CHAPTER

Magnetically based nanocarriers in drug delivery

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9.1 INTRODUCTION

In almost all of the biochemical processes we need to separate/purify biochemicals (i.e., peptides, proteins, enzymes, carbohydrates, nucleic acids, etc.). Sedimentation, centrifugation, ultrafiltration, chromatographic techniques, and electrophoretic techniques are widely used in those kind of processes (Saiyed et al., 2003; Safarik and Safarikova, 2004). But there are some difficulties, such as incomplete precipitations, non-specific seperations, chemical unstability, high investments, and/or high operational costs. Hence, scientists have focused on improving alternative novel and smart techniques for this purpose in recent decades. Magnetically responsive carrier technologies were created as alternative techniques and begun to be used in those biochemical processes. The carriers can be produced in different forms and structures (Figure 9.1). Drug-delivery systems (DDSs) are important processes, having various types of nano-sized carriers, and the scientists focused on magnetically responsive DDS in the last quarter of the twentieth century (Dobson, 2006; Misra, 2008; Duran et al., 2008; Veiseh et al., 2010a). Magnetically responsive carriers can be prepared by using magnetic micro/nanoparticles directly or any kind of carrier (especially polymeric carriers) can be modified to be magnetically responsive by loading the magnetic materials. Magnetic

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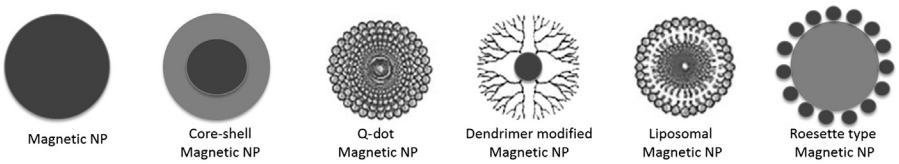

FIGURE 9.1

Common types of magnetic nanoparticles.

materials provide many benefits to the DDSs, such as targeting capability, providing contrast agent properties, especially to be used in magnetic resonance imaging (MRI) technique, and also targeted ablation therapy as hyperthermia by using excess external magnetic field during the treatment (Sun et al., 2008a).

It is possible to produce magnetically responsive carriers in the lab or it can be supplied as a commercial product in the required form from the market. The carriers are in the size range of several nm to a few mm in diameter and can be produced by using polymers, biopolymers, porous glass particles, or magnetic particles (i.e., iron oxide particles including modified forms, i.e., PEGylated) (Kohler et al., 2004). Many different types of polymeric/biopolymeric structures with different bulk and surface properties were used to produce magnetically responsive micro/nanocarriers, that is, dextran, chitosan, polyethylene imine, liposomes, phospholipids, etc. (Molday and MacKenzie, 1982; Mornet et al., 2005; Kim et al., 2007; Steitz et al., 2007; Chorny et al., 2007; McBain et al., 2007; Plassat et al., 2007; Shtykova et al., 2007; Donadel et al., 2008).

Currently, magnetically responsive nanocarriers are the most favorable in DDS research due to their unique size, which allows them to travel in all parts of the living system during treatment, especially in the case of cancer therapies (Huang and Juang, 2011). Nano-sized drug-loaded carriers called "*Nanotherapeutics*" were then created (Moorthi et al., 2011). Some of those nanotherapeutics are available in the market (i.e., Abraxane, Doxil, etc.); and others are under investigation in different phase studies (Miele et al., 2009). Those nanotherapeutics are not magnetically responsive carriers and we believe that magnetic characteristics will modulate those carriers into the smart carriers category of the DDS.

During these modulations generally iron oxide nanoparticles are used as core materials or function materials of the nanotherapeutics. These iron oxide nanoparticles are superparamagnetic materials and called SPIONs. SPIONs can be directed to a specific area thanks to their inducible magnetization and heated locally with an externally applied AC magnetic field. The magnetic properties of SPIONs make them attractive for diverse biomedical applications, such as separation techniques, MRI contrast enhancers, drug-delivery systems, magnetic hyperthermia, and magnetically assisted transfection of cells (Hofmann-Amtenbrink et al., 2009; Horák, 2005; Gupta and Gupta, 2005). In this chapter, magnetically responsive nanocarriers will be evaluated in detail.

9.2 MAGNETIC NANOPARTICLE SYNTHESIS METHODS

9.2.1 CHEMICAL SYNTHESIS OF MAGNETIC NANOPARTICLES

Several chemical-based methods, such as microemulsions, sol−gel synthesis, sonochemical reactions, hydrothermal reactions, hydrolysis and thermolysis method, flow injection synthesis (FIS), and electrospray synthesis are being used for the synthesis of magnetic nanoparticles. Control of superparamagnetic NP size, shape, and surface enables their complex synthesis procedure. First and foremost, the control of the monodispersity of magnetic nanoparticles by keeping experimental conditions well during the synthesis. A second important point is to determine the reproducible purification steps such as ultracentrifugation, size exclusion chromatography, and magnetic filtration or flow field gradient that may be needed for industrialization (Laurent et al., 2008).

9.2.1.1 Synthesis with coprecipitation technique

The coprecipitation approach is one of the simplest and most conventional ways of synthesizing magnetic nanoparticles (MNPs). Iron oxides are generally formed by this kind of application by aging the ferrous and ferric salts in aqueous media. The thermodynamics of this reaction requires the alkaline pH to be between 8 and 14 in non-oxidizing environments. Because magnetites (Fe_3O_4) are sensitive to oxidation in an oxygenated environment, they rapidly change to maghemite (γFe_2O_3). The biggest advantage of the coprecipitation technique is that very high amounts of magnetic nanoparticles can be synthesized. On the other hand, size control of the particle synthesizing is limited because of the changeable kinetic factors in the reaction due to crystal growth. There are two stages in the coprecipitation technique: firstly, concentrations of the species reach supersaturation for the formation of nucleation; secondly growth of the nuclei by solute diffusion. These two steps must be separated in order to produce monodispersed iron oxide NPs. The size and shape of the magnetic nanoparticles can be adjusted by changing pH and temperature (Laurent et al., 2008). Spherical magnetite particles between 30 and 100 nm can be synthesized by the reaction of a Fe(II) salt, a base, and an oxidant in liquid solution (Hasany et al., 2012).

In a study of Kavaz et al. (2010) by changing the Fe^{+2}/Fe^{+3} ratio, varying sizes of Fe_3O_4 nanoparticles were obtained and, in addition to Fe^{+2}/Fe^{+3} ratio, pH and ionic strength of the medium was also investigated for protein purification. Erdal et al. (2012) mentioned a study obtaining magnetic nanoparticles by changing the quantity of the alkali solution. Wu et al. (2011) synthesized 15-nm sized Fe_3O_4 nanoparticles using a method of ultrasonic chemical coprecipitation. The use of $C_{12}H_{25}OSO_3Na$ as a surfactant led to uniform dispersion and same-sized nanoparticles. Furthermore, applying ultrasound prevented aggregation of particles. In this respect, Xia et al. (2012) observed the same phenomenon (aggregation) by the help of triethanolamine ($N(CH_2CH_2OH)_3$) (TEA) as a surfactant.

9.2.1.2 Microemulsions

Nanoparticle synthesis with coprecipitation is an easy technique, resulting in a wide-ranging size of nanoparticle synthesis. Several other methods were developed for the synthesis of more uniform-sized nanoparticles. Magnetite NPs are produced by a mixture of nanoemulsion consisting of the iron source and sodium hydroxide (NaOH) (Solans et al., 2005). Acetone lysis is used for the removal of nanoparticles from the surfactant and washed with ethanol. Superparamagnetic properties and high magnetization are observed with colloidal nanoparticles. Oil and water phases contain the dissolved substances; and in addition to that, the selection of surfactant material is mainly determined by the physicochemical properties of the system (Hasany et al., 2012). Any type of surfactants (cationic, anionic, and nonionic) can be used for this method. Scaling-up the technique and adverse effects of residual surfactants may create some difficulties on nanoparticles synthesized by microemulsion methods (Ang et al., 2014). Chin and Yaacob (2007) demonstrated iron oxide NPs smaller than 10 nm by using water/oil microemulsion, which is smaller than those obtained by coprecipitation. In another study, Lee et al. (2006) showed that using iron precursor at high temperature ended up with crystalline maghemite nanoparticles. Furthermore, Sun et al. (2004) revealed that very small magnetite nanoparticles were synthesized in the same manner.

9.2.1.3 Hydrothermal and high-temperature reactions

Hydrothermal methods were used in the synthesis of Fe_3O_4 NPs and ultrafine powders (Liu et al., 2007). Reactors and autoclaves containing liquid media are subjected to 2000 psi pressure and temperature less than 200 °C and after that two routes can be followed for the formation of ferrites by hydrothermal reactions (Hasany et al., 2012). During the hydrothermal reaction process the conditions of the reaction are very important. Solvent type, temperature, and duration affect the synthesis of the products. The size of the Fe_3O_4 particles increases when the reaction duration is prolonged and higher water content aids in precipitating the bigger magnetic iron oxide particles. During the hydrothermal reaction, the size of the particles is controlled by nucleation and grain growth processes. Keeping the other parameters in a constant rate, their production rate is controlled by the temperature. At higher temperatures nucleation becomes faster than grain growth so that the size of the particle is decreased, but if the reaction duration is prolonged the grain growth will be advantageous in the reaction (Fu and Ravinda, 2012). For example, Sun et al. (2004) managed to design the iron(III) acetylacetone with diameters between 4 and 20 nm with the presence of 1,2-hexadecanediol oleic acid and oleyamine and they managed to change the hydrophobicity into a hydrophilic property by adding bipolar surfactant.

9.2.1.4 Sol–gel reactions

A sol–gel reaction approach is a more adequate application type for synthesis of nanostructured metal oxides. Hydroxylation and condensation reactions are used

for sol−gel reactions, which originates a "sol" stage of NP production (McCarthy et al., 2007). With the help of further condensations and polymerizations, three-dimensional metal oxide is constructed in wet gel. These condensation and hydroxylation reactions are generated at room temperature, so another heat treatment is necessary to construct the final crystalline state. The structure produced during the sol stage of the sol−gel application determines the properties of the gel. The properties of the kinetics, growth reactions, hydrolysis, condensation reactions, and structure of the gel are affected by the type of solvent, temperature of reaction, nature, salt concentration of precursors, pH, and agitation (Atif et al., 2006). For example, Solinas et al. (2001) designed Fe_2O_3 nanoparticles with molar concentration between 0.25 and 0.57 by sol−gel reactions. Two factors were investigated in this study; the temperature and surface evaporation/volume ratio of sol stage. According to the report, the size of the nanoparticle is determined by the gelation process and silica matrix was used for the formation of NPs.

Rãileanu et al. (2004) synthesized $Fe_xO_y-SiO_2$ nanocomposites, utilizing a sol−gel technique; Ni substituted Co ferrite nanoparticles were formed. Minimum calcination temperature was observed as 500 °C to achieve single-phase spinel structures. No effect was reported on particle size due to changing Ni content (Rãileanu et al., 2004). In contrast, changing Ni content effected magnetization saturation (Mozaffari et al., 2014). The study of Masoudpanah et al. (2014) produced La-substituted $ZnFe_2O_4$ nanoparticles by a sol−gel autocombustion technique. La was substituted within octahedral and tetrahedral sites of crystal structure and the magnetic properties of the nanoparticles were examined. According with the increasing La content, magnetic saturation increased, decreasing inversion coefficient.

9.2.1.5 Polyol reactions

The polyol reactions are similar to sol−gel reactions but they are versatile applications for synthesizing magnetic nanoparticles in well-determined shapes and sizes. With the help of the different polyol solvents, such as PEG, the procedure offers various properties. They can be used as reducing operators or stabilization agents for the prevention of aggregation and control of particle size. During the process; the suspension is mixed and heated to the temperature of interest, which is the boiling point of polyol. An intermediate is formed in the polyol solution and metal nuclei are obtained by reduction. Submicrometer-sized particle formation is adjusted by increasing the temperature of the chemical reaction as well as activating heterogeneous nucleation (Laurent et al., 2008). There is a second method which is more suitable for the formation of magnetic nanoparticles by polyol reactions. Increasing the temperature degradation of polyol that allows more controlled sized particle formation in submicrometer size (Pileni, 1993). Abbas et al. (2014) developed a new method for silica-covered magnetic nanoparticles. In their study, polyethylene glycol was used as a stabilizer, reducing agent and binding factor with silica. Thus, with a single-step reaction, without using

surfactant, high stability against oxidation was reached as the nanoparticles were heated up to 600 °C (Abbas et al., 2014).

9.2.1.6 Flow injection synthesis

Flow injection synthesis (FIS) is a new approach based on flow injection in order to synthesize magnetic nanoparticles. This application occurs in a capillary reactor under laminar flow by continuous or segmented mixing of reagents. In comparison to a segmented or continuous method; continuous synthesis was found to be a more advantageous way of synthesizing iron oxide NPs. The synthesized magnetic nanoparticles were in the range of between 2 and 7 nm according to evaluations obtained by X-ray diffraction, electron microscopy, etc. (Alvarez et al., 2006).

9.2.1.7 Gas/aerosol phase methods

Aerosol technologies (spray and laser pyrolysis) have become interesting approaches, because these technologies have a direct and continuous rate of production capacity (Teoh et al., 2010). A solution of an organic solvent containing reducing agent and ferric salts is sprayed into a series of reactors, causing condensation of aerosol solvent and evaporation of solvent in spray pyrolysis (Zhao et al., 2012). The resulting dried residue was composed of particles whose size depended on the primary size of the original droplets. Five to 60 nm sized maghemite particles with various shapes have been synthesized using various iron precursor salts in alcoholic solution (Gonzale-Carreno et al., 1993). The volume of the reaction can be lowered by using a laser pyrolysis method. A gaseous mixture of iron precursors heated up with lasers causes production of small and uniform-sized nanoparticles. Laser prolysis conditions are adjusted so that nanoparticles sized between 2 and 7 nm are produced (Faraji et al., 2010). In the study of Harra et al. (2013), Fe_2O_3 was coated by TiO_2 particles partially or fully using a spray pyrolysis process ending up with ferromagnetic material. The help of calcination transformed titanium dioxide transformed into photocatalytic material. Furthermore, these obtained nanoparticles can be used as a photocatalyst with magnetic separation for diverse applications. Kumfer et al. (2010) constructed reduced iron oxide nanoparticles by a gas phase flame method. Magnetic and particle size specifications of iron oxide nanoparticles were invested with altering flame structure and flame temperature. Increasing flame temperature resulted in an increase in magnetic saturation, particle size, and iron fraction. The results of this study showed prospects for the future of nanotechnology in remediation environmental applications.

9.2.1.8 Sonolysis

Thermolysis or sonolysis is being used for formation of iron oxide nanoparticles from their organometallic precursors. Nanoparticle growth is limited by the addition of polymers and organic capping reagents. Ferrous salts are converted to MNPs at very high hot spot temperatures by sonication. Sodium dodecyl sulfate

(SDS) forms stable solution of Fe_3O_4 by sonolysis of $Fe(CO)_5$ (Abu Mukh-Qasem and Gedanken, 2005). Sonochemical application is used for the production of nanoparticles from organometallic precursors (Khalil et al., 2004). Metal colloids are produced with addition of stabilizers of polymers (Wu et al., 2007).

9.2.1.9 Microwave irradiation

A microwave irradiation method has several advantages compared with other conventional synthesis techniques leading to more homogeneous nucleation and shorter crystallization time of MNPs, of which the preparation step can be performed in a glass or plastic reaction container. This technique ends up with formation of uniform colloidal nanoparticles. Parsons et al. (2009) managed to synthesize iron oxide/oxyhydroxide nanoparticles using a standard microwave oven. High concentrations of the starting materials have been used for the application. $FeCl_3$ is slowly titrated with sodium hydroxide for the synthesis of magnetic nanoparticles. The controlled growth and crystalline structure of the particles were dependent on synthesis temperature. By using this technique authors also discovered that the synthesized nanoparticles have similar growth on three different axes (Hasany et al., 2012; Parsons et al., 2009).

9.2.2 GREEN CHEMISTRY

Nanoparticles are of great interest for researchers due to their size-dependent physicochemical properties, such as mechanical, biological and electrical properties, catalytic activity, antimicrobial activity, biosensing, conductivity, compared to the bulk of the same chemical's composition. There are many important applications for magnetic nanoparticles including magnetic fluidics (Jeyadevan et al., 2003), catalysis (Zhang et al., 2005; Tsang et al., 2004), biotechnology and medicine (Osaka et al., 2006; Gupta and Gupta, 2005), MRI (Mornet et al., 2006; Li et al., 2005), magnetic storage media (Hyeon,2003; Sun et al., 2000), biosensors (Miller et al., 2002), and environmental remediation (Mahdavi et al., 2013).

Magnetic nanoparticles have been synthesized physically and chemically for a long time but some of the chemical methods involve toxic solvents which could potentially generate hazardous byproducts (Sun et al., 2004; Peng et al., 2006; Herrera-Becerra et al., 2008). Therefore the use of biological systems is exploring new procedures for the formation of nanoparticles. The biological systems including microorganisms and plants have become a major focus nowadays. Among microorganism-mediated production of nanoparticles, plants (plant extract and living plant) seem to be the best candidates as they are comparatively simpler, more cost-effective and a better option for the large-scale production of magnetite nanoparticles (Bankar et al., 2010; Marchiol, 2012). The use of a biological system as green chemistry in the production of magnetite nanoparticles is rapidly developing due to ease of production and it is environmentally friendly, without the use of any toxic or expensive chemicals (Ahmad et al., 2003; Shankar et al., 2004; Ankamwar et al., 2005; Huang et al., 2007) and also saves energy (Bansal

et al., 2004). Especially the combinations of biomolecules in the plants such as enzymes, proteins, amino acids, vitamins, polysaccharides which are important for bioreduction of the magnetic nanoparticles have been investigated by researchers (Iravani, 2011).

The efficient and rapid extracellular synthesis of magnetic nanoparticles, using aqueous extracts of several plants such as carob leaves (*Ceratonia siliqua*) (Awwad and Salem, 2012), *Passiflora tripartite* (Kumar, 2014), *Sargassum muticum* (seaweed) (Mahdavi et al., 2013), canola oil (Kumar, 2014), *Camellia sinensis* (green tea) (Hoag et al., 2009: Shahwan et al., 2011; Kuang et al., 2013), oolong tea (Kuang et al., 2013; Huang et al., 2014), black tea (Kuang et al., 2013; Machado et al., 2013), sorghum bran (Njagi et al., 2011), eucalyptus (Madhavi et al., 2013; Wang, 2013; Wang et al., 2014a, 2014b), pomegranate (Machado et al., 2013; Rao et al., 2013), plantain peel (Venkateswarlu et al., 2013; Thakur and Karak, 2014), and *Tridax procumbens* (Senthil and Ramesh, 2012), using plant biomass such as alfalfa biomass (Herrera-Becerra et al., 2007), pine wood shavings (Ramasahayam et al., 2012), and orange peel (Lopez-Tellez et al., 2013), and using plant biomass as template such as soya bean sprout (Cai et al., 2010) tea waste (Lunge et al., 2014), have been reported.

9.2.2.1 Green synthesis through plant extract

In Kuang et al.'s study, three different tea extract (green tea, oolong tea, and black tea) were used to synthesize magnetic nanoparticles. The catalysis capacity of magnetic nanoparticles was investigated over oxidation of monochlorobenzene (MCB). SEM images indicate that magnetic nanoparticles have a chain-like structure. Also Shahwan et al. used green tea extract for the synthesis of magnetic nanoparticles. In their study, the nanoparticles were examined as a catalyst for the degradation of cationic dyes such as methylene blue and anionic dyes like methyl orange. Almost 100% removal of methylene blue and methyl orange was observed at an initial dye concentration of 10 mg/L and 100 mg/L, respectively. Also, they compared the green chemistry production route with a conventional method for magnetic nanoparticle preparation (Figure 9.2).

Eucalyptus globulus leaf extract was used by Madhavi et al. as a bioreducing agent to synthesize magnetic nanoparticles. They reported that oenothein B in plant extract is responsible for the synthesis and stabilization. On the other hand, Wang used eucalyptus leaf extract and synthesized nanoparticles exhibited flocculation capacity at 25 °C with an azo dye, acid black 194, therefore the nanoparticles could be used in water purification.

9.2.2.2 Green synthesis through plant biomass

Herrera-Becerra et al. (2008) reported that iron oxide nanoparticles could be synthesized by using alfalfa biomass. In this study, the role of pH as a size-limiting parameter was focused on. According to her study, higher pH values yielded smaller particles in the range of 1−4 nm with a greater proportion of the Fe_2O_3. When pH decreased to 5, larger particles were produced.

FIGURE 9.2

Magnetic nanoparticle production with plant extract. (A) Leaf extract is obtained from plant leaf in water. (B) Leaf extract is used as a bioreductant and capping agent to produce magnetic nanoparticles from Fe_3O_4 solution. (C) Nanoparticles are obtained by separation from the solution.

Ramasahayam et al. (2012) developed a new microwave-assisted method to synthesize magnetic nanocomposite using pine wood shavings. They discovered that tannin was the renewable resource. On the other hand, as a second method they used oven-drying for production, and in this way the obtained nanoparticles were used as a water purifier by removing phosphorus from water.

Iron oxide nanorods were produced by Lopez-Tellez et al. (2013) by using orange peel extract which consists of starch, cellulose, hemicelluloses, and lignin. The cellulose content of orange peel was used as a stabilizer by reducing Fe (II) metal ions. According to their study iron was deposited on the surface of the biomass and it mainly existed in the form of iron, iron (II) oxide, and magnetite.

9.2.2.3 Green synthesis through biotemplate

Cai et al. reported that superparamagnetic Fe_3O_4 nanoparticles were produced by using soya bean sprout as biotemplate at room temperature and atmospheric pressure. This study was the first in which magnetic nanoparticle production from plant biomass was used as a template (Figure 9.3). The nanoparticles were simultaneously formed on the epidermal surface and in the interior stem wall when the template was sunk in Fe_2^+ and Fe_3^+ solutions, then reacted with NaOH. After milling, magnetic separation, washing, and drying steps nanoparticles were collected.

9.2.3 MAGNETOSOMES—A BIOLOGICAL SOURCE FOR MAGNETIC NANOPARTICLES

Magnetotactic bacteria (MB) were discovered firstly by Balkwill and colleagues (Blakemore et al., 1975). Magnetotactic bacteria were found in nature with different morphotypes and dimensions such as spherical, cocci, bacilli, or spiral bacteria. The species are Gram-negative with flagella (Bazylinski, 1996). MB move

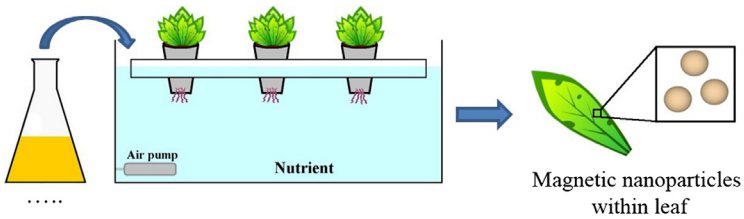

FIGURE 9.3

Magnetic nanoparticle production with plant template. By addition of aqueous solution of Fe_3O_4 to nutrient solution, Fe_3O_4 is absorbed by the plant root. After a period of time magnetic nanoparticles are formed within the plant leaf under dark light at room temperature.

Table 9.1 Application Areas of Magnetosomes

Alternative magnetic field cancer therapy	Alphandery et al. (2011)
Magnetotactic bacteria for cancer therapy	Mathuriya (2014)
Doxorubucine-loaded magnetosomes for antineoplastic effects on hepatic cancer	Sun et al. (2008b)
Navigation control strategy for magnetotactic bacteria in microchannel	Felfoul and Martel (2013)
Specific drug targeting and controlled release at tumor site	Takeyama et al. (1995)
Fluorescent bacterial magnetic nanoparticles as bimodal contrast agents	Lisy et al. (2007)

with magnetotaxis, which is the ability to sense and orient to a magnetic field, to avoid oxygen. Two common examples for these magnetotactic bacteria are *Magnetospirillum magnetotacticum* and *Magnetospirillum gryphiswaldense* (Xie et al., 2009). Magnetotactic bacteria convert Fe salts to magnetic nanocrystals *in vivo* by taking Fe salts into the cell. These magnetic nanocrystals (Fe_3O_4—magnetite or Fe_3S_4—greigite) were called "bacterial magnetosomes" (BMs) (Balkwill et al., 1980). Magnetosomes have a chain structure that indicates a sequence from the beginning toward the end of the cell (tail). MB become more sensitive to external magnetic fields by piecing together their own internal dipole moment. The magnetic moment of the magnetite is three times stronger than greigite.

Due to their properties, such as paramagnetism, nanoscale, narrow-size distribution (25–55 nm), and being bounded to the membrane, magnetosomes were thought of as alternatives for targeting drug carriers (Hoell et al., 2004). The main areas of use for magnetosomes are given in Table 9.1. Besides, magnetosomes have advantages such as:

- Limited size distribution and uniform morphology;
- Stable single-magnetic-domain particles;
- In **T**2 weighted imaging, signal attenuation is apparent;

- Membrane of magnetosomes charged negatively;
- Show better dispersion;
- Easy modification with antibody;
- Drug loading to membrane of magnetosomes (Sun et al., 2008b; Liu et al., 2010).

9.3 MAGNETIC NANOPARTICLES MODIFICATIONS

9.3.1 FUNCTIONALIZATION AND ENCAPSULATION WITH NATURAL POLYMERS

Magnetic nanoparticles have gained a great deal of interest due to their biocompatibility with low toxicity, easy surface modification, and magnetic properties (Gu et al., 2006), for researchers from a wide range of disciplines such as medicine, biology, and materials science, especially in the areas of MRI, hyperthermic treatment for malignant cells, and targeted drug delivery. Magnetic nanoparticles can bind to drugs, proteins, enzymes, antibodies, or nucleotides, therefore they can be directed to organs using an external magnetic field (Gupta and Gupta, 2005). However, there is a drawback of magnetic nanoparticles for drug-delivery applications. For instance, magnetic nanoparticles were most likely cleared rapidly by macrophages or the reticuloendothelial system (RES) before reaching the desired site (Gupta and Wells, 2005). Particles which have a large hydrophobic surface are coated with plasma components and removed rapidly from the circulation, whereas particles with a hydrophilic surface can resist the attack of plasma components and stay for a long time in the body (Gaur et al., 2000). On the other hand, nanoparticles tend to aggregate due to strong magnetic dipole−dipole interactions between particles trying to reduce the energy associated with the high surface area to volume ratio. Moreover magnetic nanoparticles without a coating on the surface, are chemically highly active, and are oxidized in air, resulting in a loss of their magnetization (Mikhaylova et al., 2004; Wa et al., 2006; Kim et al., 2006). Therefore, biocompatible surfactants, polymers, and oxide compounds with functional groups have been used to modify and stabilize magnetic nanoparticles (Santra et al., 2001). By this way, possible side effects of encapsulated drug by magnetic nanoparticles covered with a polymer shell, can be reduced or minimized (Kohler et al., 2004; Yu et al., 2006; Gupta and Curtis, 2004b).

In the literature the most common natural polymers used for coatings of magnetic nanoparticles are derivatives of dextran, starch, chitosan, alginate, gelatin, and albumin. Many natural polymers are biocompatible and have no side effect that is convenient for biomedical applications. Carbohydrates and proteins are suitable as coating agents for magnetic nanoparticles.

Lübbe et al. (2001) reported the first phase human clinical trials with drug-loaded and natural polymer-coated magnetic nanoparticles. In his study, epirubicin, which is an antracycline antibiotic especially used for the treatment of solid

tumors (Bonadonna et al., 1993), was bound chemically to magnetic nanoparticles with a particle size 100 nm in ferrofluid form. Later, magnetic nanoparticles were coated with starch. Starch has anionic phosphate groups on its chemical structure, therefore it is easy to bind epirubicin cationically via positively charged amino sugars. He stated that before the human trials no LD_{50} was found for the ferrofluid observed in preliminary animal studies (Lübbe et al., 1996a). As treatment protocol, after 45 min of intravenous infusion, a magnetic field was built up and the ferrofluid could be successfully directed to the tumors. In another study, Cole et al. (2011) prepared polyethylene glycol (PEG) modified crosslinked starch-coated magnetite nanoparticles. Nanoparticles were administered intravenously to a 9L-glioma rat model for brain tumor targeting. MRI and histological analyses visually confirmed the enhanced PEG-starch-magnetic nanoparticles delivery to tumors.

The presence of functional amino and hydroxyl groups makes chitosan a suitable candidate for the encapsulation of magnetic nanoparticles. Kuroiwa et al. (2008) and Zhu et al. (2009) stated that chitosan-coated magnetic nanoparticles can cross through cell membranes and between tight junctions of epithelial cells and easily opened chitosan mediation. Chitosan and its derivatives have been the most widely used polysaccharides. In Dung and et al.s' (2009) study, chitosan-coated magnetic nanoparticles were prepared by coprecipitation of $FeCl_2$ and $FeCl_3$ solution in ammonium medium and glutaraldehyde was used as a crosslinker. Then, nanoparticles with an average diameter of 23 nm were applied in enzyme immobilization or removal of heavy metal ions in water. In another study, polyethylene glycol- (PEG-)grafted chitosan was used to coat magnetic nanoparticles, after conjugation with chlorotoxin, a tumor-targeting agent, and a near-infrared florophore, magnetic nanoparticles could cross through the blood−brain barrier and target a brain tumor (Veiseh et al., 2009a,b).

Alginate is able to form gels in the presence of divalent cations (Sreeram et al., 2004), also alginate gels are ionotropic. They are used for matrix-supporting tissue repair and regeneration also by the US Food and Drug Administration (FDA) (Sun and Tan, 2013). Magnetic nanoparticles were synthesized by coprecipitation of ferric and ferrous ions by alkaline treatment and later coated with alginate. It was reported that the core diameter of magnetic nanoparticles was 5−10 nm, and after coating with alginate the hydrodynamic diameter was found to be around 193.8−483.2 nm. Also, T2 relaxivity of the alginate-coated SPIONs was found to be higher than SPIONs, therefore it can be used as a negative MRI contrast agent (Ma et al., 2007).

Dextran is a complex branched glucose, and widely used in biomedical applications. According to Singh and colleaques' study, four different particle formulations were prepared as uncoated magnetite, uncoated maghemite, dextran-coated magnetite, and dextran-coated maghemite, and their cytotoxicity was investigated. Only dextran-coated maghemite demonstrated genotoxicity (Singh et al., 2012). In another *in vivo* study, dextran-coated magnetic nanoparticles with a diameter

of 50 nm, were used for intraocular applications and it was stated that it was suitable and safe (Raju et al., 2011). On the other hand, dextran-crosslinked magnetic nanoparticles were prepared and the HIV-1 tat peptide was attached to the dextran that moves freely through cellular and nuclear membranes. By means of this tagging, the uptake of nanoparticles increases over 100-fold into lymphocytes when compared to untagged particles (Josephson et al., 1999; Allport and Weissleder, 2001; Wunderbaldinger et al., 2001). This idea opens up further possibilities for MRI tracking of cell transplants.

Magnetic albumin microspheres were prepared by Ma et al. (2000). In this study, bearing adriamycin (an anticancer drug) demonstrated reduced toxicity to animal cells compared to a single dose of adriamycin. Also, Chunfu et al. (2004) synthesized albumin-coated magnetic nanoparticles about 200 nm in diameter with a microemulsion method. For the production process, cotton oil as oil phase, a mixture of HSA and magnetite solution as water phase and Span-83 as emulsion agent were used. The particles were radiolabeled with ^{188}Re for targeted therapy with a labeling efficiency of about 90%.

Gelatin is a protein derived from collagen (Young et al., 2005) and has multifunctional groups (−NH2, −COOH) on the chain which makes it a suitable candidate to bind to doxorubicin to form drug−polymer conjugate (Leo et al., 1997). In an interesting work, gelatin-coated magnetic nanoparticles were used as thermo seed for hyperthermic treatment of cancer cells, that is because gelatin has shown greater affinity for bone tissue and been found to induce bone formation (Gaihre et al., 2009). In the study, the gelatin-coated magnetic nanoparticles were soaked in simulated body fluid and apatite formation on the surface was investigated. As a result, the presence of a self-assembling gelatin layer helped hydroxyapatite crystal growth.

9.3.2 FUNCTIONALIZATION AND ENCAPSULATION WITH SYNTHETIC POLYMERS

Recent developments in nanotechnology brought new processes for surface modifications of NPs. The surface of the NPs can be manipulated by coating and letting the material gain physical, optical, electronic, chemical, and biomedical properties (Issa et al., 2013; Frey et al., 2009).

Coating of the nanoparticle surfaces is one of the most efficient approaches against toxicity. The degree of surface coating has been proved to be the principal parameter in cellular uptake. Complete surface coating of MNPs prevents opsonization and rapid endocytosis, and hence prolongs plasma half-life (Jung and Jacobs, 1995). The uncovered negatively charged MNPs were reported to exhibit a toxic effect over a defined threshold dose. Additionally, uncoated MNPs are poorly soluble and tend to precipitate in aqueous media impeding blood vessels in *in vivo* studies. In this sense, numerous coatings have been proposed to reduce the

toxicity. Mahmoudi et al. (2009) demonstrated that uncovered particles cause greater toxicity than polyvinyl alcohol (PVA)-coated MNPs. Furthermore, the toxicity of non-coated particles may remarkably be reduced by alteration with surface-saturated non-coated particles. Coating maghemite particles with dimercaptosuccinic acid (DMSA) was reported as an approach which nearly prevents toxicity (Auffan et al., 2006) by eliminating direct contact of the particle and human dermal fibroblasts. The study of Park et al. (2005) demonstrated that when MNPs are embedded in chitosan to obtain magnetic chitosan particles, complete coating of MNPs with chitosan caused particles that exhibited relatively low cytotoxicity.

Encapsulation is a big challenge because of the very small sizes, high surface energy and area of the NPs (Peracchia et al., 1997). There are two conventional applications for surface encapsulation; dry and wet approaches. In dry encapsulation, physical vapor deposition, plasma treatment, chemical vapor depositions, and pyrolysis of polymeric/non-polymeric organic materials are used. On the other hand in wet encapsulation, sol−gel processes, emulsifications, and solvent evaporation applications are used. Encapsulations of NPs are useful in areas such as controlled drug, gene, or other bioactive reagent release. These controlled release applications protect the agents from degradation, help targeted delivery, and prolong active reagent release duration (Wang et al., 2004; Di Marco et al., 2007). Encapsulation of MNPs can be done either with natural or with synthetic polymers. Furthermore, coating particles with polymers improves compatibility with organic ingredients, reduces susceptibility to leaching, and protects particle surfaces from oxidation. As a result, encapsulation enhances chemical stability, dispersibility, and reduces toxicity (Stolnik et al., 1994).

These methods are simple and conventional approaches. Chitosan is the most frequently used material for encapsulation with natural polymers. On the other hand, D-,L-polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactic acid (PCL), polyvinyl alcohol, polyethylene glycol (PEG), poly-N-vinyl pyrolidone, polyethyleneimine (PEI), and polyethylene oxide (PEO) are examples of synthetic polymers (Ramachandran and Shanmughavel, 2010). The type of synthetic coating depends on the application for which MNPs will be used. Due to the development of the emulsion polymerizations such as miniemulsions, microemulsions, soap-free emulsion polymerizations, new synthesizing methods are created (Xu et al., 2004). In general, MNPs are suspended in the dispersion phase but in the presence of the MNPs, monomers are polymerized so that the polymerized magnetic nanoparticles are produced (Zhang et al., 2012). Within all emulsion polymerizations; miniemulsion polymerization is the most convenient method for producing magnetic polymeric nanoparticles. In miniemulsion, polymerization monomer droplets and MNPs come together to work as nanoreactors. Ramirez and Landfester (2003) have succeeded in encapsulating MNPs with two miniemulsion applications by three-step preparation processes.

A range of secondary surfactants around MNPs have been studied for toxicity *in vivo*. Alginic and citric acid surfactants were determined to be remarkably less

toxic than PEG starch and decanoic acid. This study demonstrated the significance of optimizing surface coating to minimize toxicity (Kuznetsov et al., 1999). Hua and coworkers have revealed that poly-(aniline-co-N-(1-one-butyric acid) aniline) (SPAnH)-coated Fe_3O_4 particles with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) bound-BCNU-3 could be concentrated at targeted sites *in vitro* and *in vivo* by an externally applied magnet and then applied to brain tumors. Magnetic targeting was found to enhance the concentration and retention of bound-BCNU-3 (Hua et al., 2011).

Xu et al. (2004) reported that nanosized iron oxide particles can be coated with polyacrylamide particles and these crosslinked MNPs can serve in biological applications. Another encapsulation technique can be carried out by polyvinyl alcohol (PVA) use. The hydroxyl groups of the polymer chain guarantee the hydrophobic property of the encapsulation, which has similar surface chemistry property to carbohydrates (McBain et al., 2008). In another study, Singh et al. (2012) investigated the non-coated magnetite, non-coated maghemite, dextran-coated magnetite, and dextran-coated maghemite for cytotoxicity, and neither of the samples showed cytotoxicity below 100 mg/mL. However, the dextran-coated maghemite exhibited genotoxicity.

Amorphous silica is very strong and widely used for encapsulation of iron oxide nanoparticles. There are several applications of iron oxide particles coated with silica which are frequently used in biomedicine. Encapsulation with silica is performed by hydrolysis of tetraethoxysilane or silic acid neutralization. Polyethyleneimine (PEI)-coated MNPs were used for *in vitro* non-viral gene delivery as well as antisense oligonucleotides and siRNA to reduce gene expression (McBain et al., 2008). Polyethylene glycol (PEG) is a common polymer for encapsulation of particles. PEG-attached or -adsorbed surfaces of nanoparticles exhibit hydrophilic and flexible properties (Dong and Feng, 2004). PEG shell provides stability in water with its non-toxic structure, while Fe_3O_4 core provides magnetic separation, targeting and MRI availability. Silver (Ag) particles provide fluorescence and antibacterial property and a combination of Fe_3O_4@Ag−PEG was used by Wang et al. (2003) as a drug-delivery system for photothermal chemotherapy. Another research including layer-by-layer deposition step pointed out that maghemite stabilization can be carried out with polymers PEI and poly (ethylene oxide)-block-poly(glutamic acid) (PEO-PGA). The first layer around the maghemite core is surrounded by PEI, and the second is surrounded by PEO-PGA (Viau et al., 1996).

9.3.3 LIGAND MODIFICATIONS AND TARGETING

The pharmaceutical industry is growing day by day, leading therapies for many diseases either by relieving pain or by completely diminishing symptoms. However, most of these fail to be specific to disease, so when they are administered intravenously, as a consequence of entering the systemic circulation, they are distributed along all tissues via the blood flow, including to

non-diseased, healthy cells, causing side effects. These side effects may be deleterious, especially in the case of chemotherapies, considering the power of the drug administered. To overcome this disadvantage, micro- and nanoparticle-based targeting studies have been undertaken. These carriers possess specific ligand for the diseased areas, providing localized targeting, reducing or completely eradicating the unwanted side effects, by decreasing their systemic distribution as well as the opportunity of administration of lower doses of cytotoxic drugs, yielding improved treatment efficacy and also an economic advantage.

Currently, nanoparticles are commonly studied as drug carriers with precise nanostructure construction (controlled drug release characteristics, high biocompatibility, ease of administration, etc.), instead of microparticles, considering the size factor, that nanosizes enable the particles to largely escape the body filters (Akagi et al., 2007; Yoo et al., 2011). The specificity of nanoparticles is one of the most important factors for all therapies including hyperthermia, diagnostic imaging, and theranostics (Veiseh et al., 2010a). Non-specific binding will produce misleading results, as well as damage to healthy tissues. To limit this binding, nanoparticles are designed to have an affinity for target sites through passive, active, and magnetic targeting techniques. Passive targeting uses the predetermined physicochemical properties of a specific nanoparticle to target migration to a specific tissue site. For example, a solid tumor tissue can be targeted by passive targeting methods, which is called enhanced permeation and retention (EPR) (Maeda et al., 2000). This term is based on the principle that rapidly growing tumor cells will be in an effort to generate new blood vessels, which are disorganized and permeable. This enables extravasation of nanoparticles out of the vessels into the tumor tissue. However, EPR is only defined to specific metastatic solid tumors, and the application depends on many factors (blood flow, vasculature, and lymphatic drainage rate) putting therapy at risk. Because passive targeting is applicable for only specific *in vivo* applications and does not guarantee internalization of nanoparticles by targeted site cells, nanoparticles should be futher modified with molecular ligands to apply active cell targeting (Zhang et al., 2002). Nanoparticle complexes are now designed with targeting ligands, complementary to specific receptors on target cells, for active targeting to only diseased area.

These target-specific therapies can be supported by a magnetic field, which is advantageous regarding accumulation of nanoparticles at a desired site. In magnetically targeted therapies, cytotoxic drugs can be attached to biocompatible magnetic nanoparticles, or can be encapsulated inside. Magnetic nanoparticles are commonly produced with Fe, Mn, Ni, Co, and their oxides, with varying size of 5−200 nm (Chomoucka et al., 2010). However, nanoparticles with a diameter of 100 nm are considered as potential medical carriers, as this size allows nanoparticles to circulate *in vivo*, escaping the filters and possibly reaching the targeted area. Targeting is achieved by appropriate design of the surface ligands such as small organic molecules (Zhang et al., 2002; Sinha et al., 2006), peptides (Veiseh et al; 2005; Montet et al., 2006b), proteins (Gunn et al., 2008), antibodies (Artemov et al., 2003; Hu et al., 2006; Huh et al., 2005), and aptamers (Schafer

et al., 2007; Yigit et al., 2007; Yigit et al., 2008). The surface ligands figure the physicochemical properties of the surface, such as hydrophobicity, surface charge and zeta potential and distribution in solution. These properties have an especially important role in determining nanoparticle−cell associations, such as cellular membrane permeability, immune responses, and localization *in vivo* (Kobayashi et al., 2014). When considering the magnetic nanoparticle surface chemistry for drug delivery, it is favored that magnetic nanoparticles have a hydrophilic character with a neutral charge with coating or ligands, and do not exceed 100 nm in size to prevent rapid clearance by RES (Gupta and Gupta, 2005; Torchilin and Trubetskoy, 1995; Duguet, 2006).

Magnetic or superparamagnetic nanoparticles (SPIONs) are commonly used in cancer studies for chemotherapy, hyperthermia, and diagnosis as contrast agents or as theranostics, mainly with direct targeting strategies for higher specificity. They are favorable agents because of their improved/promising abilities to accumulate and be visualized at the tumor site, as well as their ease of use. In 2013, Sadhukha and his colleagues developed inhalable, epidermal growth factor receptor (EGFR)-targeted superparamagnetic nanoparticles, for hyperthermia in lung cancer (Sadhukha et al., 2013). In another study, in 2013, magnetic nanoparticles targeted to uPAR receptor with the specific peptide target, showed fivefold increased accumulation at tumor site, compared to controls (Hansen et al., 2013). In 2014, a study conducted by Gallo and colleagues showed enhanced signaling abilities of iron oxide nanoparticles with specialized surface coatings and CXCR-4 targeting (Gallo et al., 2014).

One of the commonly used targeting strategies is to target the folate receptor, one of the highly expressed receptors on many different types of cancers. There are many studies for targeting the folate receptor, a very newly published study shows that MnO nanoparticles conjugated with folic acid, can be used for imaging brain tumors (Chen et al., 2014). In another study conducted by Gupta and colleagues, claims that methotrexate, another ligand for folate receptor, conjugated iron oxide nanoparticles succeeded to reduce the viability of human cervical cancer cells (Gupta et al., 2014). Another ligand is chlorotoxin, which has a high affinity for a set of lipid raft-anchored complexes that contain matrix metalloproteinase-2 (MMP-2) and chloride ion channels which are needed to sustain the glioma cancer cell's invasive nature. It was used by Gu and his colleagues, conjugated to Gd_2O_3 nanoparticles for imaging (Gu et al., 2014). In addition, aptamer ligands are used to target prostate cancer. Antibodies like transferrin, monoclonal antibody A7 herceptin (Trastuzumab), are widely used ligands in breast, colon, and brain tumors (Cirstoiu-Hapca et al., 2007; Cui et al., 2013). In addition, proteins such as lactoferrin (Gupta and Curtis, 2004a), TGF-β-insulin, ceruloplasmin (Gupta et al., 2003), pullulan (Kaneo et al., 2001), and Tat-peptide (Lewin et al., 2000) are being used to derivatize magnetic nanoparticles. There are also aptamers used to target magnetic nanoparticles to treat cancers (Herr et al., 2006).

These drug-containing magnetic nanoparticles, usually in a suspension of biocompatible ferrofluid, are injected into the patient through the systemic circulation. When the particles have entered the bloodstream, powerful, high-gradient

magnetic fields are used to accumulate the complex at the specific target site. Once the complex reaches a sufficient concentration at the target site, the drug can be released either via enzymatic activity, charge interactions, or via changes in physiological conditions such as pH, osmolality, or temperature (Alexiou et al., 2000), and be internalized by the targeted cells. This technique has important advantages over the standard, non-targeted methods of cytotoxic drug therapy. The potency of this therapy is dependent on several physical conditions, such as the field strength, gradient and volumetric and magnetic properties of the particles, as well as chemical characteristics of the particles. Because the carriers are normally administered via the circulatory system, hydrodynamic parameters, including blood flow rate, rate of capillary degradation, ferrofluid concentration, infusion route, and circulation time also have an important role, also with the parameters like distance from the magnetic field source, reversibility and strength of the drug/carrier binding, and tumor volume (Lübbe et al., 1999). Regarding all these parameters, targeting seems to be most efficient in regions with slower blood flow, closer to skin (magnetic field), and neovascular (Voltairas et al., 2002).

In addition to the type of ligand used, targeting is affected by targeting molecule density and by the size and shape of the nanoparticle. Recent studies have indicated that the density and molecular organization of bound ligands significantly influences nanoparticle binding to target cells due to the multivalency event (Hong et al., 2007), an enhanced binding avidity occurring when multiple ligands simultaneously bind with multiple receptors between two surfaces (Wright and Usher, 2001). Several nanoparticle systems have been engineered to achieve higher affinities to their cellular targets utilizing this principle (Natarajan et al., 2008; Gratton et al., 2007; Park et al., 2008). In a study in 2006, iron oxide nanoparticles were decorated with several densities of the RGD peptide, it was reported that simultaneous ligand binding could be increased with higher RGD presentation (Montet et al., 2006a). In addition, multivalency is also influenced by nanoparticle size and shape. In the study of Jiang et al. (2008) nanoparticles with sizes ranging from 2−100 nm were decorated with targeting herceptin antibodies and their ability to be localized to target cells were evaluated. Decuzzi and his colleagues showed that oblique-shaped particles, which have been decorated with targeting molecules, show better cell binding affinity compared with spherical NPs (Decuzzi and Ferrari, 2006).

There are also some limitations associated with magnetically targeted drug delivery (Pankhurst et al., 2003). One of the most important problems is the possibility of clotting in the blood vessels at the target region due to sedimentation of the magnetic nanoparticles, which may lead to serious systemic disorders. Scaling up from animal models is also difficult, regarding the increased distances between the target site and the magnet. Management of the drug release may be a problem, as once the drug is released, it is no longer attracted to the magnetic field, and control of the drug may be problematic. However, recent preclinical and experimental studies show that it is possible to overcome these limitations and use magnetic targeting to enhance drug therapies (Gallo and Hafeli, 1997).

9.3.4 MAGNETIC RESONANCE IMAGING

MRI is a method for imaging soft tissue with the highest resolution. Therefore, the technique is used for the study of all kinds of soft tissues in the body, including the nervous system; during the imaging the patients are not exposed to any ionizing radiation.

Iron oxide nanoparticles with magnetic properties can be used to get better results in MRI. There is also a commercial form of iron oxide nanoparticles called Feridex IV. These nanoparticles can be coated with a biocompatible polymer to make the surface suitable for medical applications. At the same time, the therapeutic drugs can be loaded into the shell providing controlled drug release. Thus, multifunctional nanoparticles with a double effect can be prepared.

In a study reported in 2008 by Jain et al., multifunctional MNPs were prepared to be used as both drug carrier and MR contrast enhancer. Iron oxide nanoparticles, prepared by the coprecipitation method, were covered with oleic acid and pluronic F-127. Either doxorubicine (DOX), paclitaxel (PTX), or a combination of DOX/PTX were loaded into magnetic nanoparticles. The antiproliferative activity of drug-loaded nanoparticles was evaluated on MCF-7 breast cancer cells. According to the results, the drug-loading efficiency of MNPs was between 74% and 95% and the drug release was sustained (approximately 5 days). The IC50 value for combined drug-loaded MNPs was found to be lower than the drug alone. Therefore, drug combination therapy was successfully achieved. Efficiency of MNPs as MR contrast enhancer agent was compared to a commercially available agent, Feridex IV. The T2 relaxivity ($\mathbf{r}2$) of the MNPs was found to be higher than Feridex IV. However, $\mathbf{T}1$ relaxivity ($\mathbf{r}1$) of the MNPs was lower. Therefore, oleic acid and pluronic F-127-coated and drug-loaded MNPs are more sensitive in $\mathbf{T}2$ weighted imaging (Jain et al., 2008).

9.3.5 HYPERTHERMIA

Body temperature rise is a natural defense mechanism of the body against infection and disease. Scientists conducted many hyperthermia applications in order to eliminate the disadvantages of conventional cancer treatments such as chemotherapy, radiotherapy, and also to provide a more efficient treatment. Hyperthermia, which means a rise of body temperature over 41 °C, can be created artificially by drugs or medical devices. Cancer treatment is possible by controlling the temperature rise while protecting the vital organs such as the brain and heart.

The principle of treatment is based on the physical and chemical differences between healthy and cancer cells. First and foremost, the vascular structures are significantly different in cancer tissue than healthy tissue. Vasculature of healthy tissues exhibits a fairly regular and stable structure, whereas tumoral tissue is highly irregular. While increased temperature in healthy tissue can be adjusted by the blood flow rate, the angiogenesis in some areas of the tumor tissue is sufficient, whereas in some parts it is inadequate. In the regions with poor

angiogenesis, hypoxia and acidosis are seen with low pH. This situation makes the tumor tissue more susceptible than healthy tissue. Because both temperature and metabolites are caused by cellular activity in these regions they cannot be controlled effectively and cannot be removed (Reinhold and Endrich, 1986; Hildebrandt et al., 2002; Santos-Marques et al., 2006).

Hyperthermia therapy can be applied in three ways, local, regional, and whole-body hyperthermia. In local hyperthermia application, heating of a certain small area (only tumor tissue) is provided by two electrodes. In order to prevent the temperature rise on the skin, there should be a cooling unit on the head edge of the electrode. In the regional hyperthermia application, it is aimed at heating a relatively larger area than local hyperthermia (e.g., a tissue or an organ all). Finally, in whole-body hyperthermia, which is especially studied to treat metastatic cancer, heat is applied to the whole body (Habash et al., 2006; Falk and Issels, 2001).

Hyperthermia treatment can be used alone, but when administered together with chemotherapy and radiotherapy it was observed that the treatment efficacy increased.

In a study reported in 2007 by Franckena et al., chemotherapy with cisplatin and hyperthermia therapy were applied together to 47 cervical cancer patients who had a recurrence of tumor tissue although exposed to a radiotherapy treatment in the years between 1992 and 2005. According to the studies results, it was observed that hyperthermia improve the effectiveness of cisplatin on tumor cells by increasing the cellular uptake of cisplatin. The combination of cisplatin and hyperthermia were applied to 47 patients for 6 weeks and during the application the temperature of the tumor site was raised to 40−43 °C. As a result, tumor tissues in 53% of patients were completely destroyed, and tumor tissues in 74% of patients shrunk considerably (Franckena et al., 2007).

Recently, studies are particularly concerned with developing new methods to reduce the side effects of hyperthermia. This approach led the researchers to work with nanotechnological materials which have become one of the popular research areas in recent years. There are especially lots of studies about the effect of the magnetic oxide nanoparticles on cancer treatment. It is possible to use iron oxide nanoparticles to treat cancer in various ways. In hyperthermia treatment, nanoparticles are covered with a biocompatible polymer and surfaces were modified with an antibody (ligand) for targeting cancer tissue. Polymer layers can be loaded with cancer drug. Later, nanoparticles raise the temperature of the cancer tissue and release the drug with an external magnetic field, which causes the death of cancer cells (Chicheł et al., 2007). This combination therapy of hyperthermia and chemotherapy is known as thermochemotherapy.

Polymer-coated magnetic iron oxide or magnetic nano- and microencapsulation have been used since 1970s. The aim of magnetic drug targeting is:

- To provide optimum concentration of drug at a target site;
- To increase the controlled temperature with an external magnetic field;
- To drive nanoparticles to targeted area with a magnetic field (Ciofani et al., 2009).

In a study reported in 2012 by Gao et al., methotrexate-conjugated magnetic nanoparticles (MTX−MNPs) with 30.1 ± 5.2 nm diameter were prepared and used to investigate the effects of simultaneous chemotherapy and hyperthermia in a MCF-7 breast cancer cell line. For this purpose, three experimental groups were prepared. In the first group, only the magnetic field was treated on cells and the temperature rose to 43 °C. During the experiment, the magnetic field frequency was 300 kHz and the heating time was maintained for 20 min. Finally, the relative cell viability was found to be $87.6\% \pm 10.8\%$. In the second group, MTX−MNPs without magnetic field (chemotherapy) was treated on cells and the relative cell viability was found to be $64.5\% \pm 7.2\%$. In the third group, the MTX−MNPs with a magnetic field were treated on cells and the relative cell viability was reduced to $13.3\% \pm 1.3\%$. These results show that the effect of the combination therapy are higher than chemotherapy or hyperthermia alone (Gao et al., 2012).

In a study reported in 2012 by Liu et al., bacterial magnetosomes (BMs) and iron oxide nanoparticles were compared for hyperthermia application. BMs were isolated from *M. gryphiswaldense* MSR-1. Fe_3O_4 MNPs were prepared by coprecipitation method and covered with aminosilane. Nanoparticles were exposed to an alternative magnetic field (AMF) with frequency of 300 kHz and amplitude of 110 Gs. As a result, although Fe_3O_4 MNPs raised the temperature to 50 °C, BMs exhibited a higher heating speed and temperature than MNPs (Liu et al., 2012).

9.3.6 GENE THERAPY

Gene therapies relied on viral transfers, until the detrimental side effects and aggressive nature was understood. Lately, non-viral gene transfection techniques have been developed with physics- and chemistry-based methods, which mainly use the advantage of charge interactions and energetic processes. The rationale for non-viral gene delivery lies in the safety of this approach. These methods exploit the natural uptake pathways (endocytotic mechanisms) of cells during the transfection process, without disrupting the cell membrane, resulting in high cell viabilities post-transfection (Jenkins et al., 2011). One of the most promising method of non-viral transfection is the use of carefully designed magnetic nanoparticles, which have advantages in terms of solubility, pharmacology, and stability. In addition, gene delivery with nanoparticles may be a promising approach for vaccination regarding their high resemblance to virus structure, stabilizing and protecting DNAs inside (Zhao et al., 2014). In this approach, therapeutic or reporter genes are attached to magnetic nanoparticles, they are then manipulated to the target site via a high-field/high-gradient magnetic field, ensuring rapid and efficient transfection.

Gene delivery with magnetic nanoparticles was first studied by Cathryn Mah, Barry Byrne, and their colleagues who also showed firstly *in vitro* transfection with C12S cells and then *in vivo* using an adeno-associated virus (AAV) encoding green florescent protein (GFP) linked to magnetic nanoparticles via a heparin sulfate

linker (Mah et al., 2000). After the development of this method by Mah and coworkers, Plank, Rosenecker, and others further developed the technique and coined the term "magnetofection." With the work of this group transfection time and efficiency increased considerably compared with the older studies (Plank et al., 2003). For magnetic nanoparticle-based transfection applied *in vitro*, the particle/DNA complex in a suspension is given to the cell culture where the magnetic field is produced by magnets (or electromagnets) which are placed below the cell culture. This method enhances precipitation of the complex leading transfection to take place much quicker. For *in vivo* studies, applying a magnetic field to the target site may help transfection, directing the therapeutic gene to a specific organ or desired site within the body. Generally, magnetic nanoparticles with the therapeutic gene are applied intravenously, where direct injection to the desired site may also be carried out together with high-gradient external magnets for catching the particles in the bloodstream. Once the nanoparticles are captured in the target site, they become available for uptake by the tissue. The genes inside the particles can be released via either enzymatic cleavage of the crosslinking polymers, pH-dependent reactions, or deterioration of the polymer matrix.

Also, studies were performed for the optimization of the nanoparticles. Grief and Richardson (2005) concluded that the magnetic nanoparticle-based gene delivery method is likely to be the most effective way for targeting the sites near the skin, nearby the source of the magnetic field.

These particles generally reside in magnetic iron-oxide either scattered within a polymer matrix – like silica, polyvinyl alcohol (PVA), polyethylene glycol (PEG), polyether-ester- dextran – or encapsulated within a polymer or metallic shell (Neuberger et al., 2005; Harris et al., 2003). The shell or matrix can be further modified by attachment of carboxyl groups, amines, biotin, streptavidin, antibodies, peptides, ligands, etc. Another approach to promote the intake of nanoparticles is the adapting of surface charges with cationic polymers.

The particles are usually coated with positively charged polyethyleneimine (PEI), which binds negatively charged DNA and condenses DNA because of the large number of secondary amine groups (Abdallah et al., 1996). In addition, PEI eases lysosomal escape of the complex after endocytosis by buffering the intralysosomal pH, causing the lysosome to release its contents (Akinc et al., 2005). It is now understood that nanoparticles carrying DNAs enter the cell by endocytosis through clatharin-dependent pits (Schillinger et al., 2005), which can also be beneficial for PEI–DNA complexes.

Polyethyleneimine-coated *magnetic* nanoparticles were first described in 2002 by Scherer et al., in which the authors demonstrated incorporation of DNA with superparamagnetic nanoparticles resulted in more efficient and fast (in only 10 min) transfection of some commercial transfection reagents *in vitro*. In addition to promoting targeted gene delivery, the main advantage of this technique is the rapid accumulation of the gene–particle complex onto the target site, which also saves the time and dose of DNA to have efficient transfection. Since this original work, *magnetofection* has been used to transfect a number of cell types,

including primary lung epithelial cells (Gersting et al., 2004) and blood vessel endothelial cells (Krotz et al., 2003).

MNP-guided lentiviral transduction of endothelial cells can be significantly promoted and targeted by using optimized magnetic nanoparticles. Morishita and colleagues demonstrated that the magnetic nanoparticles can increase the effectiveness of the cell-fusion vector hemaglutinating virus of Japan envelope (HVJ-E). They produced protamine sulfate (PS)-coated magnetic nanoparticles conjugated to HVJ-E, and showed the transfection was increased *in vitro* in BHK21 cells without any toxicity. In another work, they associated heparin-coated maghemite with the HVJ-E vector and injected the complexes to livers of BALB/c mice, which resulted in increased transfection levels (Morishita et al., 2005).

Pickard and his colleagues reported the powerful potential of magnetic nanoparticles to mediate gene transfer to key neural transplant cell populations such as astrocytes and neural stem cells (NSCs) (Pickard and Chari, 2010; Pickard and Chari, 2011). The group also demonstrated MNPs can be used for transfection of oligodendrocyte progenitors, whose efficiency is improved by focusing of a static/oscillating magnetic field. They then transplanted these OPCs into cerebellar tissue slices, and showed that they survive, proliferate, and differentiate into oligodendrocytes, presenting the translational potential of the magnetic nanoparticles for OPC transplantation therapies.

9.3.7 DRUG DELIVERY

Transition through the plasma membrane and intracellular delivery of therapeutic agents such as drugs, peptides, proteins, and genes has gained remarkable attention due to their significant contribution to biomedicine (Berry, 2005; Xu et al., 2006). The direct delivery of drugs and bioactive molecules without any protective structure results in easy degradation, which is a major hindrance. Thus, development of potent and proper delivery systems is needed (Tartaj et al., 2007). Nano- and micro-sized drug-delivery systems enable targeting of the desired location within the body, reducing the therapeutically effective drug concentration and diminishing the adverse side effects of drugs and enhancing their efficacy. These are the significant benefits of those drug carriers (Ritter et al., 2004).

For more than 30 years the appropriately engineered and therapeutic agent-loaded magnetic nanoparticles have become promising materials as drug-delivery systems due to their unique physical features and functional properties at the cellular and molecular levels of biological interactions (Shinkai, 2002; McBain et al., 2008). Characteristically, the magnetically active core of the magnetic nanoparticles that directs the particles to the desired and targeted area and for hyperthermia or for temperature-enhanced release of the drug, is coated with an effective shell that may carry the targeting ligand and imaging reporters (Chomoucka et al., 2010; Veiseh et al., 2010b). The type of therapeutic agent to be used specifies the localization of the drug either inside the shell structure or on the surface of the coating (Veiseh et al., 2010a,b). These tools have limited

non-specific cell interactions, controlled release, and potential in loading of a diversity of drugs. Their easily direction through the magnetic field and designing them with targeting ability are the advantages of using magnetic nanoparticles to deliver therapeutic compounds in biomedical applications (Tartaj et al., 2007).

Loading, transporting, and releasing of drugs are the main steps in using magnetic nanoparticles for drug delivery (Davis et al., 2008; Veiseh et al., 2010a). Primarily, the drug molecule must be distinguished according to the coating type of the nanoparticle and loading procedure. In addition, carrying and protecting of the drug using nanoparticles are required (Veiseh et al., 2010b). Subsequently intracellular uptake of the nanocarrier or its therapeutic payload should be accomplished (Sun et al., 2008a). Therefore, the size and surface characteristics of nanoparticles have gained importance in transportation across the cell membrane. Nanoparticles smaller than 50 nm and coated with prepotent material facilitate their crossing through the plasma membrane (Mrsny, 2007). Finally, the release of these therapeutic agents to target area in cell cytoplasm must be examined to allow the desired actions to take place and the optimal therapeutic efficacy should be determined via regulating the release mechanism and the therapeutic payload delivery (Sun et al., 2008a; Veiseh et al., 2010a).

In drug-delivery therapy the patients are subjected to an injection via the circulatory system with the magnetically based nanocarriers that are generally found as a biocompatible ferrofluid. The external, high-gradient magnetic fields are applied to accumulate the magnetic nanoparticles at a desired target site following the particles reaching the bloodstream. The enzymatic activity, changes in physiological conditions such as pH, osmolarity, or temperature and uptake by the target cells can induce the release of the drug once the nanocarrier is accumulated at the target site (Alexiou et al., 2000).

9.3.7.1 Effectiveness of the therapy

Several parameters must be taken into consideration for the effectiveness of using the magnetic nanoparticle-based delivery systems. For instance (1) the physical parameters, such as size and magnetic characteristics of the nanoparticles, field strength and geometry, and drug/gene-binding capacity; (2) physiological parameters, including depth of the target tissue, vascular supply, body weight (Neuberger et al., 2005; Sun et al., 2008a), reversibility and strength of the drug/carrier binding; and (3) hydrodynamic parameters (in intravenous or intraarterial administration) such as blood flow rate, ferrofluid concentration, infusion route, and circulation time (Lübbe et al., 1999). In addition, the location of the targeting is required to be in the site of slower blood velocity and the distance of the magnetic field to the target site is desired to be close for efficient drug-delivery systems (Dobson, 2006).

Increasing the magnetization can be used as another option to enhance the development of the drug-delivery systems (Jordan et al., 1998). The magnetic particles are preferred to be superparamagnetic to prevent the agglomeration when the magnetic field is no more active and to be situated in the circulation without

being removed by the body's natural filters, such as the liver or the immune system (Pankhurst et al., 2003).

9.3.7.2 Limitations of magnetic drug delivery

There are also several challenges that limit the success of the magnetically based targeted drug-delivery systems (Lübbe et al., 1999) including (1) accumulation of the magnetic nanocarriers in the blood vessels of the target site which may lead to embolization, (2) the removal of the magnetic field after releasing of the drug, (3) the possible toxicity of the magnetic nanocarriers, (4) the complicated explication of data obtained in animal models to humans due to several physiological parameters such as weight, blood volume, cardiac output. (Lübbe et al., 2001), (5) although small size is a necessity for magnetic carriers to gain superparamagnetic properties, directing the particles becomes difficult due to a magnetic response of reduced strength that a small size implies (Pankhurst et al., 2003), and (6) considerations of the magnetic field geometry must be taken to avoid the decrease in magnetic gradient with the distance to the target that leads to limitations in the drug delivery (Neuberger et al., 2005). These above limitations are not obstacles for magnetic carriers to be used in drug-delivery applications according to recent studies.

9.3.7.3 Magnetic nanoparticles in drug delivery

Magnetic carriers for delivery of therapeutic drug (prototype drug—adriamycin HCl) were developed and used for the first time by Senyei and Widder in the late 1970s (Senyei et al., 1978; Widder et al., 1978). Their studies were the beginning of the numerous *in vitro* and *in vivo* studies that emphasized the effectiveness of these powerful drug-delivery vehicles and even resulted in several clinical trials (Xu and Sun, 2013).

9.3.7.3.1 Chemotherapeutics

Currently, magnetic nanoparticles have been designed to attach to several chemotherapeutic agents (such as etoposide, doxorubicin, paclitaxel, and methotrexate) that enable the initiation of a therapeutic response through cytotoxic, cytostatic, or antineoplastic effects, for treatment of diseases ranging from rheumatoid arthritis to highly malignant cancer types (Schulze et al., 2005; Jain et al., 2005; Cole et al., 2011). In addition, unwanted adverse side effects of these agents can be eliminated by their integration into target-specific magnetic carriers. The interaction between the drug and magnetic nanocarriers can occur by physical complexation with hydrophobic drugs or covalent bonding with cleavable linkages for intracellular delivery (Veiseh et al., 2010a).

Two anticancer drugs, cisplatin and gemcitabine, were encapsulated into magnetically based nanoparticles by Yang et al. in 2006. These authors observed that the release behaviors of drugs varied, as cisplatin presented sustained release due to its ability of highly solubility in the oil phase and easy encapsulation, whereas

gemcitabine had rapid release behavior because of its hydrophilic character (Yang et al., 2006).

Kohler et al. investigated the effect of iron oxide nanoparticles loaded with methotrexate (MTX) frequently used as an anticancer agent for cancer treatment, on breast and brain tumor cells (Kohler et al., 2005, 2006). In one of these studies, MTX was covalently immobilized on the nanoparticle surface via a poly(ethylene glycol) self-assembled monolayer (PEG SAM) and a cleavable amide linkage. The cytotoxicity and cellular uptake levels of nanoparticle−PEG−MTX conjugate on 9L glioma cells were found to be higher than free MTX *in vitro* (Kohler et al., 2006).

There are also a small number of clinical trials of magnetic delivery systems to date. In 1996, Lubbe et al. published encouraging results for the first time for phase I studies of magnetically targeted drug-delivery systems (Lübbe et al., 1996a,b). In this work, epirubicine and nanoparticles interacted electrostatically. The effect of mechanical occlusion of the tumor with high concentrations of ferrofluid and delivery of epirubicin with low concentration through magnetic nanoparticles were investigated. As a result, epirubicin was effectively targeted and delivered to the tumor in 6 of 14 patients studied.

Koda and co-workers carried out the second clinical trial in 2002. The reserachers aimed at developing a magnetic particle carrier coupled with doxorubicin hydrochloride to conduct on 32 patients with hepatocellular carcinoma. Tumors were efficiently targeted in a total of 30 patients; 15 of these tumors remained stable or increased in size and only 5 showed progress in 17 patients (Koda et al., 2002).

A third clinical study was performed in 2004. In this study magnetically targeted doxorubucin was delivered to four patients with hepatocellular carcinomas through the hepatic artery using intraprocedural MRI. Within the results, it was observed that a selective targeting of between 64% and 91% of the tumor volume was affected by the drug in comparison to between 7% and 30% of the normal liver tissue (Wilson et al., 2004).

9.3.7.3.2 Radiotherapeutics

Radionuclides, radioactive nuclides, can also be used as cancer therapeutic agents in radiotherapy due to their ability to produce DNA-damaging free radicals and induce apoptosis (Brans et al., 2006). For enhancing the tumor uptake of the radionuclides and the effectiveness of the therapy, magnetic nanoparticles are developed and used under a magnetic field above the tumor area (Häfeli, 2004). Radioactive nuclides remain coupled with the magnetic nanoparticles during the treatment and this enables a reduction in the problems that occurred after the drug release from the carrier. For instance, when the radionuclide attached magnetic carrier is directed and localized at the tumor cell environment, it is not necessary for tumor cells to take up the agent. The effect of the radiation will reach the surrounding tumor tissue without detaching from the carrier. In 1995, Häfeli et al. investigated the effect of targeting of a magnetic carrier coupled to a β-emitter

(Y-90) in cell culture and animal studies. Using radionuclide with a magnetic field increased the radioactivity by 73% ± 32% compared to without a magnetic field (6% ± 4%) (Häfeli et al., 1995). Following this work, the efficiency of this type of system has been emphasized by the same group applying both yttrium-90 and rhenium-188 with magnetic carriers *in vitro* and animal models (Häfeli et al., 1997, 1999, 2001).

Recent studies have also revealed that conjugation with antibodies and peptides facilitated the direction of radionuclides away from healthy organs (Brans et al., 2006). So far, radioisotopes like ^{188}Re have also been used for intratumoral delivery by magnetic nanoparticles (Zhang et al., 2004, 2005; Cao et al., 2004; Liang et al., 2007). Particularly, SPIONs have been examined as radionuclides (Hamoudeh et al., 2008) and modified by histidine to enhance the attachment of radionuclides through chelate formation. With the same strategy, Chunfu et al. (2004) used albumin to functionalize and coat the prepared SPIONs with a diameter of 200 nm and reported labeling efficiency of approximately 90% was achieved. Following studies demonstrated that polyacrylamide (Zhang et al., 2005) or a silica shell (Cao et al., 2004) were also effective in coating magnetic particles for radiolabeling. In 2007, the same group synthesized amino-functionalized SPIONs to conjugate with Hepama-1, a specific monoclonal antibody against liver cancer cell line, SMMC-7721, to radiolabel with rhenium-188. As a result, ^{188}Re labeled immunomagnetic nanoparticles were found to be significantly effective in killing the cancer cells (Liang et al., 2007).

9.3.7.3.3 Biotherapeutics

In recent years, small interfering RNA (siRNA)-based RNA interference (RNAi) and oligodeoxynucleotide (ODN)-based antisense therapies have become promising strategies for the treatment of numerous diseases by demonstrating their effectiveness with silencing the specific gene (Juliano et al., 1999; Brigger et al., 2002; Guo et al., 2010). However, their intracellular activity depends on several parameters, such as protecting from enzymatic degradation by nucleases, crossing through plasma membrane, and being taken up by the target cells (Piao et al., 2013). To overcome these limitations recent studies have revealed that, thus far, various carriers have been developed for the effective delivery of siRNA and ODN and magnetic nanoparticle system is one of those carriers (Schillinger et al., 2005). Coating the MNPs with cationic polymers facilitates the complexation of negatively charged nucleic acids and endosomal release by enhancing acidification of endosomal vesicles (Huth et al., 2004).

Cationic polymer-coated transfection kits, called magnetofections, are available commercially and have been used effectively in laboratories for *in vitro* and *in vivo* applications (Schillinger et al., 2005; Mykhaylyk et al., 2007). The magnetofection principle is based on the use of magnetic fields to concentrate and penetrate nucleic acids (DNA, siRNA, dsRNA, shRNA, mRNA, and ODN) into the target cells (Plank et al., 2011). Currently there are several types of formulations

in the market optimized for different applications. For instance, OZ Biosciences (France) has developed PolyMag Neo, SilenceMag, NeuroMag, and ViroMag for DNA, siRNA primary neurons transfection, and viral applications, respectively.

In a study of Medarova et al. (2007) magnetic nanoparticles were also developed for silencing green fluorescent protein (GFP) production in a GFP-expressing xenograft tumor mouse model. In addition, they also performed imaging studies of siRNA delivery in tumors by MRI and near-infrared *in vivo* optical imaging (NIFR), simultaneously. Within this work, researchers accomplished a result indicating the feasibility of vivo tracking of MNP taken up by tumor with MRI and optical imaging.

Kumar et al. (2010) also designed SPIONs with specific ligand peptide (EPPT) targeting tumor-specific antigen uMUC-1 and a synthetic siRNA silencing antiapoptotic gene, *BIRC5*. A preferential tumor uptake of the nanodrug, a significant decrease in tumor growth rate, and visualization of the system by MRI and near-IR optical imaging were observed after giving the nanodrug to the mouse models intravenously.

Agrawal et al. (2009) synthesized dendrimer-conjugated magnetofluorescent nanoworms called dendriworms for *in vivo* delivery of EGFR targeting siRNA and had success against brain tumors in mice.

MNPs can also be modified with various materials, such as denrimers, to enhance its delivery properties. Pan et al. (2007) used polyamidoamine (PAMAM) dendrimer to coat the nanoparticles and antisense surviving oligodeoxynucleotide (asODN) to inhibit the survivin gene in particular cancer cells. According to the results, they suggest that PAMAM dendrimer-coated MNPs may serve as a promising gene transfection system promoting inhibition of cancer cell growth.

Jingting et al. (2011) revealed that magnetic iron oxide (Fe_3O_4)-dextran-anti-β-human chorionic gonadotropin (HCG) nanoparticles represented an appropriate vector for the delivery of heparinase antisense oligodeoxynucleotides (AS-ODN) and demonstrated strong resistance to degradation in choriocarcinoma tumors in mouse models.

Bioagents, such as peptides and antibodies, can function against several cell mechanisms, including activation of apoptotic/necrotic pathways, function blocking (e.g., interfering with cell adhesion, cell surface receptors, angiogenesis, or inhibiting protease and kinase action), and immune response stimulation and this makes them promising therapeutic molecules in medicine (Bhutia and Maiti, 2008).

The antibody Herceptin, also known as trastuzumab, was used often as a therapeutic agent in several studies. It recognizes the Her2/neu receptor which is overexpressed on cell surfaces of 20−30% of early-stage breast cancer tumors and regulates cell proliferation. The interaction of Herceptin with Her2/neu results in the inducing of cell death (Ross et al., 2004).

In a study that Huh et al. (2005) carried out Herceptin was used as a mAb targeting agent. In this study, researchers conjugated fluorescent dye-labeled

Herceptin to magnetic nanocrystals and this probe enabled both *in vitro*, ex vivo and *in vivo* optical detection of cancer by selective targeting of human cancer cells as well as with MRI.

Ito et al. (2004) produced magnetite nanoparticle-loaded liposomes and interacted with Herceptin for combination of antibody therapy and hyperthermia. They obtained a highly therapeutic effect causing antiproliferation of breast cancer cells.

Others have also revealed that Herceptin-modified magnetic nanoparticles have provided targeting and biotherapeutic characteristics to the nanoparticles (Funovics et al., 2004; Sakamoto et al., 2005).

Moreover, the epidermal growth factor receptor variant III (EGFRvIII)-attached magnetic nanoparticles increased the antitumor effect in *in vitro* glioblastoma cells and in an *in vivo* mouse glioma model (Hadjipanayis et al., 2010).

Successful delivery of chlorotoxin (CTX), a peptide ligand for Cl^-channels, blocks the channels and gains a significant role of inhibition of specific cancer types, through magnetic nanoparticles performed for brain tumors such as gliomas. In this study more enhanced cellular uptake and greater invasion inhibition rate were observed with CTX-loaded nanoparticles than without using nanoparticles (Veiseh et al., 2009b).

Veiseh et al. (2010b) developed a magnetic nanovector comprised of superparamagnetic iron oxide nanoparticle core coated with polyethylene glycol (PEG)-grafted chitosan, and polyethylenimine (PEI). Moreover, they demonstrated the combination effect of CTX and green fluorescence protein (GFP) siRNA in glioma cells by the nanovector modified with those agents and resulted in an improvement in tumor specificity and potency.

Magnetic liposomal nanoparticles were synthesized for the delivery of the transforming growth factor (TGF) β1, a cytokine that enhances the formation of bone and cartilage and therefore is effective in the treatment of articular cartilage defects, by Tanaka et al. (2005). They observed that the administration of the magnetoliposomes containing TGF β1 into the site of a cartilage defect in a rabbit model under magnetic force was found to be significantly more effective in the treatment.

9.3.7.3.4 Drug delivery with magnetosomes

BMs have primary amino groups on the surface and can be modified or interact with drugs via this group. Some drugs such as doxorubicin, epirubicin, mitomycin, and bleomycin contain an amino group per molecule. BMs can be linked to these drugs by using crosslinking agents. Protein drugs, nuclei acid drugs, radioactive isotopes, and chemotherapeutic drugs can be loaded onto BMs (Sun et al., 2011).

In a study reported in 2007 by Sun et al., doxorubucin, a cancer drug, was linked onto BMs with glutaradehyde. Cancer-suppressant effects of the DOX-loaded BMs (DBMs) were evaluated *in vitro* with H22, HL60, and EMT-6 cells and *in vivo* with BABL/c mice. Tumor tissue was formed with H22 cells in BABL/c mice *in vivo* studies. According to the results, DBMs inhibited the cell

proliferation and were more effective than free DOX. DBMs, DOX, and BMs were injected into tumor-bearing mice. While DOX and BMs suppressed the tumor in mice at 78.6% and 4.3% rates, the DBM suppression rate was reported as 86.8% (Sun et al., 2007).

In a study reported in 2013 by Deng et al., bacterial magnetosomes were isolated from *Magnetospirillum magneticum* AMB-1. Cytosine arabinoside (Ara-C), was used for acute leukemia treatment, and was linked to membrane by crosslinking of genipin (GP). The sizes of Ara-C-linked BMs (ABMs) are 72.7 ± 6.0 nm and zeta potential are -38.1 ± 9.1. Drug loading rate is $47.05\% \pm 0.64\%$ to BMs. Initial burst release was not observed during drug release from nanoparticles and Ara-C could be released 80% within 3 months (Deng et al., 2013).

9.4 CONCLUSIONS

The synthesis of magnetic nanoparticles covers an expansive range of compositions and versatile sizes. Several types of monodispersed spherical nanocrystals with adjustable particle sizes and compositions have been synthesized by a wide range of chemical synthesis applications: coprecipitation, microemulsions, thermal decomposition, sol−gel reactions, polyol processes, flow injection synthesis, sonolysis, and electrochemical and aerosol methods. Depending on the aim of the nanoparticle, synthesis of high-quality MNPs in a controlled environment by these methods has both advantages and disadvantages. By developing new approaches for having a result of homodisperse population of magnetic nanoparticles with controlled size and composition and aimed function exhibits detailed understanding of the synthesis mechanisms. Challenges with the nucleation and growth during the formation of the nanoparticle have to be accomplished in coming years with the development of more powerful methods. In spite of these numerous challenges, MNPs are accepted as the most promising tools to attain the desired sensitivity and efficacy required for future medical diagnostics and therapeutics. These current rapid developments in the synthesis and surface modification of MNPs have enabled the use of these NPs for more effective diagnosis and therapy.

The importance and potential of magnetic nanoparticles in various industrial processes as well as biomedical applications is becoming more predominant. For applications, using environmentally friendly materials for the production of nanomaterials as an avenue of green chemistry have gained huge interest, especially for the production of magnetic nanoparticles. The use of plant or extract as stabilizer or reducing agent to give successful results to overcome the side effect of other methods agents is promising. Furthermore, the green chemistry approach is economically favorable due to higher production amount and lower costs. Therefore, this method provides a promising way for sustainable production of magnetic nanoparticles as well as other metal nanoparticles.

As has been highlighted, to synthesize RES-evading particles, it is important to develop protection strategies especially to stabilize the naked magnetic

nanoparticles. For many applications, especially coating with natural polymer, against nanoparticle degradation is the oldest and simplest method. Natural polymers are non-toxic, biocompatible and, biodegradable. The combination of inorganic nanoparticles with organic materials for biotechnological and biomedical applications overcomes the limitations of nanoparticle occupation. Apart from this, it can also be used for further surface functionalization with ligands or drugs. Therefore, derivatization of the particles with natural polymers may block opsonization and help targeting therapy with magnetic nanoparticles.

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