



Magnetic Nanoparticles: An Overview for Biomedical Applications

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Abstract: The use of magnetic nanoparticles has greatly expanded for numerous biomedical applications over the past two decades due to their high surface area, size-dependent superparamagnetic properties, precision tunability, and easy surface modification. Magnetic nanoparticles can be engineered and manipulated with other nanoparticles and functional compounds to form multi-modal systems useful in theragnosis. However, superior biocompatibility, high loading efficacy, regulated drug release, and in vitro and in vivo stability are necessary for the efficient incorporation of these nanoparticles into physiological systems. In recent years, considerable advancements have been made and reported both in synthesis and application, given the broad range of biomedical-related prospective uses of magnetic nanoparticles. Here, in this review, we have highlighted some essential works, specifically related to the application of magnetic nanoparticles in drug delivery, magnetic hyperthermia, magnetic resonance imaging, magnetic particle imaging, biosensors, and tissue engineering.

Keywords: magnetic nanoparticles; biomedical applications; synthesis; characterization; hyperthermia; magnetic resonance imaging

1. Introduction

Nano-sized materials or particles and their formulations, due to their exclusive physical and chemical properties, are being extensively explored by scientists and researchers around the world for various industrial (material fillers, solar cells, superconductors, cosmetics, catalysis, etc.) and biomedical applications [1-3]. Among various organic, inorganic, and hybrid nanomaterials, magnetic nanoparticles have drawn special attention due to their interesting properties, such as large surface area, small size, ease of surface functionalization, and superparamagnetism. Apart from their applications in energy storage [4], magnetic fluids [5], environmental remediation [6], gyroscopic devices [7], catalysis [8], and magnetic inks [9], magnetic nanoparticles have been proven to exhibit noteworthy applications in the biomedicine field. These magnetic particles have been used for targeted delivery of drug/therapeutic agents [10], transfection of various viral/non-viral vectors and nucleic acids (referred to as magnetofection) [11], magnetic separation [12], magnetic field/light-induced hyperthermia [13], diagnostic imaging as contrast agents [14], tissue engineering [15], and various biosensing/immunosensing applications [16]. A number of formulations of these magnetic particles have been approved by the U.S. Food and Drug Administration for imaging and therapeutic applications, and some are under early clinical trials [17].

Magnetic hyperthermia and photothermal therapy have emerged as promising candidates for cancer therapy as they provide minimally invasive and site-specific treatment that ensures the minimization of the damage caused to normal tissues [13]. Preclinical models have shown the immense potential of these therapies in preventing metastasis [18]. Photothermal therapy is mostly used in combination with other forms of treatments and



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). therapies, most commonly chemotherapy. The combination of different modes of therapy has often shown synergism which would enhance efficiency and reduce dosage [19]. Magnetic resonance imaging is a reliable diagnostic technique that is non-invasive and gives detailed anatomical images. It utilizes contrast agents to enhance the contrast between the target site and the surrounding tissues [14]. Ferrite nanoparticles have been shown to be efficient contrast agents as they can influence the relaxation process and enhance the sensitivity and detection ability [20]. Among various magnetic nanoparticles, owing to their high biocompatibility and chemical stability, iron oxide nanoparticles have been exploited the most for various biomedical applications. Pure-metal-based magnetic nanoparticles, despite their high saturation magnetization values, are not suitable for in vivo applications due to their high toxicity and need to be properly surface-functionalized or protected [21,22]. In addition to stabilizing the magnetic nanoparticles, these protective materials can be further surface modified with other ligands or nanoparticles for desired applications. This review aims to give the readers an overview of the promising use of magnetic nanoparticles and their formulations for various biomedical applications. In the following sections, the composition, properties, and synthetic routes commonly adopted, followed by characterization techniques available for magnetic nanoparticles, are discussed briefly. Finally, we have concisely highlighted and discussed the applications and research undertaken over the years of these magnetic particles, particularly for magnetically-guided drug delivery, magnetic hyperthermia, photothermal therapy (PTT), magnetic resonance imaging (MRI), magnetic particle imaging (MPI), biosensors, and tissue engineering.

2. Composition and Properties of Magnetic Nanoparticles

Multiple types of magnetic nanoparticles have been fabricated. These nanoparticles are generally made up of pure metals (Fe, Co, Ni), metal alloys (CoPt, FePt), metal oxides, or ferrites [23,24]. Iron oxide nanoparticles (IONPs) are the most studied and explored. They are composed of crystalline magnetite (Fe₃O₄) or maghemite (γ -Fe₂O₃). Magnetite corresponds to an inverse spinel structure, where the anions (oxygen) form the cubic close-packed lattice, with Fe²⁺ occupying the octahedral holes, half the Fe³⁺ occupying the octahedral holes [25,26]. Maghemite is considered as Fe²⁺ deficient magnetite [27]. Ferrites are basically metal oxides with a general formula MFe₂O₄ and have a spinel structure. M is a divalent cation such as Fe²⁺, Co²⁺, Ni²⁺, Mn²⁺, Cu²⁺, Zn²⁺, etc. There must be Fe³⁺ in the chemical formula of ferrites. These transition metal-doped ferrite nanoparticles find a lot of applications in materials chemistry [28,29]. Co, Cu, Ni, and Zn doped ferrite nanoparticles have been readily used for the purpose of catalysis of reactions involving organic compounds [30].

The size, surface charge, intrinsic magnetic properties, stability in an aqueous medium, and toxicity must be taken into consideration in these nanosystems for biomedical applications. These physicochemical properties ultimately decide the in vivo fate of the particles. Generally, small-sized nanoparticles, typically less than 20 nm, are desirable. At such small sizes, ferri- and ferromagnetic nanoparticles attain a single magnetic domain, having only one large magnetic moment value. The particles exhibit superparamagnetic properties with high magnetic moments, fast response in the presence of an external magnetic field, and show negligible remnant magnetization and coercivity in the absence of an external magnetic field [17,31]. The superparamagnetic material loses its magnetization as soon as the magnetic field is withdrawn. This ensures a safe removal from the body as there is no risk of unwanted aggregation of the nanoparticles. However, these particles in a small size range with large values of surface-to-volume ratio tend to aggregate due to their high surface energy. Furthermore, the overall size of the nanoparticles must be small enough to avoid splenic filtration but sufficiently large to prevent renal clearance [32]. Naked or uncoated iron oxide particles are unstable and tend to lose their magnetism due to their oxidation in the air. Thus, to retain and enhance their stability in a physiological environment, magnetic nanoparticles must be properly surface-modified or coated. The coating material also alters the final surface charge of the magnetic nanoparticles. The

nanoparticles must be able to evade capture by RES (reticuloendothelial system) and have prolonged blood circulation time for their sufficient accumulation at the target site. Positively charged particles non-specifically bind to cells and have a small blood half-life value, while negatively charged particles are readily eliminated by the liver. Therefore, particles with a neutral surface or covered with neutral coating agents are preferred [33,34].

The stability (both compositional and colloidal) and biocompatibility of magnetic nanoparticles are critical determinants of their biomedical applications. For the stability of nanoparticles, their composition, size, shape, and surface-coating play major roles. It is generally known that smaller nanoparticles (below 10 nm in diameter) with adequate surface coating using inert polymers such as dextran or polyethylene glycol (PEG) remain both compositionally (does not erode) and colloidally (does not agglomerate) stable. Moreover, spherical nanoparticles are more stable than their anisotropic counterparts owing to relatively small surface area and uniform surface coating. Hot colloidal methods that lead to nanoparticle synthesis in a non-aqueous medium, followed by their aqueous dispersion using surface passivation with inert hydrophilic polymers, lead to better surface coating as compared to those synthesized directly in an aqueous media [35,36].

Superparamagnetic nanoparticles (temporary magnets) are better suited for biomedical applications than their ferromagnetic counterparts (permanent magnets), as in the former, the magnetism can be externally controlled. For most biomedical applications, it is desired that the particles remain mostly non-magnetic and become magnetic only within a specified spatio–temporal window during imaging and/or therapy [17]. Moreover, as superparamagnetic nanoparticles are usually smaller than ferromagnetic ones, they have better pharmacodynamics, biodistribution, and avenues of excretion.

Magnetic nanoparticles composed of transition metals such as Co, Ni, Mn, etc., are generally not favored for biomedical applications due to their high toxicity. In a free state, they induce the generation of reactive oxygen species (ROS), which results in oxidative stress and ultimately damages the cells. [37] Therefore, they need to be functionalized with biocompatible inorganic/organic materials. Iron oxide nanoparticles are generally considered biocompatible as iron ions obtained upon their metabolism can be added to the iron storage system present in the body [38]. The toxicity of magnetic nanoparticles is also dose-dependent. Magnetic nanoparticles are generally non-toxic at small dosages. However, at higher dosages, they can show toxic effects (e.g., iron ion-induced cell death via ferroptosis). Therefore, the required dosage of magnetic nanoparticles to suit a particular biomedical application is a key factor that determines their biocompatibility. Required dosage, in turn, is dictated by the targeting as well as the functional efficiency of the nanoparticles. Therefore, pure-phase (e.g., magnetite only) magnetic nanoparticles with higher saturation magnetization and/or MR relaxation time are better suited than mixed phase (e.g., magnetite and maghemite mixture) nanoparticles with lower functional parameters. Efficiently targeting diseased sites using externally applied magnetic fields or surface-attached biorecognition agents also lowers the required dosage of the nanoparticle, thus enhancing their biocompatibility [39].

3. Synthesis Methods

Magnetic particles that have dimensions in the nano range are desirable for various biomedical applications. Their properties, namely structural, magnetic, crystalline, and catalytic, depend upon the synthetic procedure. Further, functional magnetic nanoparticles made up of a magnetic core with appropriate protective coating or surface functionality are generally synthesized. Various single and multi-step synthetic procedures have been reported for the synthesis of the magnetic core of the required nanoparticle (Figure 1).



Figure 1. Synthesis methods commonly employed for the preparation of magnetic nanoparticles.

Co-precipitation or wet-precipitation is the oldest and most commonly used method to synthesize magnetic nanoparticles that have an average diameter below 50 nm. Simple as well as mixed metal oxide nanoparticles are obtained by using a stoichiometric mixture of aqueous salt solutions of two metal ions, and the particles are precipitated out upon the addition of an appropriate base (such as NaOH or NH_4OH). However, particles with irregular morphologies and broader size distribution are obtained by this method [40,41]. Highly crystalline monodisperse particles smaller than 30 nm can be obtained by the thermal decomposition method at high temperatures. In this method, an organometallic precursor in a non-polar boiling solvent is decomposed in the presence of a stabilizing agent such as 1-octadecene, hexadecyl amine, oleic acid, etc., at high temperatures. The commonly used organometallic precursors are metal carbonyls (such as Fe(CO)₅), Fe(Cup)₃ (Cup = N-nitroso-N-phenylhydroxylamine) or metal acetyl acetonates $[M^{n+}(acetylacetonate)_n]$ (where M can be Fe, Co, Mn, Ni, or Cr; n = 2, 3) [42–44]. The only limitation associated with this method is that hydrophobic particles coated with organic molecules are obtained, which need to be further functionalized with hydrophilic materials. Metallic nanoparticles can be obtained by heating volatile metal compounds in an inert atmosphere by the chemical vapor condensation (CVC) technique. Metal oxide nanoparticles can be obtained from these metal particles by their further oxidation. However, specialized instrumentation facilities are required, and the commonly used precursors, such as $Fe(CO)_5$, are toxic [45]. Hydrothermal synthesis at high temperatures (>100 °C) and high pressures (>1 atm), generally carried out in an autoclave, is another method to synthesize magnetic nanoparticles. This is a versatile one-pot synthesis method and can also be used to obtain surface-functionalized particles [46]. Nanoreactors or reverse-microemulsion systems (water-in-oil systems) stabilized by surfactants can be used to synthesize magnetic nanoparticles, and by varying the water: surfactant ratio (w_0 value), the size of the particles can be controlled [47,48]. Coating materials can also be added during synthesis using this method. In addition to these synthetic routes, electrochemical synthesis, combustion, laser-pyrolysis techniques, sol-gel, microwave-assisted and microbial synthesis, among others, have been reported [14,49–51]. Table 1 summarizes some of the synthesis methods along with their merits and demerits.

Synthesis Method	Procedure	Merits	Demerits	Ref.
Co-precipitation	magnetic particles precipitated out from stoichiometric mixture of metal ions aqueous solution by addition of base	hydrophilic NPs; simple and scalable broader size distribution		[40]
Thermal decomposition	organometallic precursor decomposed in non-polar boiling solvent at high temperatures in presence of stabilizing agent	monodisperse and size-controlled NPs; high yield	hydrophobic non-biocompatible NPs; toxic solvents	[42]
Hydrothermal	magnetic nanoparticles synthesized from aqueous metal solutions at high temperatures (>100 °C) and high pressure (>1 atm) in presence of an oxidizing agent	crystalline and water dispersible NPs of high purity	slow; large reaction time	[46]
Chemical vapor condensation	heating volatile metal precursors in an inert atmosphere	easy to prepare; small size NPs	precursors are toxic; specialized instruments required	[45]
Reverse microemulsion	particles formed inside hydrophilic core of micelles formed from surfactant molecules	simple room temperature synthesis; size controllability	low yield	[48]
Microwave	microwave-assisted heating of metal salt solutions in presence of oxidizing agent/base	homogenous heating and fast process	small scale production; expensive equipment	[49]
Electrochemical	magnetic particles synthesized from dissolution of sacrificial metal anode	size controllability by varying current density and potential	small scale production; broad size distribution	[52]

Table 1. Summary of methods employed	l for synthesis of magnetic nanoparticles.
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Magnetic core nanoparticles owing to their large surface area and energy, are sensitive to agglomeration and oxidation. The surface of the particles is rapidly oxidized in ambient conditions, which has a dramatic effect on their magnetic properties. In order to protect the particles from oxidation and forming clusters, they are generally encapsulated or surface functionalized by a biocompatible material. These materials also help in altering the surface charge of the nanoparticle and provide chemical functionalities for bioconjugation, affecting their biodistribution and pharmacokinetics. Several natural (dextran, albumin) and synthetic polymers (PEG, PAA) are mostly used to coat the surface of magnetic nanoparticles, either during the synthesis or after the synthesis [53–55]. However, the thickness of the coating can have a negative effect on the magnetic response of the particle [56]. Other coating materials include inorganic molecules such as silica, gold, and carbon [57–59]. Liposomes, which are phospholipid bilayer membrane vesicles, can encapsulate a large number of magnetic cores and deliver them simultaneously [60].

4. Characterization

Post-synthesis-prepared magnetic nanoparticles must be thoroughly characterized by using a variety of characterization techniques before employing them for various biomedical applications. The surface morphology and size of the magnetic nanoparticles can alter their physicochemical properties. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) are most commonly used for the determination of particle size and morphology of nanoparticles/nanomaterials [61]. TEM can also be used to visualize and measure the thickness of the coating material on the magnetic core particles, which is not possible using SEM [62]. The atomic force microscopy (AFM) technique is often employed for determining the size, morphology, and surface roughness of the nanoparticles [63]. Dynamic light scattering (DLS) or photon correlation spectroscopy (PCS) is used to find the hydrodynamic diameter and size distribution (polydispersity) of the particles [64]. Various analytical techniques such as energy-dispersive X-ray spectroscopy (EDS), X-ray fluorescence (XRF) spectroscopy, X-ray photoelectron spectroscopy (XPS), atomic absorption spectroscopy (AAS), and inductively coupled plasma mass spectroscopy (ICP-MS) are employed to determine the elemental composition of the magnetic particles [65]. Mössbauer spectroscopy can be used to differentiate between two types of iron oxide nanoparticles (magnetite and maghemite) and can also determine their relative concentrations in a sample [66]. The spatial distribution (elemental mapping) of elements in the nanoparticles can also be obtained using EDS analysis. The crystalline structure and phase purity of magnetic core nanoparticles can be ascertained using X-ray diffraction (XRD) spectroscopy. The sharp peaks obtained in the XRD pattern can be used to determine the size of particles using the "Scherrer equation" [65]. The selected area electron diffraction (SAED) patterns can be obtained using a TEM instrument and are often used to evaluate the crystal structure and other lattice parameters. Techniques such as XPS, zeta potential measurement, and Fourier-transform infrared (FT-IR) spectroscopy are useful in the surface characterization of nanoparticles [40]. XPS can identify as well as quantify every element (except H and He) and determine its exact electronic state present on the surface of the particle. The surface charge of the particles is determined by measuring the zeta potential. FT-IR spectroscopy is employed for identifying surface functionalities (mainly organic). These surface characterization techniques are useful in confirming the presence of coating or attachment of functionalities on the surface of the magnetic core [67]. In addition to determining thermal stability, thermal gravimetric analysis (TGA) can also determine the binding efficiency and coating formation on the particle's surface [68].

The magnetic properties are generally evaluated using a vibrating sample magnetometer (VSM) and superconducting quantum interference device (SQUID). VSM produces a hysteresis curve depicting changes in magnetization/magnetic moment of nanoparticles on varying the external magnetic field. Superparamagnetic nanoparticles have high saturation magnetization values with coercivity and remnant magnetization values equal to or very close to zero. Thus, a VSM curve passing through the origin with no open loop is obtained for superparamagnetic particles. The saturation magnetization value can reduce upon coating or surface functionalizing the particle [69,70]. SQUID monitors change in magnetization as a function of temperature and applied magnetic field. SQUID (up to 10^{-10} emu) is more sensitive as compared to VSM (up to 10^{-6} emu) [71]. Other less commonly used techniques for magnetic characterization are electron paramagnetic resonance (EPR) spectroscopy, the physical-property measurement system (PPMS), and Kerr spectroscopy based on the magneto-optic Kerr effect (MOKE) [72–74]. Table 2 provides a summary of the above-mentioned characterization techniques.

Table 2. Commonly used techniques for characterization of magnetic nanoparticles.

Characterization Technique	Application
Transmission electron microscopy (TEM)	morphology, size distribution, thickness of coating
Scanning electron microscopy (SEM)	surface morphology, size distribution
Atomic force microscopy (AFM)	size, morphology, surface roughness
Dynamic light scattering (DLS)	hydrodynamic size, polydispersity
Energy dispersive X-ray spectroscopy (EDS)	elemental composition, elemental mapping
X-ray fluorescence spectroscopy (XFS)	qualitative and quantitative elemental analysis
Atomic absorption spectroscopy (AAS)	qualitative and quantitative elemental analysis
Inductively coupled plasma mass spectroscopy (ICP-MS)	elemental composition
Mössbauer spectroscopy	differentiating between magnetite and maghemite
X-ray diffraction (XRD) spectroscopy	crystalline structure
Selected area electron diffraction (SAED)	crystal structure, lattice parameters
Zeta potential	surface charge
Fourier-transform infrared (FT-IR) spectroscopy	surface functionality, presence of coating
Thermal gravimetric analysis (TGA)	thermal stability, presence of coating
Vibrating sample magnetometer (VSM)	change in magnetization as function of external magnetic field
Superconducting quantum interference device (SQUID)	change in magnetization as function of temperature and external magnetic field
Electron paramagnetic resonance (EPR) spectroscopy	characterization of magnetic phases
Physical-property measurement system (PPMS)	direct or alternating current (DC/AC) magnetization measurement as a function of temperature and/or applied magnetic field
Kerr spectroscopy	imaging differences in magnetization on the magnetic material's surface

5. Biomedical Applications

Magnetic nanoparticles have been widely used in industrial and analytical purposes for some time. Lately, they have also been explored for applications in biomedicine owing to their biocompatibility and low toxicity. There has been a tremendous advancement in the biomedical use of magnetic nanoparticles, some of which have even made it to clinical trials [75]. As already mentioned, the properties of these nanoparticles can be easily controlled by the synthesis process, so these can be designed according to a particular biomedical requirement as these properties decide the pharmacokinetics and the distribution. The ease of controllability allows them to be very versatile when it comes to their biomedical applications. They can act as a delivery agent, providing magnetically-guided delivery, or can themselves act as theragnostic agents. They can be used as a contrast agent in magnetic resonance imaging and can function as a therapeutic agent for magnetic field or/and light-induced hyperthermia. In this review, we have described some of the important biomedical applications of magnetic nanoparticles in the following sections.

5.1. Magnetically-Guided Drug Delivery

The idea of magnetically-guided delivery simply means that the movement of magnetic nanoparticles in the body can be controlled by the influence of an external magnetic field. This concept came into existence over three decades ago. These nanoparticles loaded with a drug can be internalized at the target site and can be easily removed using an external magnetic field once the treatment is complete [76]. This reduces the dosage of the drug, which in turn reduces the side effects and toxicity to normal cells. This whole idea was employed for the very first time in the 1940s for the treatment of wastewater [77]. The metallic nanoparticle core is usually coated with another material in order to disperse them in an aqueous medium and prevent their agglomeration [78]. The most commonly used coating materials are: (a) Organic polymers, (b) Surfactants, (c) Metals (such as gold or silver), (d) Inorganic materials (such as silica or carbon), (e) Bioactive structures (such as proteins, ligands, or liposomes), etc. [79]. The first step in the process is the loading of the drug into these magnetic particles, which is performed on the coated layer on the magnetic nanoparticle. Then, these drug-loaded particles are injected into the bloodstream, and they are then carried to the target site using an external magnetic field. The particles easily accumulate in tumors and other inflammatory sites owing to the enhanced permeability and retention (EPR) effect [80].

5.1.1. In Vitro Studies

Kettering et al. synthesized IONPs coated with starch. The uptake of NPs was studied in the presence and absence of magnets in the breast carcinoma cell line. The number of nanoparticles taken up by the cells was quantified by measuring the iron content. It was observed that appreciably more nanoparticles accumulated in the tumor cells under the influence of a magnet [81]. Similarly, Chorny et al. synthesized magnetic nanocarriers by precipitating iron oxide-based ferrofluid using calcium oleate. These carriers were loaded with antioxidant enzymes (SOD and CAT). The carriers were analyzed for their uptake in the presence and absence of a magnetic field, and the cells were studied for the amount of iron accumulated in them. It was observed that while there was not any substantial uptake in the absence of a magnetic field, a 10-min magnetic field exposure effectively internalized particles in the cells. These nanocarriers also promoted stability to the loaded enzymes by protecting them from proteolytic degradation [82].

Carbon nanotubes (CNTs) were synthesized by Chen et al., which were co-loaded with iron oxide nanoparticles and CdTe quantum dots. These carriers effectively transferred doxorubicin (DOX, an anticancer drug) under the influence of an external magnet into the cells (HeLa cell line) (Figure 2) [10]. Hybrid hollow nanogel comprising iron oxide nanoparticles was synthesized by Chiang et al. by assembling the citric acid-coated particles with a graft polymer. This system was loaded with doxorubicin, and it was effectively transferred to HeLa cells with the help of an external magnet [83].

An attempt was made to overcome the blood–brain barrier (BBB) using magnetic targeting by Thomsen et al. Iron oxide nanoparticles were chosen as the carrier, and it was observed that there was an appreciable increase in the amount of drug that crossed the BBB in an in vitro model with the help of an external magnet [84]. In a recent study by Mushtaq et al., cobalt ferrite nanoparticles were coated with polymers, and the polymer layer was loaded with doxorubicin. It was observed that the release was considerably higher in the presence of a magnetic field. Moreover, the nanoparticles were proven safe after the in vitro studies [85].

The in vitro studies mentioned so far only employed a two-dimensional cell culture. Child et al. studied the effect of the magnetic targeting potential of nanoparticles in the cells that were grown in a three-dimensional model. Their findings confirmed the application of magnetically-targeted delivery in the three-dimensional model, which is more relatable to the in vivo system [86].



Figure 2. (a) Scheme for preparation of DOX-Fe₃O₄@CNT-HQDs-Trf Conjugates. (A) Hollow cavity of CNTs filled with Fe₃O₄ NPs (Fe₃O₄@CNT); (B) Coated with poly (sodium 4-styrenesulfonate) (PSS); (C) Exterior decorated with poly(allylamine) (PAH) capped SiO₂-coated quantum dots (HQDs) to give Fe₃O₄@CNT-HQDs; (D) Surface further decorated with transferrin (Trf) to give Fe₃O₄@CNT-HQDs-Trf; (E) Further loaded with DOX giving DOX-Fe₃O₄@CNT-HQDs-Trf. (b) Illustration of magnetically-guided delivery of prepared nanosystem. (c) Fluorescence microscopy images of HeLa cells incubated with (A) Fe₃O₄@CNT-HQDs, (B) Fe₃O₄@CNT-HQDs-Trf, and (C) DOX-Fe₃O₄@CNT-HQDs-Trf under applied magnetic field; (D) HEK293 cells directly labeled with DOX-Fe₃O₄@CNT-HQDs-Trf. (i) bright-field images, (ii) fluorescent images, (iii) merged images. Reproduced with permission from Ref. [10]. Copyright 2012 American Chemical Society.

5.1.2. In Vivo Studies

One initial study by Widder et al. involved magnetic nanoparticles loaded in albumin microspheres along with the drug Adriamycin. This carrier allowed a very high accumulation of the drug at the target site in the mice model with the help of a magnet. The accumulated amount was 100 times higher than the free drug [87]. In a study by Lübbe et al., iron oxide nanoparticles were coated with starch, and 4'-epidoxorubicin was chemically linked to it. The system was injected into nude mice through I.V. (intravenous) injection and was effectively directed towards the target site using a magnet with a strength of 0.5–0.8 T [88].

Mitoxantrone-loaded magnetic nanoparticles were used by Jurgons et al. to study magnetically-guided delivery in the rabbit model. The particles were attracted to an experimental tumor, which was completely abrogated [89]. A similar system was used by Tietze et al. to compare the biodistribution and efficacy of mitoxantrone when taken up magnetically as compared to when injected free in the rabbit. There was a significant improvement in both when magnetic targeting was employed [90].

Liposomes have been used to deliver paclitaxel, but these are prone to opsonization by the phagocytes in circulation and by macrophages in the liver and the spleen. This problem was addressed using magneto-liposomes by Zhang et al. The mice were first injected with breast tumor cells, and the magneto-liposome formulation was administered through an intravenous injection. The particles were guided to the site using a circular permanent magnet. The results confirmed that the particles effectively accumulated in the tumor site with appreciable efficiency and reduced side effects [91]. In another study by Béalle et al., magneto-liposomes with a high content of magnetic nanoparticles (as high as 30% of the volume fraction) were synthesized; they were named ultra-magnetic liposomes. They effectively portrayed magnetically-guided drug delivery in vitro as well as in vivo models [92].

IONPs coated with a polymer were used to target brain tumors by Chertok et al. The polymer was starch or gum Arabic, which was modified using polyethyleneimine (PEI). This formulation was injected into rats bearing orthotopic gliosarcoma through the intracarotid route. The particles effectively accumulated in the tumor owing to magnetic targeting [93]. Aryan et al. employed superparamagnetic iron oxide nanoparticles to successfully deliver therapeutic agents to the desired branch in a 3D carotid bifurcation using an external magnetic field. They concluded that the particle size and the magnet configuration were the main parameters that need to be fine-tuned to achieve optimum efficiency for magnetic drug delivery [94].

Mah et al. developed a new method for delivery of recombinant adeno-associated virus 2 (rAAV) using magnetic microspheres reversibly conjugated with rAAV vectors for targeted gene therapy. Magnetic microsphere-bound rAAV and free rAAV were injected intramuscularly in the right gastrocnemius of 129/SvJ mice. rAAV vector delivery via magnetic microspheres showed greater transduction efficiencies than delivery via a free vector alone [95].

In another work by Shen et al., a very complex nanoformulation was made comprising folic acid (FA), iron oxide, CdTe quantum dots, a tetrapeptide, chitosan, and camptothecin (CPT). CPT is an anticancer drug, quantum dots are a fluorescent probe, and folic acid is a targeting ligand (as folate receptor is overexpressed in several cancers). Chitosan acted as a gelation material that combined iron oxide and quantum dots to form a hybrid nanogel. The tetrapeptide (GFFG: Gly-Phe-Phe-Gly, or LGPV: Leu-Gly-Pro-Val) acted as a linker to connect folic acid to the core. This hybrid formulation was administered in sarcoma-bearing mice. The particles were effectively trapped inside the tumor using a magnetic field and were selective toward cancer cells (Figure 3) [96].



Figure 3. (**A**) Schematic diagram of chitosan-based luminescent and magnetic nanoparticles (CL-MNPs) with tetrapeptide (GFFG/LGPV) conjugated to folate; CLMNPs-tetrapeptide-FA composites.

(**B**) VSM curves for Fe₃O₄ NPs (MNPs), CLMNPs, CLMNPs-GFFG-FA, and CLMNPs-LGPV-FA at 305 K in an applied magnetic field. (**C**) TEM image of CLMNPs showing Fe₃O₄ NPs and CdTe quantum dots in chitosan matrix. (**D**) Sarcoma-bearing Male S-180 Kunming mouse guided by a magnet after being injected with CLMNPs-GFFG-FA (200 μ g/mL in PBS) through the tail vein. (**E**,**F**) Fluorescence microscopy images ($\lambda_{exc} = 488$ nm, 10×) of tumor slices influenced without ((**E**), control) and with (**F**) magnet. Reproduced with permission from Ref. [96]. Copyright 2012 American Chemical Society.

The accumulation of magnetic nanoparticles with the help of a magnetic field is easier for targets that are closer to the surface. As the distance from the surface increases, it becomes more difficult to maneuver the nanoparticles. Yellen et al. addressed this problem by inserting magnetic implants in the body of an organism near the target site [97]. Another attempt was made to improve magnetic targeting in deep tissues by Pouponneau et al. Micro-carriers loaded with iron-cobalt nanoparticles and doxorubicin were administered to rabbits, and then they were kept inside an MRI scanner. The path towards the target site was pre-programmed, and the imaging gradient coils moved the nanoparticles around in the body. It was inferred that by employing the scanner, magnetic targeting was effective at any depth [98].

5.2. Magnetic Hyperthermia

Hyperthermia is heat generation that is used to heat the nearby cells and kill them. It can be used as a localized form of therapy which is gaining a lot of attention lately. Heat therapy is a highly effective treatment of cancer as tumor cells have a higher metabolic rate, and as a result, they are more sensitive to heat than normal cells [99]. When magnetic nanoparticles are exposed to an alternating magnetic field, energy is absorbed and then released in the form of heat by either hysteresis losses or relaxation mechanisms (Neel or Brownian) [100]. The heat generated depends upon the nanoparticle (size and composition) and the magnetic field (frequency and amplitude) [101]. For ferromagnetic materials, heat is generated through hysteresis losses, but these are almost negligible for superparamagnetic materials. For superparamagnetic material, the heat generation is due to Neel and Brownian relaxation mechanisms. If the particle size of a superparamagnetic nanoparticle is less than 20 nm, then heat is produced due to Neel relaxation, while for bigger-sized particles, Brownian relaxation and hysteresis losses produce heat. The optimum size range that could be used for treatment should be around 10–100 nm, and the product of frequency and the amplitude of the external magnetic field should not exceed the value 5×10^9 Am⁻¹s⁻¹ to ensure a safe treatment in the patients. IONPs, owing to their biocompatibility, non-toxicity, and high saturation magnetization, have been well explored for hyperthermia applications employing core-shell formulations [102].

In one study by Huong et al., IONPs were coated with a polymer, O-Carboxyl methyl chitosan, and loaded with (a) curcumin or (b) curcumin and folate. The heat generation capacity of the system was evaluated using an external magnetic field (Amplitude = 60 Oe, and Frequency = 236 kHz). Both the formulations were injected into the mice harboring sarcoma-180 tumors, and the dosage was kept at 20 μ g/kg. Both systems effectively generated heat, and a better biodistribution was observed when the folate was attached to the particles [103].

Citric-acid-coated iron oxide nanoparticles were synthesized by Hardiansyah et al. and were loaded in liposomes, along with the anticancer drug doxorubicin. The MTT assay established that the magnetic nanocarriers do not cause any cytotoxicity in the cell line L-929. The particles showed a synergistic effect between chemotherapy and magnetic hyperthermia when studied in the cell line CT-26 (colorectal cell line) [104].

Cobalt ferrite nanoparticles (CFNPs) have also been explored from the perspective of hyperthermia and are known to display efficient heat generation even better than Fe_3O_4 . They have high saturation magnetization and magnetic anisotropy [105]. Silica-coated

CFNPs of two different sizes (14 nm and 40 nm) were synthesized. The heat generation was found to be size-dependent, and it was better for the smaller size [13]. Citrate-coated CFNPs were synthesized by Kahil et al., and the heating efficiency was evaluated in three different media (water, saline solution, and a cell suspension). There was a temperature increase of 47 °C in the saline medium within about 13 min at a particle concentration of 8 mg/mL (Frequency = 198 kHz, Field Intensity = 9.4 kAm⁻¹). The cell viability dropped to zero when the exposure time was 30 min [106].

In another study by Oh et al., DMSA (meso-2,3-dimercaptosuccinic acid) was used as a coating on CFNPs. These particles were loaded with doxorubicin, and their hyperthermia, as well as magnetically-targeted delivery potential, was explored. The release of the drug was higher when an external magnetic field was involved. The temperature increased to about 42–45 °C when the cells (MDA-MB-231) were exposed to the magnetic field (Frequency = 307 kHz, Field Intensity = 30 kAm⁻¹) for 20 min. The combination therapy of doxorubicin and CFNPs provided significant cytotoxicity [107].

A comparison was made between cube-shaped iron oxide (IONCs) and cobalt ferrite (Co-Fe NCs) nanoparticles with respect to in vivo tumor regression and heat production efficacy by Balakrishnan et al. Co-Fe NCs showed enhanced cytotoxic effects due to intrinsic cobalt toxicity, mild hyperthermia, and their unique ability to form growing chains at tumor sites under the influence of AMF (alternating magnetic field) (Figure 4) [108].

Ferrite composites have also been studied and employed for hyperthermia applications. A mixture of cobalt-doped and manganese-doped ferrite nanoparticles were coated with a graft copolymer by Iatridi et al. These particles were exposed to a magnetic field with a strength of 250 Oe and a frequency of 765 kHz, and a temperature of 44 °C was attained with an exposure time of about 10 min. The potential of these composite nanoparticles for magnetic resonance imaging was also evaluated, and they showed promising results [109].

A multimodal nanoformulation was made with the core of CFNPs, and coating of polydopamine and zeolitic imidazole framework by Yang et al. The system was loaded with two anticancer drugs; doxorubicin was loaded in the mesoporous cobalt ferrite core, and camptothecin was loaded in the shell framework. The delivery of drugs was stimulated by exposure to a laser light of 808 nm, and complete tumor regression was observed in HepG2 tumor-harboring mice after 9 h post-injection upon laser and magnetic-field exposure. These particles showed promising results in magnetic resonance imaging contrast enhancement as well [110].

Manganese ferrite nanoparticles (MFNPs) have also been used for various biomedical applications, including magnetic hyperthermia. They have a large magnetic spin magnitude which in turn gives larger relaxivity; this improves heat generation on exposure to the alternating magnetic field. In a study by Iqbal et al., silica-coated MFNPs were synthesized and coated with silica, and their usability in hyperthermia was evaluated. The nanoparticles were taken at a concentration of 2.6 mg/mL, and the temperature rose to a maximum value of 42 °C when the field of strength of 5.5 kAm⁻¹ was employed [111].

In another recent study by Ghutepatil et al., MFNPs were coated with amino-functionalized silica. The particles were very small, about 13 nm, and superparamagnetic in nature. The magnetic field used had a frequency of 265 kHz, and the strength varied in the range 167.6–335.2 Oe. The best results were obtained when the field strength was highest, and a specific absorption rate (SAR) of 261.53 Wg⁻¹ was obtained. The biocompatibility study of these particles was performed using the cell line L929. The results established that the particles were safe to use as they displayed no cytotoxicity [112].



Figure 4. Comparison of in vivo potency of IONC and Co-Fe NC. (**A**) Timeline depiction of treatment strategy adopted. The particles were injected on day 0 (0.7 mg IONCs or 0.7 mg Co-Fe NCs) followed by hyperthermia treatment (HT) using clinically approved AMF (frequency = 110 kHz; field strength = 20 kAm⁻¹). (**B**,**C**) TEM images of IONCs (18 ± 3 nm) and Co-Fe NCs (17 ± 2 nm), respectively. (**D**) Infrared images of treated mice on days 1, 2, and 3 (HT1, HT2, HT3). Black and white arrows indicate the temperature of the skin (T_{skin}) and tumor (T_{tumor}), respectively. (**E**) Δ T plot (Δ T = T_{tumor}-T_{skin}). (**F**) The Tumor growth curve indicates a greater reduction in tumor volume for animals treated with Co-Fe NCs. Reproduced with permission from Ref. [108]. Copyright 2020 John Wiley & Sons.

5.3. Photothermal Therapy (PTT)

Photothermal therapy is another localized and non-invasive therapy for cancer in which the heat is generated upon exposure to light (near-infrared, NIR) of suitable wavelength and power density. The common photothermal agents that have been studied and explored are gold nanoparticles, graphene, and carbon nanotubes. One shortcoming all these agents suffer is that their distribution is not specific, and they are not selective in accumulating inside the tumor. These need to be made selective towards the target, and in addition, an imaging moiety also needs to be attached to follow their trajectory in the body. Iron oxide has been used to give targeting potential to these photothermal agents and effectively accumulate them inside the tumor with the help of an external magnetic field. Several studies reported that these magnetic nanoparticles are made as the core, and the photothermal agents are attached to it.

The heating effects of Fe₃O₄ have been studied upon light exposure without the incorporation of any of the above-mentioned photothermal agents. Shen et al. observed that just after a 5 min exposure to the NIR laser, the temperature rose from 25 °C to 80 °C. Carboxymethyl chitosan-coated Fe₃O₄ was intravenously administered to mice. The particles could easily accumulate in the tumor with the help of a magnet. The tumor reached a temperature of 52 °C when irradiated with a laser of 1.5 Wcm⁻² for 5 min and was destroyed after two days. These particles also have the additional advantage of magnetic resonance imaging [113].

Different morphologies of iron oxide were synthesized by Chu et al.: spherical, wirelike, and hexagonal. All these were coated with a polymer (carboxy-terminated polyethylene glycol-phospholipid) to enable good water dispersion and evaluated as photothermal agents. Three different lasers were employed, and the temperature rise was studied as a function of concentration and laser wavelength. The different morphologies were compared for their heat-generating efficiency. These nanoparticles were employed for in vitro studies using the Eca-109 cell line (esophageal cancer cells) and employed for in vivo studies using a mice model [114].

In another study by Chen et al., a thermal decomposition method was utilized to synthesize highly crystallized iron oxide nanoparticles, which were coated with the polysiloxane-based copolymer. When this formulation was irradiated with an 885 nm laser with a power density of 2.5 Wcm⁻², a temperature increase of 33 °C was observed. The formulation was administered in a mice model bearing SUM-159 tumors. The same conditions were employed to irradiate the tumor, and complete tumor regression was achieved in a period of three weeks. There was not any relapse of cancer when observed for three months post-treatment. The effect of lattice arrangement on heating efficiency was also established [115]. The potential of other ferrite nanoparticles should also be explored for photothermal therapy.

In another study by Espinosa et al., the bimodal nature of iron oxide nanoparticles was exploited by employing a magnet as well as light-induced hyperthermia (Figure 5). Iron oxide nanocubes were chosen for this study as they are known to be very efficient magnetic hyperthermia agents. The aqueous dispersion of particles was simultaneously exposed to both an 808 nm laser and an alternating magnetic field. The heating efficiency was five times that obtained from magnetic hyperthermia (MHT) alone. The magnetic hyperthermia efficiency in the cells is known to be inhibited by the phenomenon of intracellular confinement during in vitro studies. This was overcome by simultaneous laser excitation, and the efficiency was as high as 15 times when both magnet and light were employed at the same time. This dual treatment ensured complete apoptosis-mediated cell death. For in vivo studies of the solid tumor in mice model, individual therapy only provided a partial reduction in tumor growth while the dual-mode resulted in full tumor regression [116]. This sort of synergism has also been observed in other forms of combination therapy employing various modes of treatment, such as chemotherapy, photothermal therapy, magnetothermal therapy, and photodynamic therapy. Magnetic nanoparticles already have many multiple applications in targeted delivery as well as in therapy. This efficiency and usability of these particles can further be increased by loading a suitable theranostic agent on them and observing the enhancement in treatment and reduction in side effects and toxicity.



Figure 5. (**A**) TEM image of iron oxide nanocubes. (**B**) Schematic illustration of experimental device consisting of a magnetic coil and 808 nm laser for combined magnetic hyperthermia and photothermal therapy. (**C**) Thermal images acquired of cells by infrared camera after in vitro treatment. (**D**) Thermal images of mouse (in vivo study) acquired by infrared camera after treatment. (**E**) Tumor growth curve for a period of 8 days. Reproduced with permission from Ref. [116]. Copyright 2016 American Chemical Society.

5.4. Magnetic Resonance Imaging (MRI)

In radiology, magnetic resonance imaging is a valuable imaging technique utilized for the clinical diagnosis of diseases or anomalies. Hydrogen atoms (protons), which are ubiquitously present, are affected differently by the applied magnetic field based on their position and the atoms by which they are surrounded in the body. These protons are made to align and precess around a strong magnetic field followed by perturbation using a radiofrequency pulse. The perturbed protons come back to their equilibrium state by two independent relaxation pathways, T_1 (longitudinal or spin–lattice) and T_2 (transverse or spin-spin) relaxation. The variation in these processes is monitored and used to generate images showing a contrast between tissues of different anatomy/pathology. Exogeneous contrast agents, which generally shorten (sometimes increase) relaxation time, are generally employed to improve visibility and obtain clearer images. Traditional gadolinium (Gd)based contrast agents are paramagnetic and show relatively low relaxivities values. At low levels, Gd-chelates can shorten T_1 relaxation time, while high levels are required for T_2 shortening, which can be toxic [117,118]. Superparamagnetic nanoparticles are a relatively new class of MRI contrast agents and can shorten both T1 and T2 relaxation time at low concentration/dose in comparison to paramagnetic agents. They can be size-tailored, appropriately functionalized, and adjusted according to the target tissue. These nanoparticles show more pronounced T_2 relaxation due to the large difference in susceptibility of the surrounding medium and particles. The protons diffuse through this magnetic field gradient, resulting in the dephasing of magnetic moments, leading to shortened T₂ relaxation time. Thus, they can be used to generate negative/hypointense contrast using T_2 pulse sequences. However, the thickness of the coating material on nanoparticles can hinder the T₁ shortening as it requires close interaction between the contrast agent and protons [32]. Superparamagnetic iron oxide nanoparticles (SPIONs) have been extensively examined as MRI contrast agents for the diagnosis and management of tumors. Kim et al. synthesized chitosan-coated SPIONs, which showed enhanced

MRI contrasts in vitro in comparison to Resovist, a commercially available MRI contrast agent [14].

In a study by Chee et al., they designed a library of 86 short peptides and ligands to functionalize iron oxide nanoparticles to form ultra-small superparamagnetic iron oxide nanoparticles (USPIONs). They chose the ligand whose long-term stability in physiological conditions, general binding to living cells, and lack of cytotoxicity at high doses provided IONPs with the best properties for application in vivo (Figure 6). When compared to commercial MRI contrast agents (Magnevist and Resovist), IONPs functionalized with this ligand demonstrated a significant improvement in the contrast between the liver tumor and healthy liver tissue [119].



Figure 6. (**A**) Schematic illustration of preparation of peptide-coated USPIONs as MRI contrast agents. (a) Phase transfer of oleic acid coated USPIONs from chloroform to aqueous medium by ligand exchange with TMAOH; (b) Peptide coating by ligand exchange; (c) Determining best-performing peptide-coated USPION (i.e., 2PG-S*VVVT-PEG4-ol coated USPION) by funnel selection process for in vivo MRI application. (**B**) In vivo MR images of NCr nude mice in upper (coronal) and lower (transverse) plane after 0, 0.5, and 1 h of intravenously injecting 2PG-S*VVVT-PEG4-ol coated USPIONs. (**C**) Quantitative analysis of liver contrast in NCr nude mice after accumulation of peptide-coated USPIONs (dose: 1.0 mg Fe per kg). (**D**) In vivo MR images in upper and lower plane of orthotopic xenograph liver tumor model after 0, 0.5, and 1 h of intravenously injecting 2PG-S*VVVT-PEG4-ol coated USPIONs. (**E**) Quantitative analysis of contrast-to-noise ratio (CNR) after accumulation of peptide-coated USPIONs. (**E**) Quantitative analysis of contrast-to-noise ratio (CNR) after accumulation of peptide-coated USPIONs (dose: 2.0 mg Fe per kg). (TMAOH: Tetramethylammonium hydroxide; 2PG = (PO₃H₂)-O-CH₂-CO-; S* = Ser(PO₃H₂); PEG4-ol = $-NH-(CH₂-CH₂-O)_{3}-CH₂-CH₂-OH;$ T: Threonine; V: Valine). Reproduced with permission from Ref. [119]. Copyright 2018 American Chemical Society.

Lymphotropic SPIONs were shown to correctly detect and identify otherwise occult lymph-node metastases by Harisinghani et al. The SPIONs were able to enter the lymph nodes by interstitial-lymphatic fluid and, in association with high-resolution MRI, revealed small nodal metastases, which cannot be diagnosed by conventional MRI. This non-invasive method has important implications for staging and treatment of prostate cancer in comparison to the dissection of lymph nodes by laparoscopic surgery, which is the standard procedure for diagnosing prostate cancer [120].

Iron oxide nanoparticles have been shown to cross the BBB. Two SPIONs, Ferumoxide (incompletely dextran-coated iron oxide nanoparticles), which is approved by the U.S. FDA for hepatic imaging, and Ferumoxtran-10 (completely dextran-coated iron oxide nanoparticles), were evaluated by Varallyay et al. for the delineation of intracranial tumors and compared with conventional Gd-chelates for MRI in humans. Ferumoxides did not show any significant T_1/T_2 signal intensity changes due to incomplete coating resulting in rapid opsonization and short blood half-life. However, Ferumoxtran-10 showed imaging enhancement comparable to Gd-chelate [121].

Magnetic nanoparticles have also been explored for their potential as MRI contrast agents for cardiovascular disease imaging. VCAM-1 (vascular cell adhesion molecule 1) or CD106 (cluster of differentiation 106), an immunoglobin superfamily protein expressed on endothelial cells stimulated by cytokines, actively participates in the development of atherosclerosis and thus can act as a biomarker. Second-generation VCAM-1 targeting fluorescent magnetic nanoparticles, VINP-28 (VCAM-1 internalizing nanoparticles) made up of iron oxide core were synthesized by Nahrendorf et al., which were able to selectively target macrophages and endothelial cells that expressed VCAM-1 [122].

Magnetic nanoparticles can also serve as molecular MR imaging agents, which involves the characterization as well as the quantification of various biological processes at the molecular and cellular levels. Cell migration, enzyme activities, and apoptosis have been investigated using these particles. Feridex (ferumoxide), which is used for hepatic/liver imaging, is rapidly taken up by healthy liver cells (Kupffer cells), which appear to be hypointense in MR images. Whereas, due to the lack of Kupffer cells in the presence of liver tumor, the signal intensity is not altered in affected areas [123].

Magnetic nanoparticles, in conjunction with other imaging agents such as radioisotopes and fluorescent molecules, can serve as multimodal imaging systems. These multimodal systems can improve diagnostic quality by combining the strengths of individual imaging techniques. In a study by Xie et al., dopamine iron oxide nanoparticles embedded in the HSA matrix and labeled with ⁶⁴Cu-DOTA (radioactive diagnostic agent) and Cy5.5 (fluorescent dye) were tested in the U87MG xenograft mouse model for in vivo triple-functional MRI/PET/NIRF multimodal imaging (Figure 7) [124].

Magnetic nanoparticles have also been explored for applications in theranostics. Hayashi et al. employed a core–shell iron oxide nanoparticle cluster containing DOX for MRI-guided magnetic thermochemotherapy. The anticancer drug DOX was released from the core–shell nanosystem in response to AMF. The heat generated upon the application of AMF softened the shells, thereby facilitating drug release. The release of DOX continued even after the removal of AMF for continuous chemotherapy. The core–shell nanosystem accumulated in the abdominal tumors of the mice, facilitating visualization by MRI. This nanosystem exhibited higher therapeutic efficacy than chemotherapy and magnetic hyperthermia alone [125].

In another study by Gao et al., polypeptide nanoparticles loaded with the anticancer drug cisplatin and SPIONs were developed for stimulus-responsive MRI-guided chemo-ferroptosis combination therapy. In vivo investigation showed preferential accumulation in the tumor location by T₂-weighted MRI, and this system released cisplatin and Fe²⁺/³⁺ (for ferroptosis) in response to the acidic tumor microenvironment [126]. Chan et al. synthesized multifunctional nanocomposites made up of CTAB-functionalized kaolinite carrying FePt NPs and DOX. The nanocomposites served as magnetic hyperthermia agents



as well as simultaneously visualizing and treating hepatocellular carcinoma cells in a mice model [127].

Figure 7. (**A**) Triple-functional ⁶⁴Cu-DOTA and Cy5.5-labeled iron oxide nanoparticles. (**B**) NIRF (near-infrared fluorescence) in vivo images. (**C**) In vivo PET (positron emission tomography) images. (**D**) MRI images pre-and post-injection (after 18 h). Reproduced with permission from Ref. [124]. Copyright 2010 Elsevier.

5.5. Magnetic Particle Imaging (MPI)

Currently available imaging techniques, such as X-ray, ultrasound, X-ray computed tomography (CT), and MRI, are very helpful for detecting tissue architectural changes that typically accompany cancer. However, the native contrast of tumors, especially metastatic and diffuse tumors, generally does not differ from the healthy tissues for effective diagnosis. An exciting non-invasive (radiation-free) new imaging modality, magnetic particle imaging (MPI), was introduced in 2005 by Gleich and Weizenecker. [128] MPI is a highly sensitive tracer imaging technique that can directly measure the precise location and concentration of SPIONs in vivo. The SPION electronic moment, which is 22 million times stronger than nuclear MRI moments, is imaged using MPI. It takes advantage of the non-linear magnetization of SPIONs. When a time-varying excitation field is applied during signal production, SPIONs instantly "flip" producing a signal in the receiver coil. A high magnetic gradient is used to establish a field-free point (FFP), a single point in space having a magnetic field zero, in order to localize this signal. When a time-varying excitation field is added to the imaging field of view, SPIONs outside the FFP become saturated, and only the SPIONs at the FFP retain their ability to flip. The observed signal is assigned to a spot on the image that corresponds to the precise location of FFP in space. Due to the absence of background signal from the tissues, as only the signal from the particles is received, MPI provides high-resolution images with excellent contrast and a high signal-to-noise ratio. The iron-oxide tracers employed for MPI are safer compared to the tracers used in MRI and CT [129,130].

Song et al. explored MPI by using Janus semiconductor polymer-coated Fe_3O_4 nanoparticles. These NPs demonstrated seven-fold intensity compared to commercial MRI tracers and were able to image as few as 250 cancerous cells in vivo. As compared to 10^9 cells, which is the current clinical imaging capability, this system showed significantly higher resolution and sensitivity [131].

Szwargluski et al. employed MPI for the detection of intracranial hemorrhage (cerebral bleeding) in C57BL/6 mice. They were able to identify intracranial hemorrhage within 3 min following the intravenous infusion of SPIONs (tracer) and were also able to track hematoma expansion in real-time. Based on their physical features, core size, viscosity, and temperature, the tracers can be distinguished using multi-contrast MPI. By using in vivo multi-contrast MPI, they could differentiate between coagulated and non-coagulated blood in the hematoma, which is a valuable source of information during surgical procedures [132].

A key challenge associated with magnetic hyperthermia is the unwanted accumulation of SPIONs at off-target organs, such as the liver and spleen, resulting in collateral heat damage. Tay et al. developed an image-guided theranostic platform by combining MPI with magnetic hyperthermia (Figure 8). They demonstrated the MPI-guided spatial localization of magnetic hyperthermia to tumors and minimized collateral damage to the liver at a distance of 1–2 cm [133].



Figure 8. Workflow of treatment with SPIONs accumulated in liver and tumor of a U87MG xenograft mouse. Step 1: MPI image scan showing high contrast visualization of biodistribution of SPIONs in healthy organs (liver) and region of concern (tumor). Imaging parameters are set such that SPIONs do not generate heat; Step 2: Tumor region is selected to localize hyperthermia; Step 3: The magnetic field gradients are set such that FFR (field-free region) is centered at tumor; Step 4: Heat scan performed such that heating is localized at tumor (FFR) and sparing the nearby liver. Reproduced with permission from Ref. [133]. Copyright 2018 American Chemical Society.

5.6. Biosensors

Magnetic nanoparticle-based biosensors are receiving increased interest in the field of biomedicine. In comparison to traditional biosensors, they are known to show much higher sensitivity, high signal-to-noise ratio, low detection limit, and shorter analysis time. Magnetic nanoparticles are generally functionalized with biomolecules for detecting viruses, cells, proteins, nucleic acids, enzymes, etc. [16].

In a study by Wang et al., gold-coated iron oxide ($Fe_3O_4@Au$) magnetic SERS (surfaceenhanced Raman scattering) nanotags were synthesized for simultaneously detecting two respiratory viruses (HAdV and FluA H1N1). The tags comprised three parts: a superparamagnetic $Fe_3O_4@Au$ core for magnetic and SERS activity, a double layer of DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid)) dye for strong Raman signal, and specific virus-capturing antibodies on the surface. Based on the remarkable performance of the nanotags, a new SERS-LFIA (lateral flow immunoassay) strip was further developed, which showed 2000 times more sensitivity in comparison to the standard gold strip and, therefore, can be beneficial for the early detection of respiratory viruses (Figure 9) [134].



Figure 9. (**A**) Synthesis scheme for Fe₃O₄@Au SERS nanotags modified with antibodies. (**B**) Operating procedure of prepared SERS-LFIA strip for simultaneous detection of HAdV and FluA H1N1 viruses. Reprinted with permission from Ref. [134]. Copyright 2019 American Chemical Society.

The enzyme-mimicking activity (nanozyme) of iron oxide nanoparticles has also been exploited for the development of biosensors for detecting L-cysteine, glucose, hydrogen peroxide, etc. The peroxidase-mimicking ability of these particles was used to detect circulating autoantibodies in serum/plasma samples. These autoantibodies are important biomarkers for early cancer detection. Nanoporous gold-loaded ferric oxide nanocubes (Au-NPFe₂O₃NC) were synthesized by Masud et al., which were used as colorimetric and electrochemical molecular sensors for detecting p53 autoantibody by the naked eye in serum/plasma samples [135].

In another study by Kim et al., the enzyme-mimicking catalytic activity of iron oxide nanoparticles was further enhanced by decorating platinum on the surface of the particles (Fe₃O₄-Pt/core–shell NPs or MPt/CS NPs). Human chorionic gonadotropin (hCG) is an important biomarker for pregnancy. The anti-hCG antibodies modified nanoparticles (hCG/Ab-MPt CS NPs) were integrated into LFIA strips, which showed twice the sensitivity as compared to standard gold strips [136]. Thus, LFIA strips, in combination with magnetic nanoparticles, can act as better point-of-care testing devices.

5.7. Tissue Engineering

Tissue engineering (TE), an interdisciplinary field, has been able to translate and combine fundamental principles of engineering, biology, and chemistry for the development of effective and functional materials/devices for restoring, maintaining, and replacing biological tissues [137]. One of the interesting applications of magnetic nanoparticles is tissue engineering. Magnetic nanoparticles can bind to the cell membrane or can be internalized in the cell, making it possible to regulate and control cellular functions using an external magnetic field. They can also be integrated with three-dimensional scaffolds, which are biomaterials (hydrogels, films, fibers) that are known to mimic the extra-cellular matrix of the tissue and act as a support for cells to attach and proliferate. This approach of combining magnetic particles with scaffolds has resulted in their potential for the treatment of diseases and tissue repair, including muscle, bone, cardiac, and nerve tissue regeneration [15,138]. Cells can convert physical/mechanical stimuli into biochemical reactions by a pathway called mechanotransduction. These mechanical stimuli help promote cell proliferation.

In a study by Cartmell et al., RGD (tripeptide Arg-Gly-Asp)-coated CrO_2 magnetic nanoparticles adhered to human osteoblasts, and the cells were exposed daily to a magnetic field for 21 days. After 21 days, the osteoblasts were viable and proliferated, and the bone matrix mineralized as compared to the control (without applied magnetic field) [139].

Magnetic force-based TE (Mag-TE), a novel strategy was introduced by Ishii et al. in which mesenchymal stem cells (MSC) were labeled with magnetic cationic liposomes (MCL), and with the help of magnetic force, they were able to create an MSC sheet (\sim 300 µm thickness) comprising of 10–15 layers of MSC cells. The MSC sheet was engrafted into nude mice having ischemic tissue and successfully stimulated revascularization [140].

Another study by Kito et al. reported the formation of iPS (induced pluripotent stem) cell sheets by the Mag-TE technique for reparative angiogenesis. Magnetized fetal liver kinase-1⁺ (Flk-1⁺) cells were obtained by incubating mouse iPS cell-derived Flk-1⁺ cells with MCLs and were subsequently mixed with extra-cellular matrix (ECM), which formed iPS cell sheets on applying magnetic force. The iPS sheets were implanted in nude mice and were able to accelerate the revascularization of ischemic tissues (Figure 10) [141]. Thus, magnetic nanoparticles are "functional" materials and can serve as important tools for a wide range of applications in regenerative medicine and tissue engineering in the near future.

Table 3 summarizes some other formulations of magnetic nanoparticles that have been reported for biomedical applications.

Magnetic Nanoparticles Formulation	Composition	Size (nm)	Biomedical Application	In Vitro/In Vivo Application	Ref.
Fe ₃ O ₄ -loaded mPEG-b-P(DPA-DE)LG micelles	hydrophobic SPIONs encapsulated in hydrophobic core of mPEG-b-P(DPA-DE)LG micelles; Dopamine (DPA) acted as anchor for SPIONs for facilitating self-assembly of polymer	DLS: 147 ± 3 TEM: ~120	pH-responsive nanocarrier for MRI	Rat model with cerebral ischemia	[142]
MION@CMC-DOX	MIONs stabilized and coated by biocompatible CMC layer and bioconjugated with DOX via amide bonds	DLS: 38 ± 2 TEM: 7 ± 2	combined chemotherapy and magnetic hyperthermia	HEK 293T and U87 cells	[143]
Nano-in-microparticles (NIMs)	dry powder containing SPIONs and DOX in lactose matrix	DLS: NA TEM: 1000–5000	magnetically-targeted pulmonary delivery	A549 cells; Male Balb/c mice	[144]
Zinc doped iron oxide NPs conjugated to DR4 Ab	thiolated Zn _{0.4} Fe _{2.6} O ₄ NPs conjugated with protein A through sulpho-SMCC crosslinker; protein A binds with the Fc region of DR4 Ab	DLS: 32 TEM: NA	non-invasive approach to turn "on" apoptosis cell signalling using magnetic field	DLD-1 cells; zebrafish	[145]
HMCuS/DOX@IONP-PEG	DOX loaded positively charged hollow mesoporous CuS NPs capped with negatively charged IONPs and further surface conjugated with PEG	DLS: 124.5 ± 3.8 TEM: NA	multimodal system for combined chemotherapy, MRI, and NIR-light activated PTT and PDT	MCF-7 cells; tumor bearing mice	[146]
Hyaluronic acid-modified Mn-Zn ferrite (HA-MZF) nanoparticles	Mn _{0.6} Zn _{0.4} Fe ₂ O ₄ (MZF) NP encapsulated in micelles using NH ₂ -PEG ₂₀₀₀ -PCL ₃₄₀₀ to form MZF-NH ₂ micelles and further surface modified with HA using EDC/NHS crosslinking reaction	DLS: 195 TEM: NA	receptor (CD44) mediated targeted delivery with combined hyperthermia and radiotherapy	A549 cells; Male Balb/c mice	[147]
Photosensitizer (Ce6) and cancer cell membrane (CCM) coated SSAP (SSAP-Ce6@CCM)	oleic acid coated SPIONs embedded in crosslinked matrix of styrene and AA, and further modified with PEI using carboxyl groups of AA; Ce6 adsorbed onto the surface and finally cloaked with CCM	DLS: 192 TEM: NA	PDT and dual-modal MR/NIR fluorescence imaging	SMMC-7721 tumor-bearing mice	[148]

Table 3. Some of the important magnetic nanoparticle formulations employed for various biomedical applications (NA: not available).

Magnetic Nanoparticles Formulation	Composition	Size (nm)	Biomedical Application	In Vitro/In Vivo Application	Ref.
Bisphosphonate conjugated dextran-coated Fe ₃ O ₄ (Bis/Dex/Fe ₃ O ₄) NPs	bisphosphonate (alendronate) covalently attached by EDC/NHS crosslinking to carboxyl surface modified dextran-coated Fe ₃ O ₄ NPs	DLS: NA TEM: 20	radiofrequency-induced thermolysis of osteoclasts for treatment of osteoporosis	male Wistar rats	[149]
Methicillin and SPIONs containing nano polymerosome	hydrophobic SPIONs and hydrophilic methicillin containing multi-compartment nano polymerosome made up of amphiphilic dieblock co-polymer (mPEG-PDLLA)	DLS: 83 ± 6 TEM: NA	penetration of <i>Staphylococcus</i> <i>epidermidis</i> biofilm with the aid of magnetic field	male Wistar rats	[150]
Fe ₃ O ₄ @Ag-Van MNPs	cationic PEI modified Fe ₃ O ₄ NPs were coated with Ag colloidal NPs to form Fe ₃ O ₄ @Ag NPs; vancomycin (Van) was conjugated to carboxyl modified Fe ₃ O ₄ @Ag NPs	DLS: NA TEM: 290	SERS-based biosensor for detecting and capturing bacteria	S. aureus 04018, E. coli BL21 strains	[151]
Mtb (<i>Mycobacterium</i> <i>tuberculosis</i>) antibody (MTBsAb) conjugated SPIONs	dextran-coated on SPIONs crosslinked by epichlorohydrin; treated with EDBE to generate amino groups at dextran ends followed by addition of SA to form SPIO-EDBE-SA; finally conjugated with MtbsAb	DLS: NA TEM: 3.8 ± 0.4	ultrasensitive MR imaging for diagnosis of extrapulmonary tuberculosis	C57BL/6 mice bearing Mtb granulomas	[152]
Antibody (Ab ₁) conjugated Au functionalized CoFe ₂ O ₄ (CoFe ₂ O ₄ @AuNPs@Ab ₁) NPs	primary antibodies (Ab ₁) immobilized by Au NPs functionalized CoFe ₂ O ₄ NPs	DLS: NA TEM: 80	SERS-based immunosensor for detecting NT-proBNP (biomarker for heart failure)	-	[153]
Magnetic (IONP) and upconverting (UCNP) nanobeads (MUCNBs)	IONPs and UCNPs simultaneously incorporated by wrapping in amphiphilic poly(maleic anhydride-alt-1-octadecene) polymer	DLS: 120 ± 10 TEM: NA	NIR fluorescence imaging and T_2 contrast agents	HeLa-WT and A431 cells	[154]
Calcein-liposome/virus (NoV)/Fe ₃ O ₄ nanoconjugates	APTES functionalized Fe ₃ O ₄ NPs and calcein liposome conjugated separately with NoV-specific antibody form a sandwich-like structure in presence of NoV	DLS: 1120 TEM: NA	fluorometric detection of Norovirus (NoV)	-	[155]
CuFeSe ₂ — ^{99m} Tc nanocrystals	CuFeSe ₂ nanocrystals fabricated in presence of trithiol-terminated poly-(methacrylic acid) and further labeled with radioactive ^{99m} Tc	DLS: NA TEM: 4.1 ± 0.4	Multimodal imaging (photoacoustic, magnetic resonance and computed tomography imaging) PTT	4T1 cells; 4T1 tumor-bearing BALB/c mice	[156]

Table 3. Cont.

Magnetic Nanoparticles Formulation	Composition	Size (nm)	Biomedical Application	In Vitro/In Vivo Application	Ref.
Lipid-coated Fe ₃ O ₄ mesoporous silica NPs doped with ceria NPs	ceria NPs loaded on surface of mesoporous silica NPs embedded with Fe ₃ O ₄ NPs and further encapsulated by a lipid bilayer	DLS: ~250 TEM: ~100	MRI and anti-inflammatory effect by scavenging ROS for theragnosis of intracerebral hemorrhage	RAW 264.7 cells; male Sprague-Dawley rats	[157]
Poly (amino ester) coated Fe ₃ O ₄ NPs	poly (amino ester) having carboxyl groups coated on Fe ₃ O ₄ NPs	DLS: NA TEM: 10.22 ± 2.8	viral-RNA extraction method for detection of SARS-CoV-2	-	[158]
MoS ₂ @Fe ₃ O ₄ -ICG/Pt(IV) nanoflowers	PEI functionalized MoS ₂ nanoflowers covalently grafted with Fe ₃ O ₄ NPs and further loaded with ICG and Pt(IV) prodrugs	DLS: NA TEM: 80	multimodal infrared ther- mal/photoacoustic/magnetic resonance imaging with combined PDT, PTT, and chemotherapy	L929 cells; H22 tumor-bearing Balb/c mice	[159]
Superparamagnetic (Fe ₃ O ₄) chitosan plasmid gelatin microspheres	superparamagnetic chitosan-coated Fe ₃ O ₄ NPs attached with (pReceiver-M29-VEGF165/DH5a) plasmids embedded in gelatin microspheres	DLS: NA TEM: NA	neovascularization in artificial bone scaffolds on application of magnetic field	white rabbits	[160]
Core-shell Fe ₃ O ₄ poly(NIPAM-co-DEAEMA) magnetic hydrogel composites loaded with methotrexate	Fe ₃ O ₄ NPs (core) surface modified with TMSPMA encapsulated in poly(NIPAM-co-DEAEMA) shell and loaded with methotrexate	DLS: NA TEM: 50–70	dual pH- and thermo- responsive drug delivery and hyperthermia	MCF-7 cells	[161]

(mPEG-b-P(DPA-DE)LG = methyloxy-poly(ethylene glycol)-blockpoly[dopamine-2-(dibutylamino) ethylamine-L-glutamate]; MIONs = magnetic iron oxide nanoparticles;
CMC = carboxymethylcellulose; DOX = doxorubicin; DR4 = death receptor 4; Ab = antibody; sulpho-SMCC = sulphosuccinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate; Fc
region = fragment crystallizable region; PDT = photodynamic therapy; EDC = 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; NHS = N-hydroxysuccinimide; SSAP = styrene and
acrylic acid crosslinked SPION modified with polyethyleneimine; AA = acrylic acid; PEI = polyethyleneimine; mPEG-PDLLA = diblock co-polymer of methoxypoly(ethelyne
glycol) ₅₀₀₀ and poly(D)-(L)-lactic acid _{50,000} ; SERS = surface enhanced Raman scattering; EDBE = 2,2'-(ethylenedioxy)bis(ethylamine); SA = succinic anhydride; NT-pro = N-
terminal pro-hormone; BNP = B-type natriuretic peptide; APTES = (3-aminopropyl)-triethoxysilane; ICG = indocyanine green; TMSPMA = 3-(Trimethoxysilyl)propyl methacrylate;
NIPAM = N-isopropylacrylamide; DEAEMA = 2-(N,N-diethylaminoethyl) methacrylate.

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Figure 10. (**A**) Scheme for preparing iPS cell sheets. After fluorescence-activated cell sorting, iPS cells with (Flk-1⁺) and without (Flk-1⁻) Flk-1 receptor were incubated with MCLs for 2 h. Labeled cells were then mixed with ECM in ultra-low attachment plates followed by placement on a cylindrical magnet to obtain cell sheets. (**B**) The iPS cell sheet floated on the surface of culture media with the help of a magnet. (**C**) The sheet was transferred to the magnet covered with a polyvinylidene difluoride (PVDF) membrane. (**D**) Using the same magnet, the sheet was placed on the adductor muscles of mice. Reprinted with permission from Ref. [141]. Copyright 2013 Springer Nature.

6. Conclusions and Future Prospect

In this review, we have presented a systematic overview of magnetic nanoparticles and their corresponding biomedical applications. The advent of magnetic nanoparticles has made considerable advancements, particularly in the last two decades. They exhibit the possibility of tunable composition, morphology, and size, which makes them truly diverse in terms of properties and applications. The fabrication of tailor-made complex magnetic nanoparticle formulations in conjugation with active moieties, such as drugs, enzymes, and targeting ligands, has proven to exhibit a multitude of applications. Certain peculiar properties of these particles make them very conducive for use in the field of biomedical applications: the ability to act as magnetic-targeting agents, diagnostic and therapeutic abilities, good drug load capacity, and facile surface-functionalization. One aspect that makes them particularly unique and valuable is the possibility of multimodality; the same nanoparticle formulation can be used as a drug carrier, a targeting agent, and as a theranostic agent. This reduces the overall toxicity and enhances efficacy by a great deal. Iron oxide nanoparticles have been shown to be safe and non-toxic, but the toxicity of other magnetic nanoparticles still remains a concern, and it requires extensive research to overcome it. From a synthesis perspective, there are various worthwhile study areas to pursue. Reproducible synthetic procedures for creating biocompatible formulations in a single step that are chemically stable, consistent in size, and well dispersed in aqueous media with efficient surface coatings are required for the best results in biological applications. The translation of the use of magnetic particles for humans requires special attention and effort; substantial research has been carried out regarding magnetically-induced hyperthermia and drug delivery applications in animal models, but taking it a step further to clinical trials still remains a challenging area for future research. The long-term stability and toxicity need to be evaluated in both in vitro and in vivo studies. Another challenge that should not go unnoticed is the persistent existence and noticeable ecotoxicity of certain nanoparticles. With further developments in comprehensive research on magnetic nanoparticles, their efficient clinical usage may be practically feasible in the near future.

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