uncoated spherical iron-based particles were superparamagnetic at room temperature, but the Au-coated spherical particles had a very small coercivity due to slight oxidation during the coating process. While the relative magnetization decreased, the particle moment was still greater than would be expected for pure iron oxide.

The Au also provides a good surface for subsequent functionalization with chemical or biological agents

[119]. The Au coating is not sufficiently thick to keep the particles from aggregating, though. Ionic capping ligands, which bind to the particles' surface, must also be added during nanoparticle synthesis. The ligands' electrostatic charge causes the particles to repel, countering the magnetic attraction pulling them together. The larger the magnetic particle, the stronger the force it can exert against blood flow when delivering its pharmaceutical tag. But particles must be sufficiently small to rule out any risk of clogging small capillaries, which could be just a few microns wide. Magnetic nanoparticles designed for drug delivery must also be completely biocompatible. Iron oxide particles are known to be non-toxic, and are eventually broken down to form blood haemoglobin. Au-coated ferromagnetic particles are a slightly trickier issue. The small amounts of Au would likely pass through the body eventually, and any Fe would also be metabolized [119]. Carpenter [120] prepared metallic iron particles coated by a thin layer of gold via a microemulsion. The gold shell protects the iron core against oxidation and also provides functionality, making these composites applicable in biomedicine. Zhou et al. [121] prepared gold-coated iron core-shell structure nanoparticles (Fe/Au), synthesized using reverse micelles characterized by transmission electron microscopy (TEM). The average nanoparticle size of the core-shell structure is about 8 nm, with about 6 nm diameter core and 1–2 nm shell. The magnetic measurement of the nanoparticles also proved successful synthesis of gold-coated iron core-shell structure. The nanoparticles were then assembled under 0.5 T magnetic field and formed parallel nanobands about 10  $\mu\text{m}$  long.

Several authors have reported the magnetic iron oxide nanoparticles coated with silica [122–126]. An advantage of having a surface enriched in silica is the presence of surface silanol groups that can easily react with alcohols and silane coupling agents [122] to produce dispersions that are not only stable in non-aqueous solvents but also provide the ideal anchorage for covalent bounding of specific ligands. The strong binding makes desorption of these ligands a difficult task. In addition, the silica surface confers high stability to suspensions of the particles at high volume fractions, changes in pH or electrolyte concentration [123]. Recently, Tartaj et al. have prepared submicronic silica-coated maghemite hollow and dense spheres with a high loading of magnetic material by aerosol pyrolysis [124]. Silica-coated  $\gamma\text{-Fe}_2\text{O}_3$  hollow spherical particles with an average size of 150 nm were prepared by the aerosol pyrolysis of methanol solutions containing iron ammonium citrate and tetraethoxysilane (TEOS) [125].

A w/o microemulsion method has also been used for the preparation of silica-coated iron oxide nanoparticles [126]. Three different non-ionic surfactants (Triton X-100, Brij-97 and Igepal CO-520) have been used for

the preparation of microemulsions, and their effects on the particle size, crystallinity, and the magnetic properties have been studied. By using this method, magnetic nanoparticles as small as 1–2 nm and of very uniform size (standard deviation less than 10%) have been synthesized. A uniform silica coating as thin as 1 nm encapsulating the bare nanoparticles is formed by the base-catalysed hydrolysis and the polymerization reaction of TEOS in the microemulsion.

A potential hurdle for molecular imaging with paramagnetic MR contrast agents (e.g. iron oxides) entails the anticipated sparseness of the molecular epitopes on targeted cell surfaces (e.g., nanomolar concentrations), in concert with the modest signal intensity from conventional paramagnetic contrast agents, which may result in insufficient contrast-to-noise ratio (CNR) for diagnostic imaging. However, incorporating gadolinium in the nanoparticles can solve the problems associated with iron oxide nanoparticles. For targeted paramagnetic particles carrying high payloads of gadolinium, it is possible to quantify molecular epitopes present in picomolar concentrations in single cells with routine MRI [127]. Ferrofluids containing gadolinium and iron particles in the size range 80–750 Å in diameter have been prepared by evaporating gadolinium and iron onto a mercury surface in an argon atmosphere at a controlled pressure [128]. Particles of gadolinium iron garnet,  $Gd_3Fe_5O_{12}$ , were prepared by an aerosol spray pyrolysis technique starting with solutions of gadolinium and iron nitrates [129]. The as-prepared particles were polydisperse solid spheres. Average diameters in the range 0.05–0.8 μm could be

obtained by variation of the initial solution concentration. Magnetic measurements showed bulk behaviour for the saturation magnetization, but the coercivity could be varied with particle size with a maximum near the single-domain size.

### 3.4. Surface functionalization with targeting ligands

Various biological molecules such as antibodies, proteins, targeting ligands, etc., may also be bound to the polymer surfaces onto the nanoparticles by chemically coupling via amide or ester bonds to make the particles target specific. The possibilities of targeting protein coatings are numerous. Some interesting ligands with regard to targeting cell surface receptors are provided in Table 2. Linker molecules such as 1-ethyl-3-(3-dimethylaminopropyl) carbodi-imide hydrochloride (EDCI), *N*-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), *N*-hydroxysuccinimide or *N,N'* methylene bis acrylamide (MBA) are usually used to attach the initial hydrophilic coated molecules to a protein coating aimed at cell surface attachment [130]. Various applications of magnetic nanoparticles derivatized with targeting ligands are discussed in the following sections.

### 3.5. Magnetic properties of iron oxide nanoparticles

Iron oxide particle materials are classified by their response to an externally applied magnetic field. Description of orientations of the magnetic moments in a particle helps to identify different types of magnetism observed in nature. The magnetic properties

Table 2

Table of selected proteins/targeting ligands that could be used for derivatizing magnetic nanoparticles for various biomedical applications

Protein/ligand	Functional activity	References
Transferrin	Widely applied as a targeting ligand in the active targeting of anticancer agents, proteins and genes to primary proliferating cells via transferrin receptors	[151,178,181,184]
Lactoferrin	Structurally similar to transferrin, acts as an anti-infective agent, a modulator of the inflammatory response and iron absorption and an immuno-regulatory protein	[31,152,154]
Transforming growth factor- $\alpha$ (TGF- $\alpha$ )	Promotes proliferation and differentiation of cells and may be important for normal wound healing	[248]
Insulin	A hormone that regulates blood glucose levels, is a small protein	[155]
Nerve growth factor (NGF)	Promotes neurite outgrowth and neural cell survival	[255]
Ceruloplasmin	Principal carrier of copper in plasma, which plays an important role in iron homeostasis and is also an effective anti-oxidant for a variety of free radicals	[31,152,154]
Pullulan	High water solubility, no toxicity, usefulness as a plasma expander, non-immunogenic, non-antigenic properties. Also, evidences for receptor-mediated hepatic uptake of pullulan in rats	[243,244]
Elastin	A cross-linked protein in the extracellular matrix that provides elasticity for many tissues	[245]
Albumin	The major serum protein, binds a wide variety of lipophilic compounds including steroids etc	[246]
Tat-peptide	Membrane-permeating peptide, enhances intracellular delivery	[171,222]
RGD peptide	Increases cell spreading, differentiation, and enhances DNA synthesis	[247]
Folic acid	Preferentially target cancer cells, poorly immunogenic, folate receptor facilitates internalization of particles	[198]

of these particles can be described by the dependence of the magnetic induction  $B$  on the magnetic field  $H$ . Some materials such as iron exhibit ferromagnetism, in that they can be permanently magnetized. In most materials the relation between  $B$  and  $H$  is linear:  $B = \mu H$ , where  $\mu$  is the magnetic permeability of the particles. Iron oxide particles exhibit paramagnetism if  $\mu > 1$ , and diamagnetism if  $\mu < 1$ . In vacuum,  $\mu = 1$ . Alternatively, the magnetic susceptibility  $\chi = \mu - 1$  is used. Hence, paramagnetic nanoparticles have  $\chi > 0$ , diamagnetic particles  $\chi < 0$ , and in vacuum  $\chi = 1$  [131] (Table 3).

One important advantage for the magnetic nanoparticle is their superparamagnetism that enables their stability and dispersion upon removal of the magnetic field as no residual magnetic force exist between the particles. Below approximately 15 nm, such particles are so small that the cooperative phenomenon of ferromagnetism is no longer observed and no permanent magnetization remains after the particles have been subject to an external magnetic field. However, the particles still exhibit very strong paramagnetic properties (hence the name of the phenomenon) with a very large susceptibility. In MR imaging, superparamagnetic particles made of iron oxide can be used as contrast agents. They strongly influence T1 relaxation and T2 relaxation, the latter depending strongly on the size and coating of the particles [132].

Particles whose unpaired electron spins align themselves spontaneously so that the material can exhibit magnetization without being in a magnetic field are called ferromagnetic particles. Ferromagnetism is a so-called cooperative phenomenon, as single atoms

cannot exhibit ferromagnetism, but once a certain number of atoms are bound together in solid form, ferromagnetic properties arise. When the ferromagnetic particles are removed from the field, they exhibit permanent magnetization. Upon field reversal, the ferromagnetic material will initially oppose the field change, but eventually most domains will have switched their magnetization vectors and the same inverse magnetization is attained. Ferromagnetic materials, which are ground down to particle dimensions smaller than a particular domain, are no longer ferromagnetic but exhibit superparamagnetism [133]. In case of paramagnetic particles, a magnetic field is altered by the magnetic materials present in it. If a particle contains magnetic moments that can be aligned in an external magnetic field, this will amplify the field. Such substances exhibit the property of paramagnetism. In contrast to ferromagnetic materials (ferromagnetism), no permanent magnetization remains in paramagnetic materials when they are removed from the magnetic field. Paramagnetism can be understood by postulating permanent atomic magnetic moments, which can be reoriented in an external field. These moments can be either due to orbiting electrons or due to atomic nuclei. The torque applied by an external magnetic field on these moments will tend to orientate them parallel to the field, which then reinforce it [131].

Sato et al. suggest that the loss of magnetization as the particle size decrease depends largely on the crystalline magnetic anisotropy energy constant,  $K$ . Smaller  $K$  constants display lower relative magnetization values [134]. Experimental analyses including microscopy,

Table 3  
Comparison of different characteristic features of the iron oxide nanoparticles fabricated through different methods

Characteristics of the iron oxide	Synthesis of iron oxide nanoparticles prepared through				
	Aerosol/vapour(pyrolysis) method	Gas deposition method	Bulk solution method	Sol-gel method	Microemulsion method
Size and size distribution	About 5–60 nm with broad distribution	About 5–50 nm with narrow size distribution	About 10–50 nm with broad size distribution	About 20–200 nm with broad size distribution	About 4–15 nm with very narrow size distribution
Morphology	Spherical	Spherical	Spherical (large aggregates)	Spherical with high porosity	Cubic or spherical (no aggregation)
Magnetization values	10–50 emu/g with desired magnetic property	>20 emu/g	20–50 emu/g with superparamagnetic behaviour	10–40 emu/g with paramagnetic behaviour	>30 emu/g with superparamagnetic behaviour
Advantages	High production rate	Useful for protective coatings and thin film deposition	Large quantities can be synthesized	Particles of desired shape and length can be synthesized, useful making hybrid nanoparticles	Uniform properties and also size of the nanoparticles can be modulated
Disadvantages	Large aggregates are formed	Require very high temperatures	Uncontrolled oxidation of magnetite to maghemite, diamagnetic contribution	Product usually contains sol-gel matrix components at their surfaces	Surfactants are difficult to remove, only a small quantities of iron oxide can be synthesized

X-ray diffraction and magnetometry studies suggest that the reduced magnetization is due to surface characteristics of nanoparticles. Specifically, the loss of magnetization may be due to the existence of a magnetically dead layer,  $\sim 1$  nm thick, caused by an asymmetric environment effect of the surface atoms [134]. Finally, a spin canting surface effect was suggested by investigations using Mössbauer spectroscopy, which appeared to be enhanced as the particle size and temperature were decreased [135].

Particles made from iron oxide usually behave differently in magnetic field depending on their size. It was reported previously by several researchers [136,137] that abrupt changes in magnetic properties take place when the size of the particles are reduced from micrometer to nanometer range. For example, particles have superparamagnetic behaviour when the size are sufficiently small (i.e. 6–15 nm) and they behave as ferromagnetic when the grain size is in micrometer range. It was shown by Chatterjee et al. [23] that the magnetic behaviour is dependent on the blocking temperature of the particles (blocking temperature is the transition temperature between the ferrimagnetic and superparamagnetic state and is directly proportional to the size of the particles), which in turn is dependent on the size of the particles. Particles with lower blocking temperature exhibited superparamagnetic properties, whereas the higher blocking temperature of the particles showed the ferromagnetic behaviour of the particles.

Despite the increase in superparamagnetic behaviour of the particles with decrease in particle size, several authors have reported a decline in the absolute saturation magnetization values when the size of the particles is reduced to less than 10 nm [138,139]. Surface modification of the iron oxide nanoparticles usually leads to the formation of a non-magnetic shell due to the formation of particle outer layer and the thickness of such a layer could be in the order of 1–20 nm [140]. The coating of particles with non-magnetic materials may result in decrease in  $M_s$  values. Gomez-Lopera et al. [141] have found that surface coverage of poly(lactide-co-glycolide) polymer on the iron oxide nanoparticles decreased the  $M_s$  of particles to about one-half of that of the pure magnetite and the initial magnetization–magnetic field dependence is steeper in this case. Voit et al. [142] also observed similar results. They studied the magnetic behaviour of superparamagnetic iron oxide nanoparticles in ferrofluids coated with different polymers such as sodium oleate, polyvinylalcohol (PVA) or starch. They found that surface coverage of the iron oxide with either of these polymer resulted in decreased saturation magnetization values of the particles. They also observed that the values of particle sizes calculated from the magnetization data are found to be lower than the values calculated by XRD and TEM measurements,

which may be attributed to a magnetically ineffective layer on the particle surface [143].

## 4. Applications of magnetic nanoparticles

### 4.1. Cellular labelling/cell separation

Cell labelling with ferro/paramagnetic substances is an increasingly common method for *in vivo* cell separation [144] as the labelled cells can be detected by MRI [145]. Most current labelling techniques utilize either of two approaches: (a) attaching magnetic particles to the cell surface [146] or (b) internalizing biocompatible magnetic particles by fluid phase endocytosis [147], receptor-mediated endocytosis [148] or phagocytosis [149]. One strategy for efficient and specific cell labelling of magnetic particles is to modify the nanoparticle surface with a ligand that is efficiently taken up by target cells via receptor-mediated endocytosis [148,150]. A variety of potential ligands have been conjugated to nanoparticle surfaces to facilitate receptor-mediated endocytosis of the particles, including monoclonal antibodies (Mabs) [149]. Targeting agents such as transferrin, lactoferrin, albumin, insulin and growth factors, etc., have been demonstrated to preferentially target cell surface, because the receptors for these ligands are frequently overexpressed on the surface of mammalian cells [151,152]. These receptors are not only cellular markers, but also have been shown to efficiently internalize molecules coupled to these receptors [152]. Furthermore, many of these ligands are stable, and generally poorly immunogenic.

In the absence of any system to inhibit endocytosis, most nanoparticles are endocytosed by cells and eventually sequestered in digestive vacuoles in the cell. Once the particles are endocytosed, they are probably removed from contact with specific cell surface receptors and become effectively ineffective. As a result of these events, the cells are at high risk of apoptosis from overload with particles. If the particles can be prevented from leaving the cell surface, they will remain in contact with their specific receptors and would be expected to leave the cell in a state of prolonged stimulation while protecting the cells from side effects due to endocytosis [153].

In recent studies [31,154,155], we have discovered a route to derivatizing superparamagnetic nanoparticles with various targeting proteins such as lactoferrin, transferrin, ceruloplasmin, etc., that bind strongly to surface receptors such that phagocytosis is inhibited (Fig. 6). We have prepared lactoferrin, ceruloplasmin and insulin derivatized superparamagnetic nanoparticles with cubic shape and size less than 20 nm having high magnetization values. These particles are characterized *in vitro* and their influence on human dermal fibroblasts

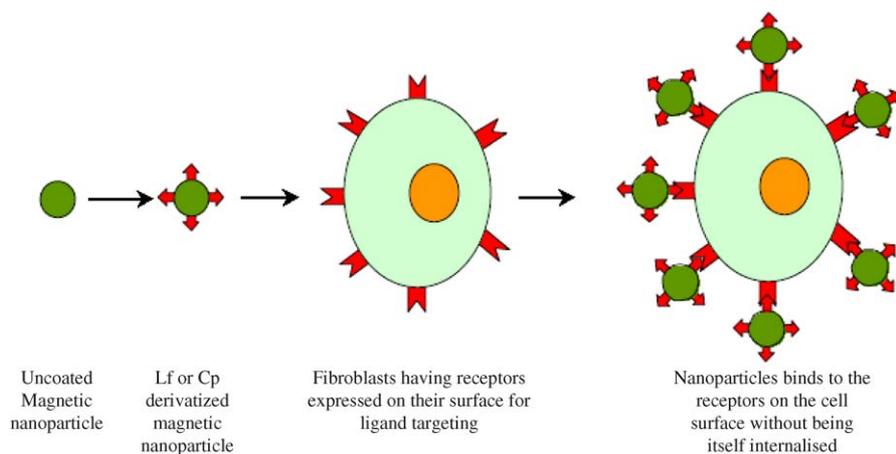


Fig. 6. Scheme of derivatization of superparamagnetic iron oxide nanoparticles either with targeting ligands such as lactoferrin (Lf) or ceruloplasmin (Cp) and their targeting to human fibroblasts. The derivatized nanoparticles act as cellular markers that are targeted at the surface receptors expressed on human fibroblasts surface without being internalized.

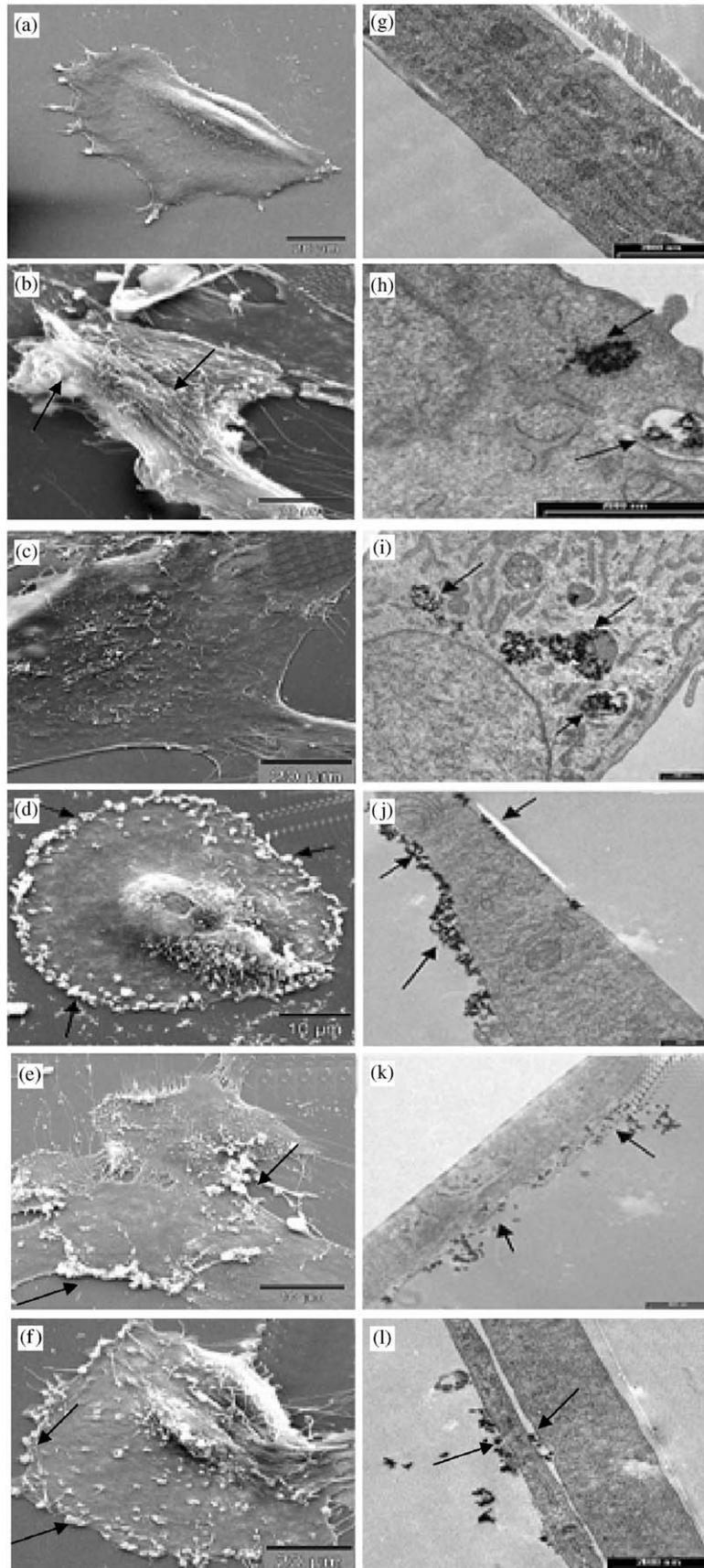
is assessed in terms of cell adhesion, viability, morphology and cytoskeleton organization using various techniques to observe cell–nanoparticle interaction, including light, fluorescence, scanning and transmission electron microscopy. The results obtained from electron microscopy studies showed that each nanoparticle type with different surface characteristics caused a distinctly different cell response (Fig. 7). The underivatized magnetic particles were internalized by the fibroblasts probably due to endocytosis, which resulted in disruption of the cell membrane and disorganized cell cytoskeleton. In contradiction, protein-coated nanoparticles attached to the cell membrane, most likely to the cell expressed receptors, and were not endocytosed. In these studies, we showed that the protein derivatization on nanoparticle surfaces makes the nanoparticles cell surface adhesive and inhibits the endocytosis. Confinement to the cell surface would provide a route that might allow removal of the particles from the cells after an appropriate residence time.

The detection of receptors for cytokines and haematopoietic factors typically of low-density associated with surfaces of cells is of great scientific interest [156]. Since cells can respond to cytokine stimulation even when the number of receptors is low, the importance of new techniques of high sensitivity for low-density receptor detection is evident. Adhesion is the basis behind many techniques for detecting immunospecific molecules on surfaces of cells and adhesive interactions can be investigated by using immunospecific particles as research tools [157,158]. Despite the predetermined affinity of probes to targets, the adhesion may be affected by steric hindrance, etc. Sometimes one-to-one binding ratio between particles and target cells is expected but only negligibly labelled cells are found [159]. The frequency of bounding effect can be evaluated by adhesion curves representing the amount

of cell-bound particles versus concentration of particles added or the number of target molecules on the surface of cells. In another approach, the cell-bound magnetic particles can be detected by measuring the magnetization of individual cells. The magnetism of cells can be determined by measuring the velocity of cells by the method of magnetophoresis in which the number of particles bound to a cell can be determined by measuring the velocity of the cell in magnetophoresis [159]. Using the method of magnetophoresis and Langmuir absorption theory, Tchikov et al. have evaluated the ligand–receptor interactions resulting from the adhesion of immunomagnetic particles to surfaces of target cells by counting the number of magnetically labelled cells [160]. As many as 200 tumour necrosis factor (TNF) receptor molecules per cell could be detected with streptavidin-coated magnetic particles coupled to biotinylated TNF as ligand. With regard to the sensitivity of the labelling systems, historically streptavidin has been the most frequently used detector for biotin because of its very high affinity constant for biotin. Sensitivity of the streptavidin–biotin complex has been shown to be greater than the antigen–antibody system [160].

However, goat anti-biotin has been shown to be a rather more sensitive detector of biotin compared to streptavidin, e.g. when conjugated to gold particles [161]. The method of magnetophoresis using these labelling systems can be of importance in detecting the cell-bound ligands, with the sensitivity comparable to the biological sensitivity of cells responding to cytokine signalling. These systems may also serve as sensitive probes for evaluating the expression of low-density receptors on cells and more generally can be employed for investigations of adhesion events in biological systems.

Recently, many cell-resistant polymeric materials and coatings have been developed and utilized for peptide



immobilization to promote highly specific cell–ligand interactions with a very low background adhesive signal from adsorbed serum and cell-borne proteins. These materials include PEG, PEG-based hydrogels, polyacrylamide, PVP, dextran and PVA, etc. [162–165].

Although PEG is highly effective in promoting a low cell-binding surface, its utility for peptide grafting is limited because each surface-immobilized macromolecule has only one site available for ligand coupling, producing low surface concentrations of ligands. On the other hand, surface-bound dextran, which has more sites for protein grafting than PEG, has been shown to reduce protein adsorption on biomaterials as well as surface-grafted PEG [166].

One recent study suggested that surface-bound dextran on biomaterial surfaces should be biocompatible because it mimics the glycocalyx on cell surfaces [167]. Another studies conducted in order to find the biocompatibility of dextran have shown that the surface-immobilized dextran on biomaterials is quite stable in most tissue environment because dextran is resistant to dextranase-mediated degradation [168]. Dextran-coated materials do not degrade enzymatically in most tissues because dextranase, the enzyme that degrades dextran, is produced by bacteria and not by tissues [169].

Wilhelm et al. [170] have modified the maghemite nanoparticles by anionic maghemite nanoparticles (AMNP) to demonstrate that it is a highly versatile system suitable either for a high-efficiency non-specific cellular uptake mediated by adsorptive endocytosis, either for specific cell recognition allowed by the nanoparticle surface modification or for binding of a specific ligand. The authors have shown that bare anionic maghemite nanoparticles, free of any dextran coating, exhibit a surprisingly high level of cell internalization that is comparable with nanoparticles modified with Tat peptide [171] or encapsulated into dendrimers [172]. They interact strongly and non-specifically with the plasma membrane due to their surface negative charge. This adsorption step, which appears to be ubiquitous, precedes the internalization step and governs the overall cell uptake. Alternatively, to induce receptor-mediated endocytosis pathway and cell-specific magnetic labelling, it is necessary to reduce the non-specific nanoparticle–cell membrane interactions and to force the recognition of the nanoparticles by the surface receptors.

Electrostatic interactions also govern the adsorption of the anionic nanoparticles onto the cell membrane [173]. It is known, however, that plasma membrane

possesses large negatively charged domains, which should repel anionic nanoparticles. Comparatively, cationic sites are scarcer on the plasma membrane, but are revealed by the possible absorption of anionic ferritin [174], smaller charged markers such as anionized hemoundecapeptide [175] and eventually negatively charged liposomes (with typical size 100 nm) [176]. Wilhelm et al. have shown that as anionized ferritin, anionic maghemite nanoparticles bind on the cell surface in the form of clusters probably because of their repulsive interactions with the large negatively charged domains of the cell surface [170]. In addition, the nanoparticles already bound on the cell surface present a reduced charge density, that may favour their aggregation with other free nanoparticles. In their studies, authors have suggested that the high efficiency of anionic nanoparticle cell uptake seems to be related first to the non-specific process of nanoparticle adsorption on the cell membrane and second to the formation of nanoparticle clusters. Non-specific adsorptive pathway can be inhibited by steric hindrance, due to the coating of the AMNP surface with albumin or with dextran [170].

The most commonly used nanoparticles are dextran coated but do not present sufficient cellular uptake to enable cell tracking, probably because of a relatively inefficient fluid-phase endocytosis pathway. One strategy was to substitute the fluid phase endocytosis pathway to the more efficient receptor-mediated endocytosis pathway by coupling dextran-coated particles with specific ligands. Significant improvements in the magnetic labelling efficiency and versatility were achieved by the attachment of a transfection agent or a small peptide, known to facilitate cell internalization on the surface of nanoparticles [177]. Some authors exploited the ubiquitous transferrin receptor to shuttle transferrin-coupled dextran-coated particles into gliosarcoma cells [178] as well as into neural progenitor cells [179]. The cell capture of transferrin-coupled nanoparticles was two to four times higher compared to the dextran-coated particles and was dependent upon the level of cell expression of the transferrin receptors. These studies demonstrated the possibility of imaging a gene expression *in vivo* using targeted superparamagnetic nanoparticles [180]. However, the uptake efficiency remained limited by the number of cell membrane receptors. Another strategy was to combine superparamagnetic nanoparticles with a transmembrane permeabilization agent, known to facilitate the translocation of a wide variety of macromolecules into cells.

Fig. 7. Scanning electron microscopy (SEM) (a–f) and transmission electron microscopy (TEM) (g–l) pictures of human fibroblasts (a, g) control; and incubated with (b, h) with uncoated magnetic nanoparticles; (c, i) PEG-coated nanoparticles; (d, j) lactoferrin-derivatized nanoparticles; (e, k) ceruloplasmin-derivatized nanoparticles and (f, l) insulin-derivatized nanoparticles. Uncoated magnetic particles show vacuoles in cell body and distorted cell membranes. PEG-coated nanoparticles were internalized in huge amounts without being toxic, while protein-coated nanoparticles were not endocytosed and were found to attach at the cellular membranes (see arrows).

Some authors [181–183] performed the graft on cross-linked dextran-coated superparamagnetic nanoparticles, of a membrane-translocating signal peptide (e.g. HIV-1 Tat protein), known to freely travel through cellular and nucleic membranes and succeed in improving the magnetic labelling efficiency. MRI imaging was used to visualize gene expression by Moore et al. [184] by using human transferrin receptor, which is an endocytic receptor, that functions to internalize transferrin that acts as marker gene.

Recently, Nam et al. [185] have developed an ultrasensitive method for detecting protein analytes using magnetic particle probes. This system is based on magnetic microparticle probes with antibodies that specifically bind a target of interest such as prostate-specific antigen (PSA) and nanoparticle probes that are encoded with DNA that is unique to the protein target of interest and antibodies that can sandwich the target captured by the microparticle probes. Magnetic separation of the complexed probes and target followed by dehybridization of the oligonucleotides on the nanoparticle probe surface allows the determination of the presence of the target protein by identifying the oligonucleotide sequence released from the nanoparticle probe. In another study, the same group has reported a method that utilizes oligonucleotides as biochemical barcodes for detecting multiple protein structures such as immunoglobulin E in one solution [186]. The approach takes advantage of protein recognition elements such as dinitrophenyl (DNP) functionalized with oligonucleotide strands and the fact that hybridization events that result in aggregation of 13 nm gold nanoparticles can significantly alter their physical properties [187].

#### 4.1.1. Detoxification of biological fluids

In an attempt to isolate living cells from biological fluids containing toxic substances by using cell surface antigens for cell nanoparticle binding, magnetic beads were coated with antibodies against epithelial surface antigens, specifically in nearly all these cases with epithelial specific antigens (ESA, clone VU-1D9) [188]. The size of these particles varied from 50 nm to a few microns, the matrix material was mostly silica and in some cases polystyrene. During incubation of a blood sample with beads coated with an epithelial specific antibody, the beads bind to the epithelial cells. The rosetted cells can then be purified by washing steps on a magnet rack. In all cases, the purity, recovery rate and condition of the isolated tumour cells depend on the number of washing steps, composition of used buffers and specification of the beads [189].

#### 4.2. Tissue repair

Tissue repair using iron oxide nanoparticles is accomplished either through welding, apposing two

tissue surfaces then heating the tissues sufficiently to join them, or through soldering, where protein or synthetic polymer-coated nanoparticles are placed between two tissue surfaces to enhance joining of the tissues. Temperatures greater than 50 °C are known to induce tissue union [190]. This is believed to be induced by the denaturation of proteins and the subsequent entanglement of adjacent protein chains [190,191]. Nanoparticles that strongly absorb light corresponding to the output of a laser are also useful for tissue-repairing procedures. Specifically, gold- or silica-coated iron oxide nanoparticles have been designed to strongly absorb light [192,193]. The nanoparticles are coated onto the surfaces of two pieces of tissue at the site where joining was desired. This technique affords methods to minimize tissue damage by using the least harmful wavelengths of light and/or lower powered light sources.

Stem cells are the body's master cells and have a unique ability to renew themselves and give rise to other specialized cell types. These cells, therefore, have the potential to be used for transplantation purposes, for example, to replace degenerated cells or repairing of a damaged tissue, providing signals so that the stem cells can yield the appropriate cell types for the development of a tissue [194]. An obstacle to developing such therapy is a lack of targeting strategies on both neural stem cells and on the signals that determine their behaviour and fate for tissue development. The superparamagnetic nanoparticles could be coupled to the cells and used to target these cells at the desired site in the body. In addition, various proteins, growth factors, etc., could be bound to these nanoparticles that might be delivered at the damaged tissue, where it would play a role in tissue development. While there is no doubt that the use of stem cells in the form of cell-based therapies offers tremendous potential for disease treatment and cures for many common diseases including diabetes, cancer, heart disease, Alzheimer's and Parkinson's disease, central to this process would be the ability to target and activate these stem cells at required sites of injury and repair using magnetic particle technology [195].

#### 4.3. Drug delivery

Another possible and most promising application of these colloidal magnetic nanoparticles is in drug delivery as carriers of drug for site-specific delivery of drugs. Ideally, they could bear on their surface or in their bulk a pharmaceutical drug that could be driven to the target organ and released there. For these applications, the size, charge and surface chemistry of the magnetic particles are particularly important and strongly affect both the blood circulation time as well as bioavailability of the particles within the body [196]. In addition, magnetic properties and internalization of particles depend strongly on the size of the magnetic particles [23]. For example, following systemic administration,

larger particles with diameters greater than 200 nm are usually sequestered by the spleen as a result of mechanical filtration and are eventually removed by the cells of the phagocyte system, resulting in decreased blood circulation times. On the other hand, smaller particles with diameters of less than 10 nm are rapidly removed through extravasations and renal clearance. Particles ranging from ca. 10 to 100 nm are optimal for intravenous injection and demonstrate the most prolonged blood circulation times. The particles in this size range are small enough both to evade RES of the body as well as penetrate the very small capillaries within the body tissues and therefore may offer the most effective distribution in certain tissues [24].

Superparamagnetic iron oxide nanoparticles of narrow size range are easily produced and coated with various polymers, providing convenient, readily targetable magnetic resonance imaging agents. Because of the large surface area to volume ratio, the magnetic nanoparticles tend to agglomerate and adsorb plasma proteins. The body's reticuloendothelial system (RES), mainly the kupffer cells in the liver, usually take up these nanoparticles due to the hydrophobic surface. Surface coverage by amphiphilic polymeric surfactants such as poloxamers, poloxamines and poly(ethylene glycol) (PEG) derivatives over the nanoparticles significantly increases the blood circulation time by minimizing or eliminating the protein adsorption to the nanoparticles [197].

In an attempt to resist the protein adsorption and thus avoid the particle recognition by macrophage cells and to facilitate the intracellular uptake by specific cancer cells for cancer therapy and diagnosis, superparamagnetic magnetite nanoparticles were surface modified with PEG by Zhang et al. [198]. By the inductively coupled plasma emission spectroscopy (ICP) measurements, the authors have shown that the uptake amount of PEG-modified nanoparticles into mouse macrophage (RAW 264.7) cells was much lower than that of unmodified nanoparticles. However, for breast cancer (BT20) cells, PEG modification facilitated the nanoparticles internalization into the cells. The possible mechanism for this uptake is that PEG can dissolve in both polar and non-polar solvents and have high solubility in cell membranes [199].

Widder et al. [200] demonstrated the utility of magnetic albumin microspheres (MM-ADR) in animal tumour models. Significantly greater responses, both in terms of tumour size and animal survival, were achieved with MM-ADR than adriamycin alone. Gupta et al. [201] demonstrated that the efficacy of magnetic microspheres in the targeted delivery of incorporated drug is predominantly due to the magnetic effects and not due to the particle's size or nonmagnetic holding. The ultrastructural disposition of adriamycin-associated magnetic albumin microspheres was also demonstrated in normal rats by Gallo et al. [202]. The transmission

electron micrographs showed extravascular transport of microspheres as early as 2 h after dosing and were observed and remained in the extravascular tissue for up to 72 h. Since the drug delivery device was retained in the vascular endothelium of the target tissue for up to 72 h, it was suggested that the microspheres might act as a depot from which the drug is released. Magnetic microspheres were designed to avoid rapid RES clearance that is problematic for other particulate carriers. Magnetic microspheres are usually injected into the arterial supply of the target organ to take advantage of first-pass organ extraction. Because these particles are smaller than 1  $\mu\text{m}$  in diameter, they are able to pass through target capillaries, prior to systemic clearance. As the magnetic particles traverse the target organ capillaries, an external magnetic field can retain the particles in small arterioles and capillaries. Retained particles may undergo extravascular uptake, which could ultimately lead to intracellular (i.e., tumour cell) drug uptake [203].

Fine ferromagnetic particles have been coated with poly(ethylene glycol)/amino or carboxyl groups to permit the covalent attachment of proteins, glycoproteins, and other ligands with the retention of biological activity. Ferromagnetic particles have also been used for various *in vivo* applications such as a tracer of blood flow, in radionuclide angiography, and for use in inducing clotting in arteriovenous malformations. Zimmermann et al. [204] were the first to propose that erythrocytes or lymphocytes containing fine ferromagnetic particles could be propelled to a desired site by an external magnetic field. It was demonstrated by Freeman and Geer [205] that iron particles could pass through capillaries when properly conditioned and later confirmed by Meyers et al. [206], who showed that iron particles could be magnetically controlled in the vascular system of experimental animals. There have been no previous investigations to examine the ability of magnetic microspheres to deliver drugs to brain tumours; however, there have been two investigations in normal rats [207]. Following administration of magnetic microspheres containing oxantrazole, the brain contained 100–400 times higher oxantrazole levels than those obtained after the solution dosage form, indicating the successfulness of drug delivery via magnetic microspheres. It was evident from these studies that under proper conditions, magnetic microspheres were capable of enhancing total brain concentrations.

A number of authors (see, e.g., Refs. [208–210]) have described the preparation of particles or liposomes containing a certain amount of magnetite or other ferrites. Some of them have focused on the field of drug transport and release: thus, the use of albumin with entrapped magnetite has been checked for the release of anti-cancer drugs like mitomicin and adriamycin [211,212]. However, albumin has the disadvantage of

provoking a possible immune response [213], so interest has been progressively focused on magnetite particles covered with a shell of polylactide/glycolide. However, the possible adverse effects of the presence of inorganic particles in the body as magnetite must also be taken into consideration. Experiments performed *ex vivo* on the toxicity of magnetite or magnetite-loaded polymeric particles have demonstrated that the latter have rather low cytotoxicity [214], and magnetite itself has much adverse effects [215]. Nevertheless, care must be taken with the particle size of the carriers: any fraction larger than 5  $\mu\text{m}$  must be avoided in order to prevent capillary blockade.

Gomez-Lopera et al. [141] have described a method for preparing colloidal particles formed by a magnetite nucleus and a biodegradable poly(DL-lactide) polymer coating. The method is based on the so-called double emulsion technique, employed to obtain polymeric spheres loaded with therapeutic drugs, to be used as drug delivery vectors. The aim of this work was to obtain, in a reproducible and rather simple way, colloidal particles that were both magnetic field responsive, and useful as drug delivery systems. In order to investigate to what extent this target is achieved, they have compared the structure, chemical composition, and surface properties of the composite particles with those of the nucleus and the coating material, simple molecules to macromolecules, cells, or other colloids. Considerable work that demonstrates that biodegradable polymers are ideal as drug carriers because of their minimum toxicity and immunological response has been performed [216–218].

The attachment of drugs to magnetic nanoparticles can be used to reduce drug doses and potential side effects to healthy tissues and the costs associated with drug treatment. Most iron oxides have a relatively short blood half-life and their primary application is for imaging of liver, spleen and the GI tract. Surface-modified iron oxide nanoparticles having long blood circulation times, however, may prove very useful for imaging of the vascular compartment (magnetic resonance angiography), imaging of lymph nodes, perfusion imaging, receptor imaging and target specific imaging [219].

#### 4.4. Magnetic resonance imaging

Superparamagnetic iron oxide nanoparticles play an important role as MRI contrast agents, to better differentiate healthy and pathological tissues. Recent developments in MR imaging have enabled *in vivo* imaging at near microscopic resolution [220]. In order to visualize and track stem and progenitor cells by MR imaging, it is necessary to tag cells magnetically. Tat protein-derived peptide sequences have recently been used as an efficient way of internalizing a number of

marked proteins into cells [221]. Lewin et al. hypothesized that biocompatible magnetic particles could be derivatized with similar sequences and that entire particles could be efficiently ferried into haematopoietic and neural progenitor cells in quantities upto 10–30 pg of superparamagnetic iron per cells [222]. Iron incorporation did not affect cell viability, differentiation, or proliferation of CD34<sup>+</sup> cells. Following intravenous injection into immunodeficient mice, 4% of magnetically CD34<sup>+</sup> cells homed to bone marrow per gram of tissue, and single cells could be detected by MRI in tissue samples. In addition, magnetically labelled cells that had homed to the bone marrow could be recovered by magnetic separation columns.

Weissleder et al. [223] have presented the evidence that transgene expression can be visualized noninvasively by MRI *in vivo*. The authors have conjugated human holo-transferrin to iron oxide nanoparticles and showed that increase in receptor levels at the cell surface can cause considerable changes in MRI signals. These superparamagnetic iron oxide nanoparticles are relatively non-toxic when administered intravenously, and similar preparations are in clinical use [224], and as the iron oxide core is biodegradable, iron oxide nanoparticle degradation theoretically will allow multiple imaging of transgene expression over time.

The availability of a universal MR marker gene to image gene expression could be particularly important in monitoring gene therapy, in which exogeneous genes are introduced to ameliorate a genetic defect or to add an additional gene function to cells, and construction and testing of such vectors is currently under way. The desired strategy can also be used to image endogenous gene expression during development and pathogenesis of disease. With advances in establishing transgenic mouse models, an animal line might be developed with an imaging marker gene under the control of a given promoter under study, so that the promoter activity can be directly visualized. The work opens an exciting avenue for developing additional and complementary strategies to image gene expression in deep organs by MRI [225].

Magnetic nanoparticles have been used to detect apoptosis by MRI by Zhao et al. [226]. Apoptosis is an active process of cellular self-destruction that plays an important role in number of disorders including neurodegenerative diseases, cerebral and myocardial ischaemia and organ rejection following transplant [227]. Therapeutic treatment of tumour cells *in vivo* results in changes in MR image contrast that are thought to reflect the morphological features of apoptosis, such as cell shrinkage and membrane blebbing [228]. The C2 domain of synaptotagmin I, which binds to anionic phospholipids in cell membranes, was shown to bind to the plasma membrane of apoptotic cells by both flow cytometry and confocal

microscopy. Administration of C<sub>2</sub>-SPION can lead to significant increases in image contrast in those regions of a tumour containing relatively large number of apoptotic cells. The authors showed that conjugation of the protein to SPION allowed detection of this binding using MRI. Specific detection of apoptotic cells using this contrast agent was demonstrated both *in vitro*, with isolated apoptotic tumour cells and *in vivo* in a tumour treated with chemotherapeutic drugs [229].

The MRI technique can detect apoptosis at an early stage in the process and has the advantages over other methods such as magnetic resonance spectroscopy (MRS) and radionuclide techniques, that it can detect apoptotic regions with relatively high spatial resolution. The SPION label is highly sensitive to MR detection and is also relatively non-toxic. SPION has been approved for clinical use as a blood pool agent for MRI [230].

#### 4.5. Hyperthermia

The use of hyperthermia (heat) in the treatment of malignant tumours is as old as medicine itself. For example, Hippocrates, the father of medicine, proposed that surface tumours should be cauterized by application of hot iron. In modern times, more advanced methods (hot water bath, pyrogens such as mixed bacterial toxins, perfusion heating, high-frequency radiation, magnetic fluid hyperthermia) were employed to heat, and hopefully destroy, tumours [231].

Magnetic induction hyperthermia, one of the therapies for cancer treatment, means the exposition of cancer tissues to an alternating magnetic field. Magnetic field is not absorbed by the living tissues and can be applied to deep region in the living body. When magnetic particles are subjected to a variable magnetic field, some heat is generated due to magnetic hysteresis loss. The amount of heat generated depends on the nature of magnetic material and of magnetic field parameters. Magnetic particles embedded around a tumour site and placed within an oscillating magnetic field will heat up to a temperature dependent on the magnetic properties of the material, the strength of the magnetic field, the frequency of oscillation and the cooling capacity of the blood flow in the tumour site. Cancer cells are destroyed at temperature higher than 43 °C, whereas the normal cells can survive at higher temperatures. Heat could be generated applying an appropriate magnetic field. The size of the magnetite crystals is submicrometric, so the powders or bulk of these biomaterials have comparable properties. These materials are not only biocompatible, but also bioactive and could be useful for bone tumours [232].

Much work using magnetic particles for hyperthermia has already been done in order to manifest a therapeutic effect on several types of tumours by performing

experiments with animals [233] or using cancerous cell cultures [234].

Choosing high-power magnetic particles combined with appropriate external magnetic field, very small amounts of magnetic fine particles in the order of tenth of milligram may easily be used to raise the temperature of biological tissue locally up to cell necrosis. It was shown that hyperthermia greatly enhances cytotoxicity of radiation and drug treatment with brain tumour cell lines, which were also confirmed by multimodel hyperthermia studies with rat, rabbits and dogs [235]. Toxicity studies revealed a maximum tolerable thermal dose of normal brain in dogs to be 44 °C, 30 min, using interstitial microwave antennas. Known side effects of hyperthermia in animal experiments are cerebral necrosis, oedema, focal haemorrhage and infarction. A breakdown of the blood–brain barrier is observed at temperatures of 42.5–43 °C, 60 min. Clinical studies performed so far have shown that interstitial brain hyperthermia is feasible and that toxicity is acceptable under careful control of the heating and limitation of the target volume.

Differential endocytosis of modified aminosilan magnetite nanoparticles into primary glioblastoma cells, but not in normal glial cells *in vitro*, has been reported previously by Jordan et al. [236]. In this study, authors observed a 10-fold higher uptake by glioblastoma cells than by normal cells. Further new aminosilan-type nanoparticles were taken up by prostate carcinoma cells but not by normal prostate cells, endothelial cells or fibroblasts *in vitro*. Preliminary data indicate that the malignant cells take up nine times more particles than normal cells. Wada et al. have proved the usefulness of dextran magnetite (DM) for the oral cancer hyperthermia. DM suspension was locally injected into the tumour-bearing tongue and tongues were heated up to 43.0–45.0 °C, by an AC magnetic field of 500 kHz. They found that the inhibition of the growth of tongue carcinoma in the four-time heating group was significantly greater than in the control group. Moreover, the survival rate was significantly higher in the heated groups than in the control group. Histological examination revealed a brown uniform DM accumulation at the stroma in the margin of the tumours. Many of the tumour cells disappeared at the site adjacent to this accumulation [237].

Heat-induced therapeutic gene expression is highly desired for gene therapy to minimize side effects. Furthermore, if the gene expression is triggered by heat stress, combined therapeutic effects of hyperthermia and gene therapy may be possible. Ito et al. [238] combined *TNF- $\alpha$*  gene therapy driven by the stress-inducible promoter, *gadd 153*, with hyperthermia using magnetite cationic liposomes (MCLs). In nude mice, MCLs induced cell death throughout much of the tumour area on heating under an alternating magnetic field. This heat

stress also resulted in a 3-fold increase in *TNF- $\alpha$*  gene expression driven by the *gadd* 153 promoter as compared with that of nonheated tumour. The combined treatment strongly arrested tumour growth in nude mice over a 30-day period, suggesting the potential for cancer treatment [238].

#### 4.6. Magnetofection

Magnetofection (MF) is a method in which magnetic nanoparticles associated with vector DNA are transfected into cells by the influence of an external magnetic field. For this purpose, magnetic particles might be coated with the polycation polyethylenimine. These complexes readily associate with negatively charged DNA since the magnetic particles are positively charged due to the polyethylenimine. Whether viral or nonviral vectors, MF has been shown to enhance the efficiency of the vectors up to several thousand times [239]. For magnetically enhanced nucleic acid delivery, MF is universally applicable to viral and non-viral vectors, because it is extraordinarily rapid, simple and yields saturation level transfection at low dose in vitro [240]. Further, since these magnetic particles do not rely on receptors or other cell membrane-bound proteins for cell uptake, it is possible to transfect cells that normally are non-permissive [239].

Krotz et al. [241] have recently used MF to enhance gene transfer to cultured primary endothelial cells. MF of human umbilical vein endothelial cells (HUVEC) increased transfection efficiency of a luciferase reporter gene up to 360-fold compared to various conventional transfection systems. In contrast, there was only 1.6-fold increase in toxicity caused by MF, suggesting that the advantages of MF outbalanced the increase in toxicity. The authors suggested that MF could be an effective tool for pDNA transfection of endothelial cells allowing high efficiencies of transfection. In another study [242], the same authors have utilized the technique of MF to enhance ODN delivery at low toxicity and procedure time in vitro and in vivo. In vitro, target knockout was assessed at protein and mRNA levels and by measuring superoxide generation after antisense MF against the p22(phox) subunit of endothelial NAD(P)H-oxidase. Antisense MF against p22(phox) significantly decreased basal and prevented stimulated superoxide release due to loss of NAD(P)H-oxidase activity by mRNA knock-out as assessed after 24 h.

#### 5. Concluding remarks and future directions

The concept of drug delivery using magnetic nanoparticles greatly benefit from the fact that nanotechnology has developed to a stage that it makes possible not only to produce magnetic nanoparticles in a very narrow

size distribution range with superparamagnetic properties but also to engineer particle surfaces to provide site-specific delivery of drugs. Magnetite due to its strong magnetic properties was used first in biology and then in medicine for the magnetic separation of biological products and cells as well as magnetic guidance of particle systems for site-specific drug delivery. The size, charge, and surface chemistry of magnetic particles could strongly influence their biodistribution. Another important point is that the magnetic properties depend strongly on the size of the magnetic particles. In the last decade, the activities in the clinical applications of magnetic carriers and magnetic particles have been very high, because the needs of better diagnostics procedures on one side and better treatment modalities are, on the other hand, strongly increasing.

The most relevant biomedical applications for these particles may be targeted at healthcare for the aged peoples and in particular at diseases of the musculoskeletal system. Many of these diseases are characterized by severe inflammation, disability and pain and the better control of inflammation is an important goal to which magnetic nanoparticles may contribute to a great extent. Precise delivery of anti-inflammatory drugs to the exact area of inflammation is a desirable end since this could lead to reduced drug dosages, elimination of side effects on the other healthy tissues and increased rapidity of action. Application of external magnetic fields to the area of inflammation, e.g. a joint, while the particles are to be targeted may provide an important component of such treatments. If successful, this type of treatment could be used to treat autoimmune diseases, direct immunosuppressive drugs to where they are needed in transplant patients and patients with tumours. Attachment of specific antibodies to the particles may allow particles to target specific cell types i.e. macrophages having receptors expressed on their surfaces. Inflammation could then be modified by using magnetic fields to focus the macrophages or divert them away from tissue if appropriate. This kind of application also includes other biological applications, e.g. cell separation, in which the improvement of the success rate is of importance for the classification and further surely handling of cells. Successful development in this area will aid the growth of the biomedical industry as well as improving the quality of life in the population.

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