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Iron Oxide Nanoparticles and Nano-Composites: An Efficient Tool for Cancer Theranostics

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Abstract

In recent years, functional Iron oxides nanoparticles and nano-composites have gained a special traction in the field of nano-biomedicine, owing to their multifunctional capabilities that includes the inherent magnetic resonance imaging, magnetic bioseparation, cargo delivery and magnetic hyperthermia behavior. Interestingly, there are various forms of iron oxides available, with each form having their own specific characteristics. The different polymorphic forms of iron oxides are obtained through various synthetic routes and are usually surface modified to prevent their oxidation. The chapter shall encompass the synthesis and surface modification of Iron oxides nanoparticles, physicochemical properties, and theranostic application of the magnetic iron oxide nanoparticles in cancer. Also, the future directions of Iron oxide nanoparticles and nano-composites towards the achievement of clinically realizable nanoformulation for cancer theranostic applications were highlighted.

Keywords: iron oxide nanoparticles, functionalised nanoparticles, hyperthermia, MRI contrasting ability, tumor ablation, tumor environment, nano-carrier, clinical translation

1. Introduction

Over the past few decades, nanomaterials have gained a special interest and are being investigated widely for various applications, owing to their unique characteristics [1]. Until now, several nanoparticulate systems have been studied to demonstrate their potential to detect, diagnose and treat cancer effectively with high degree of specificity and affinity to target cells, in comparison to the other onco-therapeutic approaches [1–3]. Iron oxides are being investigated widely in the field of nano-biomedicine, owing to their greater degree of variability and versatility [4]. The multifunctional capabilities of iron oxide nanoparticles including tumor labelling, magnetic bioseparation, biological entities detection, transfections, *in vivo* cell tracking, tissue repair, clinical diagnosis, targeted drug delivery, magnetic hyperthermia and altered drug pharmacokinetics can be achieved via surface modification and bioconjugation [5, 6]. Iron oxides are found in many forms, namely magnetite (Fe_3O_4), hematite ($\alpha\text{-Fe}_2\text{O}_3$), maghemite ($\gamma\text{-Fe}_2\text{O}_3$), wustite (FeO), bixbyite type

(β -Fe₂O₃), ϵ -Fe₂O₃ [6, 7], hydroxides, oxide/hydroxides [7] and ζ -Fe₂O₃ [8]. Each polymorphic form of Iron oxides has their own specific features [4]. Among these various forms, magnetite and maghemite are widely studied and found to be promising due to their proven biocompatibility [6] and unique magnetic properties [9]. With recent progress in nano-platforms, majority of studies are focused on the development of magnetic systems with long circulation half-life, high specific absorption rate, low Curie temperature [Wei Wu] and shortened transverse relaxation times, i.e., T₂ and T₂^{*} [9, 10].

In general, the magnetic behavior of nanomaterials is greatly influenced by various features, such as size, size distribution, shape, polymorphic form, surface modification and purity [11–13]. Iron oxide nanoparticles of various polymorphic forms are synthesized through various approaches, including co-precipitation [14, 15], sol-gel method [16], sol-gel cum reverse-micelle technique [16], thermal decomposition [17], sonochemical [18], microwave heating [19, 20], hydrothermal [21–23], microemulsion [24–26], green synthesis [27–28], bacterial synthesis [29], laser pyrolysis [30], and electrochemical synthesis [31]. For effective practical applications, the nanoparticles and nano-composites must be readily aqueous dispersible, stable and biocompatible with fascinating magnetic properties and interactive surfaces. The particle characteristics of Iron oxides such as size, shape and surface charges serves as a determining factor in achieving biological distribution and elimination [32]. Multiple studies evidence that the particle characteristics play an important role in the toxicity, i.e. smaller particles showed increased toxicity than larger particles [33–35].

2. Synthesis and surface functionalization of iron oxides

In the last two decades, several synthetic approaches have been followed to synthesize iron oxide nanoparticles with controlled size and morphology, biocompatibility and monodisperse nature [32, 36]. The schematic depiction of commonly employed chemical approaches is shown in **Figure 1**.

Of many approaches, co-precipitation is widely used for the synthesis of iron oxide due to their process simplicity [37]. At the same time, it is necessary to consider several factors such as precursor salts used, ratio of Fe²⁺/Fe³⁺ ions, nature and type of surfactants used, reaction parameters (pH and ionic strength of media, reaction temperature, stirring rate, drop rate of salt/base solution) and chemical processes employed in the co-precipitation technique to achieve the iron oxide nanoparticles with desirable crystal structure and morphology, monodispersity, and attractive magnetic properties [32, 38]. Peternele et al. synthesized magnetite and maghemite nanoparticles by precipitating the mixture of chloride salts of Fe²⁺ and Fe³⁺ (1:2 ratio) using 1.5 M NaOH or 25% Ammonia under vigorous stirring. The sequences of addition of base strongly affect the formation of phase and size of the magnetite nanoparticles. Further, much smaller sized maghemite was obtained by oxidizing prepared magnetite nanoparticles due to the Ostwald ripening effect. The nanoparticles obtained using ammonia displayed uniform size and were monodisperse in nature [37]. Cui et al. synthesized magnetite, maghemite and hematite using a common epoxide precipitation route involving an ethanolic solution of ferrous chloride tetrahydrate and a gelation agent propylene oxide. Initially, the nucleation of magnetite was observed due to the oxidation of Fe(II) precursor. From this step, the magnetite solution can be transformed into Fe₃O₄, γ -Fe₂O₃ and α -Fe₂O₃ nanoparticles through centrifugation-followed by vacuum drying, sol-to-xerogel formation followed

Figure 1.
Schematic depiction of commonly used chemical techniques.

by oxidation at 150°C and, atmosphere controlled evaporation of sol at 150°C, respectively [39, 40]. In another study, Cui et al. synthesized nanoparticles of other polymorphic forms such as lepidocrocite and goethite just by regulating the pH of the obtained greenish precipitate comprising an ethanolic solution of ferrous chloride tetrahydrate and a gelation agent propylene oxide using ammonia, followed by air oxidation under room temperature [41].

In several studies, the microemulsion and reverse micellization has been employed for the preparation of iron oxide nanoparticles with controlled morphology, but at the

same time the aggregation of nanoparticles are high and requires several washing, and stabilization [32]. For instance, Lee et al. synthesized magnetite nanoparticles by adding the hydro-ethanolic Fe (II, III) salt precursor solution into sodium dodecylbenzenesulfonate (SDBS) dispersed xylene solution under vigorous stirring for 12 h. Hydrazine was added to the solution maintained at 90°C, and the solution was refluxed for 5 h to obtain nanoparticles. The size of the nanoparticles can be tuned by modifying the relative amounts of precursor and the polar solvent-surfactant ratio [42]. In another study, Jung et al. applied the reverse micelle technique to synthesize uniform and monodisperse Fe₃O₄ nanoparticles for sensing and drug targeting applications. Herein, the precursor and surfactant immobilized in the organic phase were added to the aqueous phase comprising stabilizer and stirred for several hours to form stable reverse micelles of sub 3 nm size. The formation of large irregular and small worm-like nanoparticles was observed in the absence of stabilizer [43].

In order to achieve iron oxide nanoparticles with high-quality monodisperse nature, thermal decomposition approach has been used widely. However, this requires relatively higher temperature and involves complex operations. Hyeon et al. prepared maghemite nanocrystallites with a high degree of crystallinity and monodisperse nature by subjecting Iron pentacarbonyl-oleic acid complex to thermal decomposition. Initially, the complexation was carried out by transferring the Iron pentacarbonyl into a hot mixture containing oleic acid and octyl ether and maintained at 100°C for 1 h. The resulting mixture was cooled and treated with mild oxidant trimethylamine oxide. Following the addition of trimethylamine oxide, the solution was again heated to 130°C for 2 h under an inert (Ar) atmosphere. Further, the temperature was slowly increased to 300°C and refluxed for 1 h to obtain the maghemite nanocrystallites [44]. Later, Lassenberger et al. used a slightly modified thermal decomposition technique to synthesize the monodisperse oleic acid stabilized Iron oxide nanoparticles [45], which does not involve the use of mild oxidants. The particle size of nanocrystallites is directly linked to the concentration of the complexation agent and the heating rate employed in the synthesis process [44, 45]. Zhou et al. successfully synthesized various morphologies of monodisperse Fe₃O₄ nanoparticles by varying the ratio of Iron oleate/sodium oleate, reflux temperature, and heating rate [46]. The biggest problem with the nanoparticles obtained via thermal decomposition route is their limited solubility in aqueous environments. Thus, phase transformation is required to render them water soluble [45].

A simple hydrothermal process can be a better alternative for the preparation of monodisperse, dislocation-free, highly crystalline iron oxide nanoparticles, as it does not require high temperature. The use of surfactants in the hydrothermal process could limit the growth of nanoparticle size, while retaining the crystallinity and magnetic properties close to that of the iron oxides prepared without surfactants and bulk iron oxides [6, 32]. Ozel et al. synthesized Iron oxides of varying crystallinity and size ranging from 12 to 49 nm by varying the reaction temperature (60–180°C) and reaction time (1–48 h) [47]. Further, Torres-Gomez et al. reported the synthesis of various γ -Fe₂O₃ nanostructures such as quasi-spherical, octahedral and truncated cubes by varying the reaction temperature from 120 to 160°C [22]. Ellipsoid 3D superstructures, plate-like nanostructures and irregular structures of α -Fe₂O₃ were obtained by employing varying proportions of Fe³⁺ precursor, surfactants and solvent under varying temperatures [48]. Xu et al. used urea to synthesize template-free rod-like β -FeOOH structures by varying the reaction temperature and time [49]. Also, urea in combination with ammonia was added to ferric chloride solution and autoclaved at 150°C for 6 h to obtain β -Fe₂O₃ [50]. Various reports highlight that

depending on the type of the reducing agent and surfactants used in the hydrothermal process, different iron oxide phases and nanostructures such as spherical, polyhedral, nanocubes, octahedral, truncated cubes, hollow spheres, nanorods etc. [6, 22, 23, 32, 47, 49, 51, 52] can be obtained.

Sonochemical technique is a competitive alternative, to prepare ultrafine, monodisperse iron oxide nanoparticles with unusual properties. Usually, iron oxides prepared through this route are amorphous and possess low magnetization with supermagnetic behavior below magnetic transition temperature [6]. Hassanjani-Roshan et al. demonstrated the effect of ultrasonic power and reaction temperature on the particle characteristics such as crystalline/amorphous behavior and particle size of α -Fe₂O₃. The particle can be transformed into a highly crystalline form by subjecting them to higher temperature, following the sonochemical synthesis [53]. A cube-like Fe₃O₄ nanoparticle with different particle sizes ranging from 20 to 58 nm was obtained by varying the molar concentration of the precursor and the reducing agent [54]. According to the LaMer model, the local supersaturation should be higher to achieve the smaller particles. Ludtke-Buzug et al. state that the maximum local supersaturation is higher at lower ultrasound frequency [55].

Recently, green chemistry and biological methods are being used for the preparation of iron oxide nanoparticles owing to their safety, low cost, non-toxic and eco-friendliness approach. However, the consistency of synthetic process is highly influenced by the source of the green and biological reducing agents [40]. The microbial-mediated approach demands a lengthy incubation time for the synthesis of iron oxide nanoparticles [56]. Whilst, the plant-mediated environment-friendly approach requires less time and can be used for the synthesis of various iron oxide nanostructures like spherical, needles, cubical, dendrites, cylindrical, and so on [56] with appreciable biological activities. Many studies utilize plant extracts with mild reducing capability along with bases like sodium hydroxide [57, 58], sodium carbonate [59], glycine [60] to synthesize stabilized iron oxide nanoparticles, as this can avoid the use of environmentally-toxic stabilizing agents. Few studies reported the successful synthesis of Iron oxide nanostructures using the extracts like grape berry ferment [61], flower extract of *Avecinnia marina* [62], and leaf extract of *Bauhinia tomentosa* [27] alone. The synthesis techniques of Iron oxide nanoparticles with their characteristic size are depicted in Table A1.

Avoiding agglomeration while retaining the stability is the most crucial challenge every magnetic material undergoes. In last two decades, considerable efforts have been devoted for the passivation of iron oxide nanoparticle surface using organic and inorganic materials, to avoid agglomeration, and to have improved solubility, stability and biocompatibility. The surface functionalization of iron oxide nanoparticles could be achieved by coating the iron oxide core with shell material (or) by dispersing the iron oxide core over the shell material (or) through chemical interaction of iron oxides with shell material (or) through bioconjugation reactions (or) by coating a shell-core structure with shell material. The shell materials could be organic or inorganic materials with functional properties. The commonly employed functional materials in the passivation of iron oxide surface include organic small molecules (drugs, fatty acids, polyol, dyes, vitamins, cyclodextrins), surfactants (dextran, polyvinyl alcohol), biological molecules (proteins, nucleic acids, antibody, cells, enzymes, microbes), silanes (*p*-aminophenyltrimethoxysilane, 3-aminopropyltriethoxysilane), polymers (synthetic and natural origin), silica, metals and non-metals (gold, silver, carbon, etc.), metal oxides and metal sulphides [6]. In recent times, with the advancement in the polymer technology, several stimulus-induced targeted iron oxide-drug

nanoconjugates are developed. Inspired by the different pH conditions of tumor environment, several pH-sensitive systems are being explored to minimize the release of chemotherapeutic drugs in the blood and normal tissues [61, 63–66]. Also, the thermo-responsive systems are being explored simultaneously to have control over the drug release, i.e., the release of drug is initiated only at the hyperthermia temperature [66, 67–70]. Interestingly, other stimulus-mediated systems such as enzyme-mediated [71, 72], light-mediated [71, 73], ultrasound-mediated [71, 74] are also being explored.

3. Physicochemical properties of iron oxides

3.1 Crystallographic properties and polymorphism

Iron (III) oxides exhibit distinct physical properties due to its polymorphic forms that has same molecular formula but with different crystallographic structures. Iron (III) oxides occur in various polymorphic forms such as α -Fe₂O₃, β -Fe₂O₃, γ -Fe₂O₃, ϵ -Fe₂O₃, and ζ -Fe₂O₃. The polymorphic forms are characterized by crystal structure as: (i) rhombohedrally-centric hexagonal α -Fe₂O₃ ($R\bar{3}c$); (ii) body-centric cubic β -Fe₂O₃ ($Ia\bar{3}$); (iii) inverse spinel type cubic γ -Fe₂O₃ ($Fd\bar{3}m$) with oxygen vacancies in octahedral site; (iv) orthorhombic ϵ -Fe₂O₃ ($Pna2_1$); and (v) monoclinic ζ -Fe₂O₃ ($I2/a$). All polymorphic form of Iron (III) oxides can be converted into other phases in response to temperature and pressure. The transformations of phases are interdependent on the precursors, pH value, ionic strength, intrinsic properties of nanomaterials such as crystal structure, particle characteristics, matrix characteristics in which particles are incorporated, reaction parameters and the nature of the treatment applied (e.g. thermal/pressure/both) [6, 16, 75]. However, under ambient conditions, a thermodynamically stable α -Fe₂O₃ phase is usually formed directly from β -Fe₂O₃, ϵ -Fe₂O₃, and γ -Fe₂O₃. Temperature treatment plays a major role in the formation of α -Fe₂O₃ phase from the other phases such as β -Fe₂O₃ (~ 773 K) [76], γ -Fe₂O₃ (~ 973 K) [77] and ϵ -Fe₂O₃ (>1473 K) [78] phases. In some cases, like hollow β -Fe₂O₃, the phase can be transformed directly into γ -Fe₂O₃, and depending on the interparticle interactions and size of the nanoparticles, γ -Fe₂O₃ may exhibit both direct and indirect transformations (via ϵ -Fe₂O₃) into α -Fe₂O₃. Recently, a stable ζ -Fe₂O₃ was formed by exposing β -Fe₂O₃ above 30 GPa pressure [75, 76]. The other predominantly investigated polymorphic form includes the face-centric cubic Fe₃O₄ (FeO·Fe₂O₃, II and III oxidation states) with tetrahedral and octahedral sites. Like other Iron oxides, Iron (II, III) oxides also tend to undergo phase transformation to other Iron (III) oxide polymorphs under ambient conditions, due to their poor stability in oxygen environment [6]. It is well accepted that the crystalline phase stability of nanoparticles are much dependent on the surface stress, surface strain and surface energy. Recently, much focus has been kept on Cubic Wustite (FeO, space group $Fm\bar{3}m$), which under ambient conditions remains in metastable state and undergo oxidation to other polymorphic form of iron oxides [6, 79–80]. Like iron oxides, iron (III) oxyhydroxides also exists in various forms namely an orthorhombic goethite (α -FeOOH, space group $Pnma$), monoclinic akaganeite (β -FeOOH, space group $I2/m$), orthorhombic lepidocrocite (γ -FeOOH, space group $Cmcm$) and hexagonal feroxyhyte (δ -FeOOH) [81]. Ferrihydrite are another polymorphic form of iron (II) oxides that are relatively abundant in the natural systems and can be readily transformed into the goethite phase when there is a

rapid oxidation process [82]. Among various iron (III) oxide polymorphs, ϵ -Fe₂O₃ particles are observed only in the nanosize and they are size dependent [83]. An amorphous form of Fe₂O₃ was observed in the particles with less than 5 nm in diameter, wherein the oxygen octahedra are randomly oriented around the Fe (III) ions. However, it is difficult to distinguish the ultrafine particles of amorphous iron oxides and other polymorphs experimentally [84].

3.2 Magnetic properties

Magnetic properties of the nanomaterials play a key role in MRI contrasting ability, magnetically-induced heating, externally-targeted drug delivery and bio-sensing applications. The γ -Fe₂O₃ polymorph exhibits ferrimagnetic and superparamagnetic behavior with a curie temperature of 928 K [16, 76], whilst, the ferromagnetic ϵ -Fe₂O₃ possess highest coercivity [76, 84] with a curie temperature close to 500 K [85]. Interestingly, the magnetic order of ϵ -Fe₂O₃ nanoparticles does not get vanished even at 500 K and this different ferromagnetic state persists up to 850 K [86]. A weak ferromagnetic behavior was reported in α -Fe₂O₃ phase with a Morin transition at ~ 269 K, i.e., a transition of antiferromagnetic state from weak ferromagnetic state [76] and a curie temperature of 950 K [16]. The paramagnetic β -Fe₂O₃ becomes magnetically ordered below the Neel temperature (~ 110 K) and exhibit antiferromagnetic state. Similarly, the recently identified ζ -Fe₂O₃ exhibited an antiferromagnetic nature below Neel transition temperature of ~ 69 K [76]. A paramagnetic transition of amorphous Fe₂O₃ at temperatures above the Neel temperature of 80 K was concluded based on the interpretation on Mössbauer data [84]. In some cases, non-ideal magnetic behavior could be observed in iron oxides due to wide range of blocking temperatures, aroused from wide range of particle size distribution [6]. Wustite (FeO) is generally stable above 560°C and possess antiferromagnetic nature with a Neel temperature of about 200 K [79]. Among all Iron (III) oxyhydroxide, δ -FeOOH is the only polymorphic form which showed significant magnetization at room temperature with ferrimagnetic behavior (T_c —450 K) [87] and significant relaxation properties [88]. The smaller crystals of Feroxyhites (δ -FeOOH) showed speromagnetic behavior between 80 K and 300 K [87]. β -FeOOH is usually paramagnetic at room temperatures and below Neel temperature they exhibit antiferromagnetic property [89]. The bulk α -FeOOH and γ -FeOOH displayed a Neel transition at 252 K and 53 K respectively [90]. The magnetic property of Ferrihydrite is size dependant, which displayed antiferromagnetic behavior below 4 nm, and above 4 nm ferrimagnetic behavior was observed [91]. The magnetic properties of iron oxide nanoparticles are greatly influenced by the oxidation and aggregation. The oxidation of iron oxide nanoparticles could lead to the loss of magnetic properties. In contrast the aggregation of particles may lead to mutual magnetisation that is usually aroused by the interaction of magnetic field of one nanoparticle with the neighboring nanoparticle [32].

3.3 Chemical properties

Iron oxide nanoparticles are highly prone for oxidation, particularly in the atmospheric air, and hence require a thin and non-interactive protective coating that has minimal effect on its characteristic physical properties, especially its magnetic properties [32]. Many studies also concluded that the naked iron oxide nanoparticles tend to agglomerate owing to their high surface energy, surface area-to-volume ratio,

magnetic interactions and van der Waals forces [32, 92]. The agglomeration of the particles not only increases the particle size, but also enhances strong magnetic dipole-dipole attractions, that make the particles ferromagnetic. In general, the hydrophobic character and the huge surface area-to-volume ratio render the iron oxide nanoparticles toxic, insisting the need for the modification of particle surface, to make them hydrophilic and biocompatible [32].

4. Theranostic applications of magnetic iron oxide nanoparticles in cancer

4.1 Tumor imaging

Among various imaging modalities, magnetic resonance imaging acquires a rapid image of *in vivo* tumor environment owing to their high spatial resolution. The unique magnetic properties with shorten relaxation times of iron oxide nanoparticles could enhance the sensitivity of T₂ and T₂* contrasting ability in magnetic resonance imaging (MRI) [9]. The imaging sensitivity and specificity of iron oxide nanoparticles could be enhanced by modifying the surface of iron oxides with small molecules, peptides and antibodies, whose receptors are overexpressed on the surface of tumor cells. Yang et al., modified the poly(amino acid)-coated iron oxide nanoparticle surface with Her-2/neu antibody for the detection of HER2/neu positive breast cancer cells and observed an enhanced MRI contrasting ability with significant T₂ relaxation time. Over many years, a well-known antibody herceptin is used clinically against the HER2/neu receptor [93]. Lee et al., demonstrated the detection of small tumors using the herceptin conjugated iron oxide nanoparticles [94]. Though antibodies show promising future for onco-imaging and therapy, their steric effects limits their conjugation with iron oxides. Further, the decrease in specificity of antibody-iron oxide conjugate was observed due to the interaction of Fc receptors of normal tissues with the antibody. Recently, single chain antibodies (scFv) have gained more traction owing to their small molecular weight, antigen binding ability, non-immunogenicity and low cost. Some scFv's such as scFvEGFR10 and sm3E have been conjugated to the iron oxide nanoparticles for the reduction of T₂ relaxation time and enhanced MRI capability. Yang and his co-workers conjugated amino-terminal fragment (ATF) peptide and near infrared dye Cy5.5 into the iron oxide-amphiphilic polymer conjugate to target the urokinase plasminogen activator receptor that is over expressed in human tumor cells and stromal cells where the tumor is associated. Herein, the study reported that the imaging probe is capable to detect small tumors ranging from 0.5 to 1 mm³. The other peptides that are generally used to achieve multi-modal imaging capability include EPPT₁ peptide, Luteinizing hormone release hormone, Folic acid, Vitamin FA and Arg-Gly-Asp [95–97] and chlorotoxin peptide [98]. The iron oxides could also possess T₁ relaxation, when the particle size is greatly reduced, i.e. less than 5 nm. This phenomenon is observed due to the decrease in magnetic moment exerted with decreased particle size. The Iron (II, III) oxides below 5 nm are considered to be responsive MRI contrast agents due to its ability to be used as both T₁ and T₂ contrasting ability. Under tumor environment, the iron oxides are released from the carrier providing the T₂-T₁ switching ability during imaging [95–97]. Despite of the establishment of *in vitro* imaging potential of tumor targeted iron oxides, they are not widely used in clinical practice owing to their low specificity and sensitivity *in vivo*. Also, for successful clinical use, the targeted iron oxides must be able to monitor tumor metastasis and therapy response. The low sensitivity of single imaging modality

has evolved the multimodal imaging technique which involves the combination of MRI contrast imaging with photothermal imaging [95].

4.2 Cancer labelling and sorting

Cell labelling and sorting is a key technique in the field of oncology and stem cell research. Usually, cells are labeled using ferro or superpara or paramagnetic materials in the process of *in vivo* cell separation, and the labeled cells are visualized using the magnetic resonance imaging based protocol. In principle, two approaches are employed for cell labelling using iron oxides; (i) one by directly attaching the magnetic iron oxide nanoparticles to the cell, and (ii) the other by internalizing the iron oxide nanoparticles into the cytosol of cell using receptor-mediated endocytosis or fluid phase endocytosis or phagocytosis [99–101]. Cell labelling must not compromise the proliferating capability, motility, and cellular functions for effective stem cell trials or therapies. Arbab et al. labeled CD34+ hematopoietic and mesenchymal stem cells with ferrumoxides-protamine sulfate complex and reported the unaltered expression of phenotypic markers and differentiating capacity [102]. The cell viability remained intact when labeling concentration of iron oxide nanoparticles was used. In contrast, at high concentrations, the cellular viability decreased. The uptake of iron oxide into stem cells can be enhanced by modifying the core with dextran, citrates, HIV tat peptide, unfractionated heparin, and aminosilane. The modification of the iron oxide core exerts various biological activities in labeled cells. For instance, there is an increased cell proliferation in iron oxide-ferucarbotran labeled mesenchymal stem cells. The target-specific molecules/Iron oxide nanoconjugate can precisely label the cells *in vivo* [103]. Chen et al. demonstrated the MRI imaging potential of Herceptin bound-dextran-coated iron oxides in HER2/neu receptors expressed cancer cell lines [104]. In another study, the contrasting ability of folic acid bound-PEG coated iron oxides in the tumor cells expressing folate receptors [105].

The magnetic behavior of iron oxides allows the isolation of specific cells from the biological suspensions like blood, apart from its diagnostic ability. In non-invasive magnetic-activated cell sorting, the antibody-bound iron oxides bind specifically to the specific antigens present on the surface of target cells. The bound fraction can be separated from the unbound fraction by applying a magnetic field. For instance, circulating tumor cells are captured, and analyzed for staging cancer, selection of therapy and monitoring treatment [106]. Mi et al. developed a low cytotoxic Herceptin-functionalised conjugated oligomer-based Iron oxide-silica nanoparticle system for magnetic-activated sorting and fluorescence-activated detection of circulating tumor cells at its metastatic stage [107]. Du et al. bioengineered D-tyrosine phosphate decorated iron oxides that can be dephosphorylated to tyrosine coated iron oxides by the overexpressed alkaline phosphatases in the surface of cancer cells. The tyrosine-coated iron oxides upon dephosphorylation have been attached to the tumor cells and captured using a small magnet [108].

4.3 Cancer immunotherapy

Immunotherapy is a robust strategy to eliminate cancer cells. For effective cancer immunotherapy, the immune system must be activated against tumor antigens. The immunotherapeutic approaches include cell-based immunotherapy, monoclonal antibodies (mAb) for checkpoint blockade and cancer vaccines. In cell-based immunotherapy, the migration, expansion, and depletion of immune cells are tracked to

understand the complex cellular and molecular mechanism involved in the immune system [100]. The schematic representation of various applications of iron oxide nanoparticles is depicted in **Figure 2**.

4.3.1 Cell-based immunotherapy

The T cells have the ability to differentiate into various forms based on the interaction of specific tumor antigens on the antigen-presenting cells. Real-time T-cell trafficking using MRI can improve the T cell-based immunotherapies by achieving better localization [109]. For instance, the three-dimensional MRI visualization of *in vivo* T-cell trafficking to target tumors and the temporal regulation of T-cells within the tumors has been demonstrated using ova-specific CD8+ T cells labeled cross-linked iron oxides (highly derivatized). In addition, a similar proliferating potential, cellular interaction, and cytotoxic profiles was observed for both labeled and unlabelled cells [110]. Liu et al. developed PEG conjugated fluorescent dyes coated iron oxide for achieving dual-mode imaging such as MRI and Fluorescence. The

Figure 2.
Application of iron oxide nanoparticles/conjugates in cancer immunotherapy.

human and murine T cellular functions were not altered following the injection of these nanoparticles [111]. The labeling of T cells is the biggest challenge due to their non-phagocytic nature. However, significant internalization of Iron oxides can be achieved using the transfection agents such as polyethyleneimine, poly-L-lysine, lipofectamine [100].

Macrophages eliminate foreign particles and cellular wastes by secreting cytokines and initiating phagocytosis. Reprogramming or polarizing the tumor-associated macrophages (TAM) in the tumor environment shall overcome the difficulty of penetration of M1 macrophages from the outside environment. Iron oxides on their own activate the macrophages through metabolic degradation. In a study, the response to CD47 monoclonal antibodies by tumor-associated macrophages (TAM) has been monitored using ferumoxytol-enhanced MRI. The study showed that phagocytosis of TAM had been activated due to the inhibition of the interaction between SIRP α and CD47 by CD47 mAb [112]. In another study, 3-methyladenine was incorporated into hollow iron oxide nanoparticles to promote the immune response by reprogramming the TAM into M1-type macrophages. The nanoparticle system upregulates the NF- κ B p65 while inhibiting the expression of P13K γ to promote an immune response. The mouse model revealed a synergistic effect of polarization of macrophages by 3-methyladenine and iron oxide nanoparticles [113]. An artificial reprogramming of macrophages was reported in a study using hyaluronic acid-coated iron oxide nanoparticles. In this study, the iron oxide nanoparticles and macrophages from RAW 264.7 mouse were incubated together and injected into 4 T1 tumor-bearing mice. The results indicated the capability of these artificially programmed macrophages in polarization of TAM and enhanced tumor effect [114].

Dendritic cells play a key role in activating cytotoxic T lymphocytes and regulating adaptive immune response by presenting the tumor antigens into the draining lymph nodes. The immune response is highly dependant on the migration of activated dendritic cells to lymph nodes [103, 115]. The incorporation of iron oxides into a dendritic cell does not impair the viability of cells [103] and demonstrate the tracking of dendritic cells with MRI [100]. The antigens are loaded into immature dendritic cells, as the phagocytic ability is higher than the mature dendritic cells. The internalization of iron oxides takes place via endocytosis for the particles ranging from 20 to 200 nm [100]. Iron oxide nanoparticles bound to oval albumin showed increased expression of TNF α , IL-6, and IFN- γ in murine dendritic cells. Further the assessment of immunotherapeutic capability in mice revealed a dramatic reduction in the tumor [116, 117]. De Vries et al. reported the enhanced accuracy of magnetic resonance tracking of iron oxides-loaded dendritic cells over scintigraphic imaging. Herein, the iron oxide nanoparticles are phagocytized into the autologous *ex vivo* cultured dendritic cells without altering the phenotype and functional properties [115]. Dendritic cells loaded with iron oxide/zinc oxide core-shell nanoparticles exhibited a T₂-weighted signal reduction in the lymph node of C57BL/6 mice. However, the dendritic cells loaded with zinc oxide showed a migration towards the lymph nodes [100].

4.3.2 Cancer vaccines

Cancer vaccines play a crucial role in presenting the tumor antigens to activate specific T cells against the tumor cells. Recently, biomimetic nano vaccines encompassing tumor cell membranes with tumor antigens are used for targeting the immune or tumor cells. The vaccination capacity can be increased by introducing iron oxides with intrinsic magnetic properties. For instance, Zhang et al. developed a

biomimetic magnetosome to expand and stimulate antigen-specific cytotoxic T-cell and to track and effectively guide them into tumor tissues [118]. Further, Wang et al., demonstrated antitumor activity and immunogenic cell death with low systemic toxicity using Ce6-loaded magnetic/mesoporous organosilica nanoparticles concealed with cancer cells [119]. In another study, Long CM et al. developed magneto-vaccination using iron oxide labeled GM-CSF secreting cells mixed with tumor to stimulate the immune system by inducing the T cell tumor targeting factors proliferation [120].

4.3.3 Checkpoint blockade

The immune checkpoint is the molecular interactions between cancer cells and immune cells. The ability of tumor cells to evade the surveillance of the immune system is the common problem associated with the T cell-mediated approach, as many healthy cells like certain tumor cells encompass inhibitory checkpoint programmed cell death protein 1 ligand 1 (PD-L1) that can inactivate the T cells by binding to the inhibitory checkpoint PD1 protein expressed on T cell surface [100]. The immune response shall be enhanced by targeting inhibitory checkpoint molecules such as proteins and antibodies. Under physiological conditions, the checkpoint inhibitory molecules are highly prone to degradation and hence required to be encapsulated into a robust delivery system. In a study, the immunoswitch design was demonstrated using antibodies-loaded dextran-coated iron oxide nanoparticles for the inhibition of immune checkpoints. The antibodies against PD-L1 and 4-1BB were used to stimulate T cells. In this study, the tumor-bearing mice (C57BL/6) was initially treated with adoptively transferred T cells, followed by the administration of antibodies-loaded dextran-coated iron oxide nanoparticles or free individual antibodies. The results suggest the targeting of multiple checkpoints, as targeting only one checkpoint did not result in size reduction of tumors. Also, highlights the multifunctional utility of iron oxides for checkpoint inhibition [121]. In another study, the Iron oxide-coated folic acid-functionalised-disulphide-polyethylene glycol-conjugated polyethylenimine nanoparticles were developed for the delivery of siRNA to inhibit PD-L1 protein. The nanoparticles exhibited higher transfection ability, MRI contrasting ability, and the high cellular uptake downregulate the PD-L1, which in turn affected the T-cells cytokine-secretion level [122]. The schematic representation of immune checkpoint regulation using immunoswitch nanoparticles is shown in **Figure 3**.

Figure 3.
Schematic representation of immune check point regulation using immunoswitch nanoparticle.

4.4 Tumor ablation therapy

In tumor ablation therapy, the use of non-contact magnetic heating (above 42°C) has gained a special attention and are being utilized in clinical practice in few hospitals as an adjuvant therapy. The magnetic heating is achieved through hysteresis loss and relaxation losses under varying alternating magnetic field and radio frequencies. The efficiency of magnetic heating is directly linked to the size, shape and concentration of the magnetic nanoparticles, the strength and frequency of alternating magnetic field and cooling rate in biological tissues. The major obstacle in the use of Iron oxides for non-contact heating of tumor tissues is their low heating power. Magnetite and maghemite nanoparticles are widely employed for magnetic hyperthermia applications due to their well-established biocompatibility. However, these nanoparticles possess relatively low coercivity and require high applied frequency, usually ranging from 400 kHz to 900 kHz, to effectively heat the media in which it is dispersed. In contrast, studies indicate that ϵ -Fe₂O₃ nanoparticles exhibit hyperthermia potential at low applied frequency of about 20 kHz to 100 kHz. Though many magnetic materials reported to have high heating power, their concern over safety has limited their use in clinical practice, and encourages the optimisation of structural features of iron oxide nanoparticles for enhanced clinical hyperthermia potential [123].

Kolosnjaj-Tabi et al. demonstrated a mild hyperthermia efficacy of PEG-coated iron oxide nanocubes in a magnetic field of 23.8 kA/m and 111 kHz until the particle resides in the interstitial extracellular location. The hyperthermia efficiency of the nanoparticles was lost after cellular internalization and capture in the liver and spleen. However, the hyperthermia effect destabilize the tumor stroma to enhance the drug penetration [124]. The cancer theranostic agents are widely employed for the effective control of tumor owing to their potential diagnostic cum treatment approach. The magnetic hyperthermia efficiency and MRI T₂ contrasting ability of iron oxide nanoparticles were demonstrated using the fourth-generation dendrimer coated iron oxides [125]. Hayashi et al., developed iron oxide nanoclusters for combined MRI cum hyperthermia, as the individual iron oxides (<10 nm) are prone to leakage from capillaries. In this study, the surface of Iron oxide nanoclusters is modified with polyethylene glycol and folic acid to enhance their accumulation within the tumor environment of mice, following the intravenous administration. The mice that underwent local heating for 20 minutes reduced the tumor volume to about 1/10th of the control mice, indicating the hyperthermia efficiency of the iron oxide nanoclusters [126]. Lin et al. developed a multifunctional pegylated albumin nanocomplex comprising Iron oxide and a hydrophobic dye (IR780). The photothermal effect and MR imaging of nanocomplex were demonstrated on a cancer colon model and tumor-bearing mice, respectively [127]. The combined photothermal effect (NIR-induced) and cancer imaging (MRI and fluorescence) were demonstrated using a novel dumbbell-like Gold-Iron oxide nanoparticle by Kirui et al. [128]. A similar kind of bimodal cancer imaging with a photothermal effect was reported in hyaluronan-targeted iron oxides to bring out the photothermal efficiency and cell staining potential of the iron oxides apart from its MRI contrasting ability [129]. Espinosa et al. demonstrated a complete tumor regression using the dual-mode hyperthermia and photothermal therapeutic potential of iron oxide nanocubes [130].

4.5 Drug delivery applications

The successful delivery of therapeutic agents into the tumor environment with minimal toxicity to surrounding tissues is the biggest challenge, as it is often limited

by tumor heterogeneity, dense fibrotic stromal barriers and various vascular barriers including abnormal tumor blood vessels, tumor cells proliferating nests, normal blood vessels, positive intratumoural pressure [131, 132]. Iron oxide nanocarrier systems are often believed to overcome these biological barriers by altering the pharmacokinetics and tissue distribution profile via enhanced permeability and retention effect [9]. However, for clinical success, EPR effect may not be sufficiently enough as it offers only certain level of tumor targeting and non-specific biodistribution. Thus, the logical choice for tumor therapy shall be actively tumor-targeted iron-oxide nano-carrier system, which can be minimally toxic to normal tissues while having enhanced bio-availability, intracellular bio-distribution, and potent cyto-toxic effects against the tumor cells. [131, 132]. Recently, the tumor-targeted iron oxide nanoparticles are used to monitor the accumulation of drugs in the tumor site, while simultaneously estimating the drug level in the tumor tissues. The detection of MRI signal changes of drug loaded iron oxides can provide a track over drug delivery, estimated drug levels in tissue and therapeutic response *in vivo* [9].

Li et al. developed the iron oxide nanoclusters with photothermal mediated synergistic chemotherapy and chemodynamic therapy. In this study, the core iron oxides were surface modified using the paclitaxel-loaded human serum albumin and conjugated to the Arg-Gly-Asp peptides for tumor specific targeting [133]. Several studies indicate that insufficient penetration through BBB reduces the efficiency in treating glioblastoma multiforme. Norouzi developed pH-sensitive doxorubicin-loaded Iron oxide nanoparticles stabilized with trimethoxysilylpropyl-ethylenediamine triacetic acid to demonstrate their uptake in brain-derived using mouse model. The cellular uptake of nanoparticles was increased by 2.8 folds and provided an enhanced anti-tumor effect than free doxorubicin. Further, the study indicated that the penetration of nanoparticles into the brain was augmented due to the combination of cadherin binding peptide and external alternating magnetic field [134]. Hussein-Al-Ali et al. developed iron oxide/chlorambucil/chitosan nanocomposite with a particle size of about 15 nm. Controlled release of drug chlorambucil from the nanocomposite with significant anti-tumor effects on leukemia cancer cell lines was observed [135].

5. Concluding remarks and future directions of iron oxides

Since several decades, many studies have been conducted to evaluate the potential use of functionalised iron oxide nanoparticles for the delivery of anticancer drugs, yet there are several obstacles that need to be overcome for increased adoption of these nano-carrier systems into clinical practice. The various challenges that needs to be considered while developing the newer targeted iron oxide nanoparticles includes the synthesis of conjugated iron oxide nanoparticles without inducing a change in the chemical and magnetic properties, the high drug loading efficiency, regulation of circulation time [113], specificity and selectivity of towards tumor tissues, and controlled release of drug with the tumor region. The other challenges that are least explored in the development of iron oxide nanocarrier systems includes tumor uptake, biodistribution and bioelimination. The biological distribution is highly dependent on the nanoparticles size, morphology and surface characteristics, as these properties can strongly influence the particle interaction with serum proteins and cells [32]. The major obstacle in achieving the effective tumor therapy is the tumor heterogeneity resulted from the genetic mutations. This emphasis the need for personalized medicine involving both imaging and targeted drug delivery simultaneously,

signifying the concept ‘we observe what we treat’. The potent MRI capability of the iron oxide nanoparticles shall allow the visualization of events such as delivery of drug and other cargo molecules, efficacy of the undergoing treatment, gene expression and metastasis, bioelimination of iron oxides. Though, iron oxides exhibit many distinctive properties, the long-term fate of iron oxides, PK/PD studies, toxicology studies and the toxicity criteria are yet to be clearly defined. Recently, there is an intense focus on the development of multifunctional tumor-targeted drug-loaded iron oxide nano-carrier systems, as it can offer many benefits such as, tumor-specific targeting, MRI contrasting ability (track and monitor the accumulation of the nano-carrier system), combined hyperthermia and chemotherapy to tumor cells and stimulus-induced drug release (control over the drug release at the tumor-specific site). Until today, there are numerous studies reported to have such multifunctional properties in *in vitro* and *in vivo* animal models. The successful development of clinically reliable, multifunctional tumor-targeted drug-loaded Iron oxide nano-carrier systems can transform the future oncology treatment practices [32].

Appendix

Nanoparticle type	Precursor	Precipitating agent	Process and conditions	Size	Ref.
Co-precipitation technique					
Fe ₃ O ₄	FeCl ₂ ·4H ₂ O, FeCl ₃ ·6H ₂ O	1.5 M NaOH	Addition of precursor solution into alkali,		[37]
			a. stirring under air for 30 min	9.82 nm	
			b. N ₂ bubbling (100 mL/min) for 30 min	6.31 nm	
			c. kept under magnetic field for 24 h	7.21 nm	
Fe ₃ O ₄	FeCl ₂ ·4H ₂ O, FeCl ₃ ·6H ₂ O	25% NH ₄ OH	Addition of alkali into precursor solution		[37]
			a. stirring under air for 30 min	11.22 nm	
			b. N ₂ bubbling (100 mL/min) for 30 min	5.05 nm	
			c. kept under magnetic field for 24 h	5.41 nm	
γ-Fe ₂ O ₃	FeCl ₂ ·4H ₂ O, FeCl ₃ ·6H ₂ O	1.5 M NaOH	Addition of precursor solution into alkali, adjusted to pH 3.5 using HCl		[37]
			a. stirring under air for 1 h at 95°C	7.21 nm	
			b. O ₂ flux (100 mL/min) for 1 h at 95°C	7.21 nm	
			c. Oxidation at 250°C for 3 h	7.21 nm	
γ-Fe ₂ O ₃	FeCl ₂ ·4H ₂ O, FeCl ₃ ·6H ₂ O	25% NH ₄ OH	Addition of alkali into precursor solution, adjusted to pH 3.5 using HCl		[37]
			a. stirring under air for 1 h at 95°C	6.31 nm	

Nanoparticle type	Precursor	Precipitating agent	Process and conditions	Size	Ref.
			b. O ₂ flux (100 mL/min) for 1 h at 95°C	5.41 nm	
			c. Oxidation at 250°C for 3 h	5.41 nm	
Fe ₃ O ₄	FeCl ₂ ·4H ₂ O	Propylene oxide	78°C for 30 min, centrifugation and vacuum drying	7.5 nm	[40]
γ-Fe ₂ O ₃	FeCl ₂ ·4H ₂ O	Propylene oxide	78°C for 30 min, xerogel formation (100°C), followed by oxidation at 150°C	8.6 nm	[40]
α-Fe ₂ O ₃	FeCl ₂ ·4H ₂ O	Propylene oxide	78°C for 30 min, atmosphere controlled evaporation at 150°C	18.4 nm	[40]
γ-FeOOH	FeCl ₂ ·4H ₂ O	Propylene oxide	60°C for 30 min, pH 6.5, air oxidation for 5 h	40–80 nm	[41]
α-FeOOH	FeCl ₂ ·4H ₂ O	Propylene oxide Ammonia	60°C for 30 min, pH 8 to 8.5, air oxidation for 20 h	20–90 nm	[41]
Fe ₃ O ₄	FeCl ₂ ·4H ₂ O	Propylene oxide Ammonia	60°C for 30 min, pH ≥9, air oxidation for 2 h	25–50 nm	[41]
Fe ₃ O ₄	Fe(SO ₄)·7H ₂ O, Fe (NO ₃) ₃ ·9H ₂ O	NaOH	Primary nucleation was carried out by adding NaOH solution into precursor solution at 60°C under stirring and after 10 min HCl was added to the precipitate and stirred for 1 h	7.8 nm	[11]
Reverse micelle and microemulsion technique					
Fe ₃ O ₄	Fe(III) stearate	hydrazine	Organic phase—precursor, olyelamine and xylene; aqueous phase—F127 in water; reverse micelles solution heated to 90°C, hydrazine addition and stirring for 3 h	2.8 nm	[43]
Fe ₃ O ₄	Fe(III) oleate	hydrazine	Organic phase—precursor, olyelamine and xylene; aqueous phase—F127 in water; reverse micelles solution heated to 90°C, hydrazine addition and stirring for 3 h	7 nm	[43]
Fe ₃ O ₄	Fe(III) acetylacetonate Oleic acid	hydrazine	Organic phase—precursor, olyelamine and xylene; aqueous phase—F127 in water; reverse micelles solution heated to 90°C, hydrazine addition and stirring for 3 h	<2 nm	[43]

Nanoparticle type	Precursor	Precipitating agent	Process and conditions	Size	Ref.
Fe ₃ O ₄	FeCl ₂ ·4H ₂ O, Fe (NO ₃) ₃ ·9H ₂ O	hydrazine	Organic phase—SDBS, xylene; aqueous phase—ethanol, precursor salts in water; reverse micelles solution heated to 90°C, hydrazine addition and refluxed for 5 h	2–10 nm	[42]
Thermal decomposition technique					
γ-Fe ₂ O ₃	Fe(CO) ₅	Trimethylamine oxide	Complexation—precursor, octylether, oleic acid; maintained at 100°C for 1 h; treated with trimethyloxyde; heated to 130°C under Ar atmosphere; refluxed at 300°C	4–16 nm	[44]
Iron oxide	Fe(CO) ₅	—	Complexation—precursor, dioctylether, oleic acid; raised to 100°C, N ₂ atmosphere, and held for 10 min; refluxed at 290°C for 1 h	3–10 nm	[45]
Fe ₃ O ₄	Iron oleate	Isopropanol	Precursor, sodium oleate (different concentration), oleic acid dissolved in 1-octadecene or tri-n-octylamine; refluxed at 300 to 360°C (with varying heating rate from 5 to 15°C/min) for 2 to 6 h	Diverse morphology and size	[46]
Hydrothermal technique					
Iron oxide	FeCl ₃ ·6H ₂ O, FeCl ₂ ·4H ₂ O	Ammonia	The primary nanoparticles prepared by adding ammonia into precursor solution were grown in an autoclave at 60°C (12 h), 100°C (12 h, 72 h), 150°C (12 h, 24 h), 180°C (1 h, 12 h, 24 h, 48 h)	12–49 nm	[47]
γ-Fe ₂ O ₃	FeCl ₃ ·6H ₂ O	Hydrazine	The primary nanoparticles prepared by adding hydrazine into precursor/ PEG8000 solution were grown in an autoclave at 120°C, 140°C, 160°C for 4 h	Diverse morphology and size	[Torrez]
α-Fe ₂ O ₃	FeCl ₃ ·6H ₂ O	Ammonium hydroxide	The solution containing different precursor, solvent and additives was autoclaved at 160°C/180°C for 10–12 h	Diverse morphology and size	[48]

Nanoparticle type	Precursor	Precipitating agent	Process and conditions	Size	Ref.
β -FeOOH rods	FeCl ₃ ·6H ₂ O	Urea	70–110°C for 8 h	0.4–2.3 μ m	[49]
β -Fe ₂ O ₃	FeCl ₃ ·6H ₂ O	Urea, Ammonia	pH 9.66, 150°C for 8 h	50–90 nm	[50]
Sonochemical technique					
α -Fe ₂ O ₃	FeCl ₃ ·6H ₂ O	NaOH	The precursor solution was added dropwise into NaOH solution and subjected to varying sonication power (1, 3, 5, 7, 9 W) and reaction temperature for 30 min; annealed for 1 h	Amorphous/ crystalline particles	[53]
Green chemistry and biological methods					
Fe ₂ O ₃ rods	Fe(SO ₄)·7H ₂ O	Leaf extract/ NaOH	To the precursor solution, a mixture of <i>Lantana camara</i> leaf extract and NaOH was added; precipitate obtained was air-dried	10–20 nm	[57]
Fe ₂ O ₃	FeCl ₃ ·6H ₂ O	Leaf extract	To the Bauhinia tomentosa leaf extract, the precursor solution was added; precipitate obtained was air-dried	70 nm	[27]
Iron oxide	FeSO ₄ ·7H ₂ O	Henna extract/ NaOH	Henna extract was added to precursor solution maintained at 60 °C for 30 min; NaOH is added to form Iron oxides	150–200 nm	[58]
Iron oxide	FeCl ₃ ·6H ₂ O	Leaf extract/ Na ₂ CO ₃	One part of precursor solution was mixed with leaf extract of <i>Cymbopogon citratus</i> and heated to 60°C for 1 h; two parts of precursor added to the mixture and temperature was raised to 85°C; pH raised to 10 using Na ₂ CO ₃ to precipitate Iron oxide	9 $\pm$ 4 nm	[59]
Fe ₃ O ₄	FeCl ₂ ·4H ₂ O, FeCl ₃ ·6H ₂ O	Plant extract/ glycine	Hot precursor solution was added to whole plant extract of <i>Kappaphycus alvarezii</i> and Glycine; stirred for 60 min; heated upto 300°C for 15 min; annealed in air at 500°C for 2 h	10–30 nm	[60]
Fe-O	FeCl ₃ ·6H ₂ O	Flower extract	Precursor solution was added dropwise into flower extract of <i>Avecinnia marina</i> ; centrifuged; dried under vacuum at 125°C for 2 h	10–40 nm	[61]

Nanoparticle type	Precursor	Precipitating agent	Process and conditions	Size	Ref.
γ -Fe ₂ O ₃	Fe (NO ₃) ₃ ·9H ₂ O	Grape berry ferment	The pH of the precursor solution was adjusted from 2.7 to 1.5 using grape berry ferment; transferred to autoclave and kept at 200° C for 24 h; iron oxide precipitate was obtained	6–18 nm	[62]

Table A1.
Synthesis techniques of iron oxide nanoparticles with its characteristic size.

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References

- [1] Gharpure KM, Wu SY, Li C, Berestein GL, Sood AK. Nanotechnology: Future of oncotherapy. *Clinical Cancer Research*. 2015;**21**(14):3121-3130
- [2] Hartshorn CM, Bradbury MS, Lanze GM, Nel AE, Rao J, Wang AZ, et al. Nanotechnology strategies to advance outcomes in clinical cancer care. *ACS Nano*. 2018;**12**(1):24-43
- [3] Madamsetty VS, Mukherjee A, Mukherjee S. Recent trends of the bio-inspired nanoparticles in cancer theranostics. *Frontiers in Pharmacology*. 2019;**10**:1264. DOI: 10.3389/fphar.2019.01264
- [4] Soenen SJ, Cuyper MD, Smedt SCD, Braeckmans K. Investigating the toxic effects of Iron oxide nanoparticles. *Methods in Enzymology*. 2012;**509**: 195-224. DOI: 10.1016/B978-0-12-391858-1.00011-3
- [5] Farzin A, Etesami SA, Quint J, Memic A, Tamayol A. Magnetic nanoparticles in cancer therapy and diagnosis. *Advanced Healthcare Materials*. 2020;**9**(9):1901058
- [6] Wu W, He Q, Jiang C. Magnetic iron oxide nanoparticles: Synthesis and surface functionalization strategies. *Nanoscale Research Letters*. 2008;**3**:397
- [7] Rahman MM, Khan SB, Jamal A, Faisal M, Aisiri AM. Iron Oxide Nanoparticles. In: *Nanomaterials*. London, UK: IntechOpen Limited; 2011. pp. 43-65. DOI: 10.5772/27698. [Chapter 3]
- [8] Tucek J, Machala L, Ono S, Namai A, Yoshikiyo M, Imoto K, et al. Zeta-Fe₂O₃—A new stable polymorph in iron(III) oxide family. *Scientific Reports*. 2015;**5**:15091
- [9] Peng X, Chen H, Huang J, Mao H, Shin DM. Targeted magnetic iron oxide nanoparticles for tumour imaging and therapy. In: Rezai RF, editor. *Biomedical Engineering – From theory to applications*. London, UK: IntechOpen; 2011. DOI: 10.5772/22873
- [10] Sun C, Lee JSH, Zhang M. Magnetic nanoparticles in MR imaging and drug delivery. *Advanced Drug Delivery Reviews*. 2008;**60**(11):1252-1265
- [11] Janani V, Induja S, Jaison D, Abhinav EM, Mothilal M, Gopalakrishnan C. Tailoring the hyperthermia potential of magnetite nanoparticles via gadolinium ion substitution. *Ceramics International*. 2021;**47**(22):31399-31046. DOI: 10.1016/j.ceramint.2021.08.015
- [12] Jeevanandam J, Barhoum A, Chan YS, Dufresne A, Danquah MK. Review on nanoparticles and nanostructured materials: History, sources, toxicity and regulations. *Beilstein Journal of Nanotechnology*. 2018;**9**:1050-1074
- [13] Guisbiers G, Mejía-Rosales S, Deepak FL. Nanomaterial properties: Size and shape dependencies. *Journal of Nanomaterials*. 2012;**2012**: Article ID 180976
- [14] Besenhard MO, LaGrow AP, Hodzic A, Kriechbaum M, Panariello L, et al. Co-precipitation synthesis of stable iron oxide nanoparticles with NaOH: New insights and continuous production via flow chemistry. *Chemical Engineering Journal*. 2020;**399**:125740
- [15] Yaid NA, Joon YC. Co-precipitation synthesis of magnetic nanoparticles for

efficient removal of heavy metal from synthetic wastewater. AIP Conference Proceedings. 2019;**2124**:020019

[16] Sakurai S, Namai A, Hashimoto K, Ohkoshi S. First observation of phase transformation of all four Fe₂O₃ phases ($\gamma \rightarrow \epsilon \rightarrow \beta \rightarrow \alpha$ -phase). Journal of the American Chemical Society. 2009;**131**: 18299-18303

[17] Unni M, Uhl AM, Savliwala S, Savitzky BH, Dhavalikar R, Garraud N, et al. Thermal decomposition synthesis of iron oxide nanoparticles with diminished magnetic dead layer by controlled addition of oxygen. ACS Nano. 2017;**11**(2):2284-2303

[18] Dolores R, Raquel S, Adianez G-L. Sonochemical synthesis of iron oxide nanoparticles loaded with folate and cisplatin: Effect of ultrasonic frequency. Ultrasonics Sonochemistry. 2015;**23**: 391-398

[19] Aivazoglou E, Metaxa E, Hristoforou E. Microwave-assisted synthesis of iron oxide nanoparticles in biocompatible organic environment. AIP Advances. 2018;**8**(4):048201

[20] Hu L, Percheron A, Chaumont D, et al. Microwave-assisted one-step hydrothermal synthesis of pure iron oxide nanoparticles: Magnetite, maghemite and hematite. Journal of Sol-Gel Science and Technology. 2011;**60**: 198

[21] Ge S, Shi X, Sun K, Li C, Uher C, Baker JR, et al. Facile hydrothermal synthesis of iron oxide nanoparticles with tunable magnetic properties. The Journal of Physical Chemistry C. 2009;**113**(31):13593-13599

[22] Torres-Gómez N, Nava O, Argueta-Figueroa L, García-Contreras R, Baeza-Barrera A, Vilchis-Nestor AR. Shape

tuning of magnetite nanoparticles obtained by hydrothermal synthesis: Effect of temperature. Journal of Nanomaterials. 2019;**2019**:7921273

[23] Ankamwar B. Hydrothermal synthesis of rod shaped iron oxide nanoparticles. AIP Conference Proceedings. 2021;**2335**(1):080002. DOI: 10.1063/5.0046421

[24] Kekalo K, Koo K, Zeitchick E, Baker I. Microemulsion synthesis of iron core/iron oxide shell magnetic nanoparticles and their physicochemical properties. Materials Research Society Symposium Proceedings. 2012;**1416**. DOI: 10.1557/opl.2012.736

[25] Housaindokht MR, Nakhaei Pour A. Size control of iron oxide nanoparticles using reverse microemulsion method: Morphology, reduction, and catalytic activity in CO hydrogenation. Journal of Chemistry. 2013;**2013**:781595

[26] Rose LC, Suhaimi H, Mamat M, Lik TZ. Synthesis and characterization of oleic acid surface modified magnetic iron oxide nanoparticles by using biocompatible w/o microemulsion for heavy metal removal. AIP Conference Proceedings. 2017;**1885**(1):020113. DOI: 10.1063/1.5002307

[27] Lakshminarayanan S, Shereen MF, Niraimathi KL, et al. One-pot green synthesis of iron oxide nanoparticles from *Bauhinia tomentosa*: Characterization and application towards synthesis of 1, 3 diolein. Scientific Reports. 2021;**11**:8643

[28] Bhuiyan MSH, Miah MY, Paul SC, Aka TD, Saha O, Rahaman MM, et al. Green synthesis of iron oxide nanoparticle using Carica papaya leaf extract: Application for photocatalytic degradation of remazol yellow RR dye

and antibacterial activity. *Heliyon*. 2020; **6**(8):e04603

[29] Majeed S, Danish M, Mohamad Ibrahim MN, et al. Bacteria mediated synthesis of iron oxide nanoparticles and their antibacterial, antioxidant, cytocompatibility properties. *Journal of Cluster Science*. 2021;**32**:1083-1094

[30] Greculeasa SG, Palade P, Schinteie G, et al. Tuning structural and magnetic properties of Fe oxide nanoparticles by specific hydrogenation treatments. *Scientific Reports*. 2020;**10**: 17174

[31] Ramimoghadam D, Bagheri S, Hamid SBA. Progress in electrochemical synthesis of magnetic iron oxide nanoparticles. *Journal of Magnetism and Magnetic Materials*. 2014;**368**: 207-229

[32] Ali A, Zafar H, Zia M, Haq IU, Phull AR, Ali JS, et al. Synthesis, characterisation, applications, and challenges of iron oxide nanoparticles. *Nanotechnology, Science and Applications*. 2016;**9**:49-67

[33] Gilmour MI, O'Connor S, Dick CA, Miller CA, Linak WP. Differential pulmonary inflammation and in vitro cytotoxicity of size-fractionated fly ash particles from pulverized coal combustion. *Journal of the Air & Waste Management Association*. 2004;**54**: 286-295

[34] Oberdorster G. Pulmonary effects of inhaled ultrafine particles. *International Archives of Occupational and Environmental Health*. 2001;**74**:1-8

[35] Vogel CFA, Charrier JG, Wu D, McFall AS, Li W, Abid A, et al. Physicochemical properties of iron oxide nanoparticles that contribute to cellular ROS-dependent signaling and acellular

production of hydroxyl radical. *Free Radical Research*. 2016;**50**(11):1153-1164

[36] Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry*. 2019;**12**(7):908-931. DOI: 10.1016/j.arabjc.2017.05.011

[37] Peternele WS, Monge Fuentes V, Fascineli ML, Rodrigues da Silva J, Silva RC, Lucci CM, et al. Experimental investigation of the coprecipitation method: An approach to obtain magnetite and maghemite nanoparticles with improved properties. *Journal of Nanomaterials*. 2014;**2014**:682985

[38] Aragaw TA, Bogale FM, Aragaw BA. Iron-based nanoparticles in wastewater treatment: A review on synthesis methods, applications, and removal mechanisms. *Journal of Saudi Chemical Society*. 2021;**25**(8):101280

[39] Chang M, Shih YH. Synthesis and application of magnetic iron oxide nanoparticles on the removal of Reactive Black 5: Reaction mechanism, temperature, and pH effects. *Journal of Environmental Management*. 2018; **224**(2018):235-242

[40] Cui H, Liu Y, Ren W. Structure switch between α -Fe₂O₃, γ -Fe₂O₃ and Fe₃O₄ during the large scale and low-temperature sol-gel synthesis of nearly monodispersed iron oxide nanoparticles. *Advanced Powder Technology*. 2013;**24**: 93-97

[41] Cui H, Ren W, Lin P, Liu Y. Structure control synthesis of iron oxide polymorph nanoparticles through an epoxide precipitation route. *Journal of Experimental Nanoscience*. 2013;**8**(7-8):869-875

[42] Lee Y, Lee J, Bae CJ, Park J-G, Noh H-J, Park J-H, et al. Large-scale synthesis

of uniform and crystalline magnetite nanoparticles using reverse micelles as nanoreactors under reflux conditions. *Advanced Functional Materials*. 2005; **15**(3):503-509. DOI: 10.1002/adfm.200400187

[43] Jung E, Kim SW, Cho A, Kim YJ, Jeong GJ, Kim J, et al. Synthesis of sub 3 nm-sized uniform magnetite nanoparticles using reverse micelle method for biomedical application. *Materials (Basel)*. 2019;**12**(23):38-50

[44] Hyeon T, Lee SS, Park J, Chung Y, Na HB. Synthesis of highly crystalline and monodisperse maghemite nanocrystallites without a size-selection process. *Journal of the American Chemical Society*. 2001; **123**(51):12798-12801

[45] Lassenberger A, Grünewald TA, van Oostrum PDJ, Rennhofer H, Amenitsch H, Zirbs R, et al. Monodisperse iron oxide nanoparticles by thermal decomposition: elucidating particle formation by second-resolved in situ small-angle X-ray scattering. *Chemistry of Materials*. 2017; **29**(10):4511-4522

[46] Zhou Z, Zhu X, Wu D, Chen Q, Huang D, Sun, et al. Anisotropic shaped iron oxide nanostructures: controlled synthesis and proton relaxation shortening effects. *Chemistry of Materials*. 2015;**27**(9):3505-3515

[47] Ozel F, Kockar H, Karaagac O. Growth of iron oxide nanoparticles by hydrothermal process: Effect of reaction parameters on the nanoparticle size. *Journal of Superconductivity and Novel Magnetism*. 2014;**28**(3):823-829

[48] Tadic M, Trpkov D, Kopanja L, Vojnovic S, Panjan M. Hydrothermal synthesis of hematite (α -Fe₂O₃) nanoparticle forms: Synthesis conditions, structure, particle shape analysis, cytotoxicity and magnetic

properties. *Journal of Alloys and Compounds*. 2019;**792**:599-609

[49] Xu Z, Liang J, Zhou L. Template-free hydrothermal synthesis of β -FeOOH nanorods and their catalytic activity in the degradation of methyl orange by a photo-Fenton-like process. *OJINM*. 2013;**3**(4):58-65

[50] Rahman MM, Jamal A, Khan SB, Faisal M. Characterization and applications of as-grown β -Fe₂O₃ nanoparticles prepared by hydrothermal method. *Journal of Nanoparticle Research*. 2011;**13**(9):3789-3799

[51] Jung HS, Choi HJ. Hydrothermal fabrication of octahedral-shaped Fe₃O₄ nanoparticles and their magnetorheological response. *Journal of Applied Physics*. 2015;**117**(17):17E708

[52] Yang Y, Huang M, Qian J, et al. Tunable Fe₃O₄ nanorods for enhanced magnetic hyperthermia performance. *Scientific Reports*. 2020;**10**:8331

[53] Hassanjani-Roshan A, Vaezi MR, Shokuhfar A, Rajabali Z. Synthesis of iron oxide nanoparticles via sonochemical method and their characterization. *Particuology*. 2011;**9**(1):95-99

[54] Fuentes-Garcia JA, Alavarse AC, Maldonado ACM, Toro-Cordova A, Ibarra MR, Goya F. Simple sonochemical method to optimize the heating efficiency of magnetic nanoparticles for magnetic fluid hyperthermia. *ACS Omega*. 2020;**5**:26357-26364

[55] Lüdtke-Buzug K, Penxová Z. Superparamagnetic iron oxide nanoparticles: An evaluation of the sonochemical synthesis process. *Current Directions in Biomedical Engineering*. 2019;**5**(1):307-309. DOI: 10.1515/cdbme-2019-0077

- [56] Priya N, Kaur K, Sidhu AK. Green synthesis: An eco-friendly route for the synthesis of iron oxide nanoparticles. *Frontiers in Nanotechnology*. 2021;**3**: 655062
- [57] Rajiv P, Bavadharani B, Kumar MN, Vanathi P. Synthesis and characterization of biogenic iron oxide nanoparticles using green chemistry approach and evaluating their biological activities. *Biocatalysis and Agricultural Biotechnology*. 2017;**12**:45-49. DOI: 10.1016/j.bcab.2017.08.015
- [58] Chauhan S, LSB U. Biosynthesis of iron oxide nanoparticles using plant derivatives of *Lawsonia inermis* (Henna) and its surface modification for biomedical application. *Nanotechnology for Environmental Engineering*. 2019; **4**(1):8. DOI: 10.1007/s41204-019-0055-5
- [59] Patino-Ruiz D, Sanchez-Botero L, Tejeda-Benitez L, Hinestroza J, Herrera A. Green synthesis of iron oxide nanoparticles using *Cymbopogon citratus* extract and sodium carbonate salt: Nanotoxicological considerations for potential environmental applications. *Environmental Nanotechnology Monitoring & Management*. 2020;**14**: 100377
- [60] Arularasu MV, Devakumar J, Rajendran TV. An innovative approach for green synthesis of iron oxide nanoparticles: Characterization and its photocatalytic activity. *Polyhedron*. 2018;**156**:279-290
- [61] Jaison D, Gopalakrishnan C, Mothilal M. pH-sensitive natural almond gum hydrocolloid based magnetic nanocomposites for theragnostic applications. *International Journal of Biological Macromolecules*. 2020;**154**: 256-266
- [62] Karpagavinayagam P, Vedhi C. Green synthesis of iron oxide nanoparticles using *Avicennia marina* flower extract. *Vacuum*. 2018;**160**:286-292
- [63] Zhu L, Wang D, Wei X, Zhu X, Li J, Tu C, et al. Multifunctional pH-sensitive superparamagnetic iron-oxide nanocomposites for targeted drug delivery and MR imaging. *Journal of Controlled Release*. 2013;**169**(3):228-238
- [64] Lu J, Sun J, Li F, Wang J, Liu J, Kim D, et al. Highly sensitive diagnosis of small hepatocellular carcinoma using pH-responsive iron oxide nanocluster assemblies. *Journal of the American Chemical Society*. 2018;**140**(32): 10071-10074
- [65] Espinola-Portilla F, Serrano-Torres O, Hurtado-López GF, Sierra U, Varenne A, d'Orlyé F, et al. Superparamagnetic iron oxide nanoparticles functionalized with a binary alkoxysilane array and poly(4-vinylpyridine) for magnetic targeting and pH-responsive release of doxorubicin. *New Journal of Chemistry*. 2021;**45**(7):3600-3609
- [66] Amin M, Huang W, Seynhaeve ALB, ten Hagen TLM. Hyperthermia and temperature-sensitive nanomaterials for spatiotemporal drug delivery to solid tumors. *Pharmaceutics*. 2020;**12**(11): 1007
- [67] Kalele S, Narain R, Krishnan KM. Probing temperature-sensitive behavior of pNIPAAm-coated iron oxide nanoparticles using frequency-dependent magnetic measurements. *Journal of Magnetism and Magnetic Materials*. 2009;**321**(10):1377-1380
- [68] Tai LA, Tsai PJ, Wang YC, Wang YJ, Lo LW, Yang CS. Thermosensitive liposomes entrapping iron oxide nanoparticles for controllable drug release. *Nanotechnology*. 2009;**20**(13): 135101

- [69] Mai BT, Balakrishnan PB, Barthel MJ, Piccardi F, Niculaes D, Marinaro F, et al. Thermoresponsive iron oxide nanocubes for an effective clinical translation of magnetic hyperthermia and heat-mediated chemotherapy. *ACS Applied Materials & Interfaces*. 2019; **11**(6):5727-5739
- [70] Hemery G, Garanger E, Lecommandoux S, Wong AD, Gillies ER, Pedrono B, et al. Thermosensitive polymer-grafted iron oxide nanoparticles studied by in situ dynamic light backscattering under magnetic hyperthermia. *Journal of Physics D: Applied Physics*. 2015; **48**(49):494001
- [71] Espinosa-Cano E, Palao-Suay R, Aguilar MR, Vázquez B, Román JS. Polymeric nanoparticles for cancer therapy and bioimaging. In: Goncalves G, Tobias G, editors. *Nanooncology: Nanomedicine and Nanotoxicology*. AG: Springer International Publishing; 2018. pp. 137-172
- [72] Liu T, Shi S, Liang C, Shen S, Cheng L, Wang C, et al. Iron oxide decorated MoS₂ nanosheets with double pegylation for chelator-free radiolabeling and multimodal imaging guided photothermal therapy. *ACS Nano*. 2015; **9**:950-960
- [73] Harris TJ, von Maltzahn G, Lord ME, Park J-H, Agrawal A, Min D-H. Protease-triggered unveiling of bioactive nanoparticles. *Small*. 2008; **4**(9): 1307-1312
- [74] Fan CH, Cheng YH, Ting CY, Ho YJ, Hsu PH, Liu HL, et al. Ultrasound/ magnetic targeting with SPIO-DOX-microbubble complex for image-guided drug delivery in brain tumors. *Theranostics*. 2016; **6**(10):1542-1556
- [75] Machala L, Tucek J, Zboril R. Polymorphous transformations of nanometric iron(III) oxide: A review. *Chemistry of Materials*. 2011; **23**: 3255-3272
- [76] Tucek J, Zboril R, Namai A, Ohkoshi S. ϵ -Fe₂O₃: An advanced nanomaterial exhibiting giant coercive field, millimeter-wave ferromagnetic resonance and magnetoelectric coupling. *Chemistry of Materials*. 2010; **22**: 6483-6505
- [77] Kido O, Higashino Y, Kamitsuji K, Kurumada M, et al. Phase transition temperature of γ -Fe₂O₃ ultrafine particle. *Journal of the Physical Society of Japan*. 2004; **73**(7):2014-2016
- [78] Lopez-Sanchez J, Serrano A, Campo AD, Munoz-Noval A, Salas-Colera E, et al. A combined micro-Raman, X-ray absorption and magnetic study to follow the glycerol-assisted growth of epsilon-iron oxide sol-gel coatings. *Journal of Alloys and Compounds*. 2022; **892**:162061
- [79] Chen C-J, Chiang R-K, Lai H-Y, Lin C-R. Characterization of monodisperse Wüstite nanoparticles following partial oxidation. *Journal of Physical Chemistry C*. 2010; **114**(10): 4258-4263
- [80] Ullrich A, Rolle N, Horn S. From wustite to hematite: thermal transformation of differently sized iron oxide nanoparticles in air. *Journal of Nanoparticle Research*. 2019; **21**:168
- [81] Vernekar D, Jagadeesan D. Tunable acid-base bifunctional catalytic activity of FeOOH in an orthogonal tandem reaction. *Catalysis Science & Technology*. 2015; **5**:4029-4038
- [82] Heuss-Aßbichler S, Amthauer G, John M, editors. *Highlights in Applied Mineralogy*. Berlin: Walter de Gruyter; 2018. ISBN: 978-3-11-049122-7

- [83] Tadic M, Spasojevic V, Kusigerski V, Markovic D, Remskar M. Formation of ϵ -Fe₂O₃ phase by the heat treatment of α -Fe₂O₃/SiO₂ nanocomposite. *Scripta Materialia*. 2008;**58**:703-706
- [84] Zboril R. Iron (III) oxides from thermal processes—Synthesis, structural and magnetic properties, Mossbauer spectroscopy characterization, and applications. *Chemistry of Materials*. 2002;**14**:969-982
- [85] Lopez-Sanchez J, McIntosh G, Osete ML, Campo AD, Villalain JJ, Perez L, et al. Epsilon iron oxide: Origin of the high coercivity stable low Curie temperature magnetic phase found in heated archeological materials. *Geochemistry, Geophysics, Geosystems*. 2017;**18**:2646-2656. DOI: 10.1002/2017GC006929
- [86] Gracia-Munoz JL, Romaguera A, Fauth F, Nogues J, Gich M. Unveiling a new high-temperature ordered magnetic phase in ϵ -Fe₂O₃. *Chemistry of Materials*. 2017;**29**(22):9705-9713
- [87] Koch CJW, Borggard OK, Madsen MB, Morup S. Magnetic properties of synthetic Feroxyhite (δ -FeOOH). In: Schultz LG, van Olphen H, Mumpton FA, editors. *Proceedings of the International Clay Conference Denver, Virginia, United States: Clay Minerals Society*; 1985
- [88] Nishida N, Amagasa S, Kobayshi Y, Yamada Y. Synthesis of superparamagnetic δ -FeOOH nanoparticles by a chemical methods. *Applied Surface Science*. 2016;**387**:996-1001
- [89] Akharam MO, Fagbayigbo BO, Pereao O, Oputu OU, Olorunfemi DI, Fatoki OS, et al. Beta-FeOOH nanoparticles: a promising nano-based material for water treatment and remediation. *Journal of Nanoparticle Research*. 2021;**23**:8
- [90] Lee GH, Kim SH, Choi BJ, Huh SH, Chang Y, Kim B, et al. Magnetic properties of needle-like $\{\alpha\}$ -FeOOH and $\{\gamma\}$ -FeOOH nanoparticles. *Journal of the Korean Physical Society*. 2004;**45**(4):1019-1024
- [91] Hiemstra T. Surface structure controlling nanoparticle behavior: magnetism of ferrihydrite, magnetite, and maghemite. *Environmental Science: Nano*. 2018;**5**(3):752-764
- [92] Xia T, Wang J, Wu C, et al. Novel complex-coprecipitation route to form high quality triethanolamine-coated Fe₃O₄ nanocrystals: Their high saturation magnetizations and excellent water treatment properties. *CrystEngComm*. 2012;**14**(18):5741-5744
- [93] Yang HM, Park CW, et al. HER2/neu antibody conjugated poly(amino acid)-coated iron oxide nanoparticles for breast cancer MR imaging. *Biomacromolecules*. 2010;**11**(11):2866-2872
- [94] Lee JH, Huh YM, et al. Artificially engineered magnetic nanoparticles for ultra-sensitive molecular imaging. *Nature Medicine*. 2007;**13**:95-99
- [95] Zhao S, Yu X, Qian Y, Chen W, Shen J. Multifunctional magnetic iron oxide nanoparticles: an advanced platform for cancer theranostics. *Theranostics*. 2020;**10**(14):6278-6309
- [96] Weng Q, Hu X, Zheng J, Xia F, Wang N, Liao H, et al. Toxicological risk assessments of iron oxide nanocluster- and gadolinium-based T₁ MRI contrast agents in renal failure rats. *ACS Nano*. 2019;**13**:6801-6812
- [97] Ajinkya N, Yu X, Kaithal P, Luo H, Somani P, Ramakrishna S. Magnetic iron

oxide nanoparticle (IONP) synthesis to applications: Present and future. *Materials*. 2020;**13**:4644

[98] Sun C, Veisheh O, Gunn J, Fang C, et al. In vivo MRI detection of gliomas by chlorotoxin-conjugated superparamagnetic nanoprobe. *Small*. 2008;**4**(3):372-379

[99] Tefft BJ, Uthamaraj S, Harburn JJ, Klabusay M, Dragomir-Daescu D, Sandhu GS. Cell labeling and targeting with superparamagnetic iron oxide nanoparticles. *Journal of Visualized Experiments*. 2015;**105**:e53099. DOI: 10.3791/53099

[100] Chung S, Revia RA, Zhang M. Iron oxide nanoparticles for immune cell labeling and cancer immunotherapy. *Nanoscale Horiz*. 2021;**6**:696-717

[101] Shen Z, Chen T, Ma X, Ren W, Zhou Z, Zhu G, et al. Multifunctional theranostic nanoparticles based on exceedingly small magnetic iron oxide nanoparticles for T₁-weighted magnetic resonance imaging and chemotherapy. *ACS Nano*. 2017;**11**:10992-11004

[102] Arbab AS, Yocum GT, Rad AM, Khakoo AY, Fellowes V, Read EJ, et al. Labeling of cells with ferumoxides-protamine sulfate complexes does not inhibit function or differentiation capacity of hematopoietic or mesenchymal stem cells. *NMR in Biomedicine*. 2005;**18**(8):553-559. DOI: 10.1002/nbm.991

[103] Korchinski DJ, Taha M, Yang R, Nathoo N, Dunn JF. Iron oxide as an MRI contrast agent for cell tracking. *Magnetic Resonance Insights*. 2015;**8**(Suppl 1):15-29

[104] Chen TJ, Cheng TH, Chen CY. Targeted herceptin-dextran iron oxide nanoparticles for noninvasive imaging of HER2/neu receptors using MRI.

Journal of Biological Inorganic Chemistry. 2009;**14**(2):253-260

[105] Chen TJ, Cheng TH, Hung YC, Lin KT, Liu GC, Wang YM. Targeted folic acid-PEG nanoparticles for noninvasive imaging of folate receptor by MRI. *Journal of Biomedical Materials Research. Part A*. 2008;**87**(1):165-175

[106] Dadfar SM, Roemhild K, Drude NI, von Stillfried S, Knüchel R, Kiessling F, et al. Iron oxide nanoparticles: Diagnostic, therapeutic and theranostic applications. *Advanced Drug Delivery Reviews*. 2019;**138**:302-325

[107] Mi Y, Li K, Liu Y, Pu K-Y, Liu B, Feng S-S. Herceptin functionalized polyhedral oligomeric silsesquioxane – conjugated oligomers – silica/iron oxide nanoparticles for tumor cell sorting and detection. *Biomaterials*. 2011;**32**(32): 8226-8233

[108] Du X, Zhou J, Wu L, Sun S, Xu B. Enzymatic transformation of phosphate decorated magnetic nanoparticles for selectively sorting and inhibiting cancer cells. *Bioconjugate Chemistry*. 2014;**25**(12):2129-2133

[109] Cheng HW, Tsao HY, Chiang CS, Chen SY. Advances in magnetic nanoparticle-mediated cancer immune-theranostics. *Advanced Healthcare Materials*. 2021;**10**(1):e2001451. DOI: 10.1002/adhm.202001451

[110] Kircher MF, Allport JR, Graves EE, Love V, Josephson L, Lichtman AH, et al. In vivo high resolution three-dimensional imaging of antigen-specific cytotoxic T-lymphocyte trafficking to tumors. *Cancer Research*. 2003;**63**(20): 6838-6846

[111] Liu L, Ye Q, Wu Y, Hsieh WY, Chen CL, Shen HH, et al. Tracking T-cells in vivo with a new nano-sized MRI

contrast agent. *Nanomedicine*. 2012; **8**(8):1345-1354

[112] Li K, Nejadnik H, Daldrup-Link HE. Next-generation superparamagnetic iron oxide nanoparticles for cancer theranostics. *Drug Discovery Today*. 2017;**22**(9):1421-1429

[113] Li K, Lu L, Xue C, Liu J, He Y, Zhou J, et al. Polarization of tumor-associated macrophages phenotype via porous hollow iron nanoparticles for tumor immunotherapy in vivo. *Nanoscale*. 2020; **12**:130-144. DOI: 10.1039/c9nr06505a

[114] Li CX, Zhang Y, Dong X, Zhang L, Liu MD, Li B, et al. Artificially reprogrammed macrophages as tumor-tropic immunosuppression-resistant biologics to realize therapeutics production and immune activation. *Advanced Materials*. 2019;**31**(15):e1807211

[115] de Vries IJ, Lesterhuis WJ, Barentsz JO, Verdijk P, van Krieken JH, Boerman OC, et al. Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy. *Nature Biotechnology*. 2005; **23**(11):1407-1413

[116] Zhao Y, Zhao X, Cheng Y, Guo X, Yuan W. Iron oxide nanoparticles-based vaccine delivery for cancer treatment. *Molecular Pharmaceutics*. 2018;**15**(5): 1791-1799

[117] Traini G, Ruiz-de-Angulo A, Blanco-Canosa JB, Zamacola Bascarán K, Molinaro A, Silipo A, et al. Cancer immunotherapy of TLR4 agonist-antigen constructs enhanced with pathogen-mimicking magnetite nanoparticles and checkpoint blockade of PD-L1. *Small*. 2019;**15**(4):e1803993

[118] Zhang Q, Wei W, Wang P, Zuo L, Li F, Xu J, et al. Biomimetic magnetosomes as versatile artificial

antigen-presenting cells to potentiate T-cell-based anticancer therapy. *ACS Nano*. 2017;**11**(11):10724-10732

[119] Wang Z, Zhang F, Shao D, Chang Z, Wang L, Hu H, et al. Janus nanobullets combine photodynamic therapy and magnetic hyperthermia to potentiate synergetic anti-metastatic immunotherapy. *Advancement of Science*. 2019;**6**(22):1901690

[120] Long CM, van Laarhoven HWM, Bulte JWM, Levitsky HI. Magnetovaccination as a novel method to assess and quantify dendritic cell tumor antigen capture and delivery to lymph nodes. *Cancer Research*. 2009; **69**(7):3180-3187

[121] Kosmides AK, Sidhom J-W, Fraser A, Bessell CA, Schneck JP. Dual targeting nanoparticle stimulates the immune system to inhibit tumor growth. *ACS Nano*. 2017;**11**(6):5417-5429

[122] Luo X, Peng X, Hou J, Wu S, Shen J, Wang L. Folic acid-functionalized polyethylenimine superparamagnetic iron oxide nanoparticles as theranostic agents for magnetic resonance imaging and PD-L1 siRNA delivery for gastric cancer. *International Journal of Nanomedicine*. 2017;**12**:5331-5343

[123] Gu Y, Yoshikiyo M, Namai A, Bonvin D, Martinez A, et al. Magnetic hyperthermia with ϵ -Fe₂O₃ nanoparticles. *RSC Advances*. 2020;**10**:28786

[124] Kolosnjaj-Tabi J, Di Corato R, Lartigue L, Marangon I, Guardia P, Silva AKA, et al. Heat-generating iron oxide nanocubes: Subtle “destructurators” of the tumoral microenvironment. *ACS Nano*. 2014; **8**(5):4268-4283

[125] Salimi M, Sarkar S, Saber R, et al. Magnetic hyperthermia of breast cancer

cells and MRI relaxometry with dendrimer-coated iron-oxide nanoparticles. *Cancer Nano*. 2018;**9**:7

[126] Hayashi K, Nakamura M, Sakamoto W, Yogo T, Miki H, Ozaki S, et al. Superparamagnetic nanoparticle clusters for cancer theranostics combining magnetic resonance imaging and hyperthermia treatment. *Theranostics*. 2013;**3**(6):366-376

[127] Lin SY, Huang RY, Liao WC, Chuang CC, Chang CW. Multifunctional PEGylated Albumin/IR780/iron oxide nanocomplexes for cancer photothermal therapy and MR imaging. *Nano*. 2018; **2**(2):106-116

[128] Kirui DK, Rey DA, Batt CA. Gold hybrid nanoparticles for targeted phototherapy and cancer imaging. *Nanotechnology*. 2010;**21**(10):105105

[129] Yang R, Fu C, Fang J, Xu X, Wei X, Tang W, et al. Hyaluronan-modified superparamagnetic iron oxide nanoparticles for bimodal breast cancer imaging and photothermal therapy. *International Journal of Nanomedicine*. 2017;**12**:197-206

[130] Espinosa A, Di Corato R, Kolosnjaj-Tabi J, Flaud P, Pellegrino T, Wilhelm C. Duality of iron oxide nanoparticles in cancer therapy: Amplification of heating efficiency by magnetic hyperthermia and photothermal bimodal treatment. *ACS Nano*. 2016;**10**(2):2436-2446

[131] Chauhan VP, Stylianopoulos T, Boucher Y, Jain RK. Delivery of molecular and nanoscale medicine to tumors: Transport barriers and strategies. *Annual Review of Chemical and Biomolecular Engineering*. 2011;**2**: 281-298

[132] Jain RK, Stylianopoulos T. Delivering nanomedicine to solid

tumors. *Nature Reviews. Clinical Oncology*. 2010;**7**(11):653-664

[133] Li X, Wang Z, Ma M, Chen Z, Tang X-L, Wang Z. Self-assembly iron oxide nanoclusters for photothermal-mediated synergistic chemo/chemodynamic therapy. *Journal of Immunology Research*. 2021; Article ID 9958239

[134] Norouzi M, Yathindranath V, Thliveris JA, et al. Doxorubicin-loaded iron oxide nanoparticles for glioblastoma therapy: A combinational approach for enhanced delivery of nanoparticles. *Scientific Reports*. 2020;**10**:11292

[135] Hussein-Al-Ali SH, Hussein MZ, Bullo S, Arulselvan P. Chlorambucil-iron oxide nanoparticles as a drug delivery system for leukemia cancer cells. *International Journal of Nanomedicine*. 2021;**16**:6205-6216