Recent advances of high performance magnetic iron oxide nanoparticles: Controlled synthesis, properties tuning and cancer theranostics

Yi-Jun Liang1 | Jun Xie2 | Jing Yu3 | Zhaoguang Zheng1 | Fang Liu1 | Anping Yang1

1 School of Medical Engineering, Foshan University, Foshan 528000, P.R. China
2 School of Life Science, Jiangsu Normal University, Xuzhou 221116, P.R. China
3 College of Materials Science and Engineering, Zhejiang University of Technology, Hangzhou 310014, P.R. China

Correspondence
Yi-Jun Liang, Fang Liu and Anping Yang, School of Medical Engineering, Foshan University, Foshan 528000, P.R. China.
Email: liangyijun@fosu.edu.cn (Y.-J. L.); liufang2019@fosu.edu.cn (F.L.); foshanyap@fosu.edu.cn (A.Y.)

Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 81901881, 81701821; National Key Research and Development Program of China, Grant/Award Number: 2018YFA0902702; Science and Technology Project for Social Development of Guangdong Province, Grant/Award Number: 0903129; Natural Science Foundation of Zhejiang Province, Grant/Award Number: LY20E020017; Fundamental Research Funds for the Provincial Universities of Zhejiang, Grant/Award Number: RF-A2019004

Abstract
Recently, magnetic nanomaterials have emerged as multifunctional materials for a wide range of biomedical applications. Functional magnetic iron oxide nanoparticles (FMIONs) are typical magnetic nanomaterials with inherent advantages for disease diagnosis, prevention, and treatment: high specific surface area, excellent superparamagnetism, good colloidal stability, and remarkable biosafety. Therefore, FMION-based biomedicine has advanced at an unprecedented rate in recent years. However, the performance of FMIONs cannot yet meet complicated physiological circumstances. To overcome these limitations, researchers have designed and manipulated the geometric shapes, sizes, compositions, and surfaces to endow these FMIONs with high performance. This review summarizes recent advances in the controlled synthesis of FMIONs, cover corresponding theories of nucleation and growth in solution, and the tuning of FMION properties for disease diagnosis and therapy. After discussing the biomedical applications in cancer theranostics—tumor hyperthermia, magnetic resonance imaging and drug delivery system—in detail, the review concludes with current challenges and outlooks for the development of FMIONs.

KEYWORDS
cancer theranostics, Fe3O4 nanoparticles, high performance, nanomedicine, synthetic strategy

1 INTRODUCTION

As a representative frontier science and technology, nanotechnology has received increasing global attention in the present century.[1–3] Many natural substances exhibit unexpected characteristics at the nanoscale, which can be exploited in the development of novel nanomaterials for health and environmental improvements that ultimately benefit humankind.[4–6] Magnetic nanoparticles (MNPs) as the shining star which can be pure metals (e.g., Fe, Co,
metal alloys (e.g., FeCo, FePt, Fe₅C₉, FeₓSi₂) or iron oxides (e.g., FeₓOᵧ, MnFeₓOᵧ, CoFeₓOᵧ). Such MNPs are widely used in electronic devices and biomedicine. Although the pure metals and metal alloys exhibit stronger magnetism than iron oxides, they are less favored for biomedical applications due to the toxicity and instability. Consequently, superparamagnetic iron oxide nanoparticles (SPIONs, i.e., γ-Fe₂O₃ and Fe₃O₄) have become the representative non-toxic and biocompatible nanomaterials in diverse clinical practices, such as magnetic induction hyperthermia, magnetic resonance imaging (MRI), magnetic resonance imaging (MRI), biosensors, targeted drug delivery, and gene transfection.

To realize these biomedical applications, researchers have invested much effort in developing SPIONs with high performance. Popular methods found in the literature are biomimetic synthesis, physical methods, and corresponding chemical methods. Among which, chemical methods are regarded as the ideal synthetic routes for the controllable fabrication of monodisperse SPIONs and usually proceed through nucleation and growth process. In particular, nanoparticle formation largely depends on factors such as monomer concentration, reaction temperature, reaction time, and type of surfactant and has been explained by various theories, including LaMer mechanism, the Finke–Watzky two step mechanism, oriented attachment, and intraparticle growth.

The functional properties of MNPs greatly depend on their shape, size, composition, crystallinity, and surface modification. Thus, one can design high-performance MNPs for a particular application, as recently reported in biomedical applications of functional magnetic iron oxide nanoparticles (FMIONs). For example, in magnetic resonance imaging (MRI), FMIONs with different morphologies exhibit significantly different relaxivities. As another example, small SPIONs can easily escape during the long cycle time of liver and spleen phagocytosis, and can accumulate in lesions. FMIONs with high crystallinity provide a stronger magnetothermal effect, and a better therapeutic effect in magnetic hyperthermia, than lowly crystalline ones. Moreover, naked FMIONs can be coated with liposome to enhance their biocompatibility and reduce the cytotoxicity in tissue engineering. All of these strategies promise to advance the clinical application of FMIONs in future. This review tracks the recent progress of FMIONs from fabrication to biomedical applications in cancer theranostics. We first introduced some formation mechanisms of FMIONs in solution, especially cover the nucleation and growth processes. Based on the current popular fabrication methods, various strategies for improving the performance of FMIONs by shape-, size-, and surface-modifications are then discussed. Finally, the applications of FMIONs combining micro-nanobots, tumor hyperthermia, targeted MR imaging and drug delivery system are presented. For more detailed information on the preparation, physical properties, and biomedical applications of FMIONs, readers are referred to related reviews.

2 | NUCLEATION AND GROWTH MECHANISM OF FMIONS IN SOLUTION

Over the past few decades, nucleation and growth behaviors have been interpreted by classical crystallization theories of supersaturated solutions. For instance, in LaMer and Ostwald ripening models, the crystallization process is conceptually separated into nucleation and growth stages, and the particle size is assumed to vary with monomer concentration and surface energy. As shown in Figure 1, the monomer concentration gradually increases as the temperature reaches the critical point of precursor decomposition. When the monomer concentration satisfies the minimum nucleation concentration, burst nucleation occurs. At this moment, a large number of critical nuclei are generated by continuous consumption of the monomer. Eventually, the monomer concentration decreases to its nucleation threshold, and very few nuclei are formed. The as-formed crystal nuclei then grow simultaneously at the same rate. The classical growth stage is described by surface reaction and monomer-surface diffusion theory. As the growth rate of the crystal nucleus is proportional to the diffusion rate of the monomer, the nanocrystal solubility strongly depends on the nanocrystal size and its variation, which follows the Gibbs–Thomson relation. In particular, because the crystal growth process removes the smaller particles in the solution, which are soluble with a high surface energy, it improves the system stability, promoting the continuous growth of larger particles. Finally, monodispersed nanoparticles are obtained through the size-focusing effect.

Classical crystal theory predicts that molecules or atoms directly assemble into crystalline materials from solution. Metastable intermediates appear during thermodynamically stable crystalline-phase construction (Figure 2). In many systems, size uniformity of the obtained nanoparticles is thought to be mainly controlled by burst nucleation and diffusion-controlled growth. However, both the hot-injection and heat-up approaches affect the homogeneity of the particles during thermal decomposition. Hyeon’s group computationally simulated the hot-injection mediated nucleation and growth process. They pointed out that such highly supersaturated system provides the dynamic driving force of instantaneous burst nucleation and the succeeding size focusing, which is crucial for separating the nucleation and growth processes.
FIGURE 1  (A) The presentation of nucleation and growth process of monodisperse nanocrystals based on LaMer model. Nanocrystals with different size distribution could be obtained as grow with time and then isolated from the vessel by removing aliquots periodically. Reproduced with permission. [49] Copyright © 1950, American Chemical Society. (B) The supersaturation level associated with crystal nucleation and growth, $S_c$ stands for the critical supersaturation. Reproduced with permission. [52] Copyright © 2011, John Wiley and Sons

FIGURE 2  Cryogenic-Transmission Electron Microscopy (TEM) images of the nucleation and growth process of magnetite via co-precipitated approach. (A) The primary-particle and aggregated MNPs in 2 minutes, (B) in 6 minutes and (C) in 82 minutes, respectively. Yellow arrows of (B) showed the MNPs in early formed stage. (D) TEM image of the as-prepared MNPs. (E) Arrows in TEM image indicated the surface of a MNP attached with the primary-particle. Insets in (D, E): the crystallinity of the particles presented via fast Fourier transform. Scale bars, 10 nm. Adapted with permission. [27] Copyright © 2013, Nature Publishing Group
by the automatic regulation of uniform nucleation. However, the size distribution is affected by similar factors in both the hot-injection and heat-up approaches, and differs only by the way of forming high supersaturation at the initial stage. In hot injection, the initial stage occurs by rapid addition of the precursor, whereas burst nucleation occurs only when the monomer concentration exceeds the supersaturation level in heat-up approach, thereby crosses the barrier of homogeneous nucleation. Moreover, Lassen-berger et al.\textsuperscript{[54]} investigated the thermal decomposition of iron pentacarbonyl by small-angle X-ray scattering. Results showed that the size of inorganic nanostructures is closely related to the interplay between the precursor decomposition and nucleation process, or to the nuclei concentration in the initial stage of burst nucleation.

Apart from the classical theories mentioned above, Thanh and co-workers\textsuperscript{[29]} summarized additional mechanisms, such as the “Finke–Watzky” two step mechanism, digestive ripening, orientated attachment theory, the coalescence theory, and intraparticle growth theory. Actually, the Finke–Watzky two-step mechanism describes synchronous nucleation and growth process for synthesizing noble metals, such as ruthenium, platinum and rhodium, for which the nucleation step still follows the classic critical-nucleation principle. Notably, the first stage involves slow and continuous nucleation, and the subsequent growth stage is controlled by surface autocatalysis rather than diffusion. Meanwhile, coalescence theory and orientated attachment theory describe different orientations of the crystal lattice at the grain interfaces. In nanomaterial assemblage by oriented attachment, the crystal grains are automatically attached at crystal facets that match their dimensions and surface energies. Once a structural unit is composed, the short contact length of the crystal surface reduces the surface tension. As most of the crystal facets are arranged in order, the free energy of the system decreases and the system reaches a stable state.

In contrast, coalescence theory describes the growth of anisotropic crystals at certain precursor-to-surfactant ratios and heating rates. Under selective adsorption to different crystal surfaces, the growth rates vary at different crystal facets, eventually leading to crystal anisotropy.\textsuperscript{[55–59]} The intraparticle growth mechanism was proposed by Peng et al. to reveal the CdSe quantum-dots formation.\textsuperscript{[60]} Peng et al. suggested that the monomer diffusion changes along the surface and ultimately affects the particle morphology. However, to effectively restrain the diffusion movement, this process requires special circumstances, which means the energy of the monomer should be lower than the crystal-surface energy. In a word, the high-energy crystal facets are dissolved or removed without monomer diffusion, whereas the low-energy facets grow until intraparticle diffusion becomes possible.

A non-classical crystallization theory called mesocrystal theory has also been developed from biomineralization observations. Based on this theory, the nucleation and growth of nanoparticle involve a transition from a metastable intermediate to the stable structure. Moreover, the nanoscale building unit can induce long-range ordered aggregates by directional assembly of the matrix.\textsuperscript{[61]} Meso-morphic growth yields materials with unique advantages, such as unchanged osmotic pressure and high crystallinity, which are favorable for organic metal frameworks and macromolecule-mediated biomimeralization. Besides deepening our understanding of nucleation and growth of magnetic nanomaterials, these concepts provide a theoretical basis for preparing high-performance FMIONs.

3 | CONTROLLED SYNTHESIS OF FMIONS

The widely demand of FMIONs has driven the development of various synthetic approaches, including biomimetic synthesis by magnetosomal proteins or magnetotactic bacteria, mediated biomimetic synthesis, physical methods, and corresponding chemical methods. Over the decades, these approaches have realized high-quality FMIONs.\textsuperscript{[62–66]} However, although both biomimetic synthesis and physical methods meet the requirements of high-purity FMIONs in principle, the shape and size distributions of FMIONs are difficult to control in practice, and the simple, controllable and economical demands of large scale-up preparation are hardly met. These drawbacks can apparently be overcome by popular chemical methods, which have already demonstrated their potential in FMION preparation.\textsuperscript{[67,68]}

3.1 | Biomimetic synthesis of FMIONs

Biomimetic synthesis obtains magnetosomes through the biomineralization behavior of microorganisms with special functions. This approach has recently been promoted as a green and environmentally friendly synthesis of nanoparticles with high crystallinity.\textsuperscript{[69,70]} Magnetotropic bacteria belong to a class of anaerobic bacteria that form magnetosomes (mainly composed of Fe₃O₄) inside their lipid vesicles. Under the action of the Earth’s magnetic field, the magnetosomes move towards the oxygen-poor regions and are arranged longitudinally, acting as a compass that navigates the movements of the bacterial cells. Although magnetotactic bacteria have a long natural history, they were discovered in 1958 by Bellini and were known to researchers in 2009.\textsuperscript{[71]} Recent studies have identified the magnetosomal proteins in magnetotactic
bacteria as magnetosome-associated membrane mineralization protein Mms6, MamC, MamD, MamG, and others. Among these proteins, Mms6 plays an important role in regulating magnetosome formation during the biomineralization process. Combining in situ pH titration with co-precipitation, Rawlings and co-workers studied the function of Mms6 during the biomimetic synthesis of magnetic Fe₃O₄ particles at room temperature. They found that ferrous bivalent transporters on the magnetosome membrane transport Fe(II) ions into the magnetosome membrane while transporting protons out of the magnetosome membrane, thereby adjusting the pH of the magnetosome. Although Mms6 is the most abundant protein in magnetosomes, its regulation mechanism remains largely unclear, so magnetosome formation through biomineralization is highly contended.

Recently, Siponen et al. revealed the structural characteristics of MamP, a protein that dominates magnetosome synthesis in magnetotactic bacteria. MamP has a unique protein-folded “magnetic chromium” structure that attracts iron atoms (see Figure 3). By oxidizing Fe(II) ions to Fe(III) ions, MamP finally obtains the magnetic Fe₃O₄ nanoparticles. In vitro cell experiments yield only the Fe(II) ion; conversion to Fe(III) requires the protein activation of MamP. More importantly, proteins such as MamP and MamE can synergistically control the oxidation and reduction functions. Therefore, the biomimetic route is expected to enhance the performance and yield of FMIONs. Other functional bacteria, such as Actinobacter spp., Escherichia coli, Geobacter spp., and fungi (Verticillum spp.) can synthesize bacterial maghemite nanoparticles or bacterial iron sulfide nanoparticles when incubated with iron-containing metal precursors under appropriate conditions. Thus, biomimetic synthesis can improve our understanding of biomineralization and provides new opportunities for bio-nanomagnets in medical applications.

### 3.2 Physical methods for FMION preparation

The common FMION preparation methods are high-energy ball milling, laser ablation, metal sputtering and evaporation, flame pyrolysis, and electrodeposition. High-energy ball milling reduces the activation energy by exploiting the mechanical energy of milling, thereby resulting in greatly improves the magnetism of as-prepared FMIONs. De Carvalho et al. synthesized 12-56 nm-sized magnetite nanoparticles by high-energy ball milling, and found that milling time is crucial for achieving high-purity particles with adjustable size. Especially, increasing the milling time to 96 hours reduced the typical size of the magnetite nanoparticles to 12 nm. Pulsed laser ablation is another suitable approach that obtains pure magnetic nanoparticles with no or few
chemical precursors. Recently, Svetlichnyi et al. [81] prepared FMIONs at atmospheric pressure using nanosecond pulsed laser ablation. The subsequent annealing treatment removed the nitrides from the sample, and synchronously increased the size and remanent magnetization of the as-prepared NPs. Although the current physical methods obtain FMIONs with high purity and excellent performance, they cannot satisfy the requirements of commercial preparation because they are costly and cannot precisely control the particle size and morphology.

3.3 Chemical methods for FMION preparation

The popular chemical methods for FMION preparation are the sol–gel technique,[82] micelle synthesis,[83] co-precipitation,[84] solvothermal synthesis,[85] thermal decomposition,[86] and the electromagnetic field-assisted technique.[87] All of these methods form high-quality, monodisperse FMIONs. Rather than an exhaustive discussion of all approaches, we discuss only representative chemical methods for FMION synthesis.

3.3.1 Co-precipitation methods

Co-precipitation is a facile and convenient chemical synthesis method for FMIONs. An alkaline solution (such as NaOH) is added to an aqueous mixture of Fe(II)/Fe(III) salts at the appropriate molar ratio, and the reaction proceeds at room temperature or higher under an inert atmosphere. The reaction is described by Equation (1). In the co-precipitation process, the size, morphology, and dispersion of the as-prepared FMIONs depend on the reaction parameters such as the Fe(II)/Fe(III) ratio, pH of the reaction system, and the reaction temperature.[88] However, naked Fe3O4 nanoparticles are easily oxidized to γ-Fe2O3 under certain acidic conditions when exposed to air (Equation (2)). Valenzuela et al. [89] prepared superparamagnetic magnetite nanoparticles with a mean diameter of 10 nm via co-precipitation, and found that the morphology is closely related to the stirring velocity. Rani and co-workers [90] investigated the effect of pH on the structure, optics, and magnetism of Fe3O4 nanoparticles. They reported that at low temperature, metamagnetic transitions arose from the surface spin disorder and mixed magnetic phases of the nanoparticles.

\[ \text{Fe}^{2+} + 2\text{Fe}^{3+} + 8\text{OH}^- = \text{Fe}_3\text{O}_4 + 4\text{H}_2\text{O} \quad (1) \]

\[ \text{Fe}_3\text{O}_4 + 2\text{H}^+ = \gamma - \text{Fe}_2\text{O}_3 + 2\text{Fe}^{2+} + \text{H}_2\text{O} \quad (2) \]

Although co-precipitation approach can realize a low-cost commercial preparation under mild reaction conditions, the relatively poor crystallinity, wide size distribution, and easy agglomeration of the products easily generate adverse effects that limit their further biomedical application.

3.3.2 Thermal decomposition

Typically, thermal decomposition is performed under an inert atmosphere, and involves a mixture of metal precursors (typically a metal acetylacetonate M(acac)_n [M = Fe, Mn, Co, Ni, Zn] or metal carbonyl M(CO)_n), a surfactant (e.g., oleic acid, oleylamine, or octadecylene), and a high-boiling-point organic solvent (e.g., dibenzyl ether or dioctyl ether) for obtaining high-performance FMIONs. Monodisperse nanoparticles with controllable morphology and size are obtained by adjusting the type of solvent, surfactant ratio and heating strategy of the thermal decomposition in an efficient way.[24,28,31] Notably, uniform nanocrystals can be obtained by two synthetic modes: hot-injection and heat-up.[52,91] In fact, the hot-injection approach was originally used for preparing semiconductor nanocrystals. Murray [92] et al. synthesized a series of CdE (E = S, Se, Te) quantum dots from dimethyl cadmium (Me2Cd), dimethyl sulfide silicon ((TMS)2S), trialkylphosphine telluride, and trioctylphosphine selenide (TOPSe) precursors in trioctylphosphine oxide solvent. From a similar combination of iron organometallic precursor in the presence of oleylamine (OAm) and octadecene (ODE) with a stabilizing agent (oleic acid, OA), the authors synthesized superparamagnetic iron/iron oxide core/shell nanoparticles.[93] These synthetic protocols share two common aims: to improve the decomposition rate of the metal precursor, and to induce uniform nanocrystal formation within a relatively short reaction time.

Meanwhile, the heat-up strategy is a universal method for synthesizing monodisperse nanocrystals. The precursor–surfactant mixture is heated from a relatively low temperature, and the nanocrystals are gradually formed through the heat-up process.[94,95] Sun et al. [96] prepared monodisperse FePt NPs in OA and OAm surfactants, and revealed how the molar ratio of precursors affect the morphology and size of the nanoparticles. In a subsequent study, they thermally decomposed different acetylacetonate metal salts to obtain two Mn2+ and Co2+ doped ferrite nanocrystals (i.e., MnFe2O4 and CoFe2O4).[97] Along a similar synthetic route, Hyeon and co-workers prepared uniform iron oxide nanocrystals from iron pen-tacarbonyl Fe(CO)5 mixed with OA in dioctyl ether.[98] The Hyeon group also reported the laboratory scale preparation of highly uniform FMIONs from environmentally friendly, nontoxic iron chloride precursor...
After a single reaction, they obtained almost 40 g of monodispersed FMIONs without requiring a size-sorting process (Figure 4). High-quality FMIONs prepared by the heat-up approach are reported in similar papers.

### 3.3.3 Electromagnetic field-assisted technique

The temperature gradients that occur in conventional conducting heating process may cause the uneven heating during the reaction. Furthermore, as heat conduction is non-reaction selective and extremely susceptible to the environment, it can hardly achieve stable and controllable preparation. To maintain uniform heating, energy compensation techniques such as extending the reaction time or raising the temperature are needed to overcome the energy barrier, and to effectively separate the nucleation and growth stages. To our knowledge, the nucleation and growth processes of thermal decomposition are time- and temperature-dependent. Hyeon et al. found that while aging at 260°C for 1 day, as-formed MNPs were polydispersed with poor crystallinity, whereas after aging at the same temperature for 3 days, they presented as monodispersed MNPs. In sharp contrast, no MNPs were formed after aging at 240°C and 200°C for 1 and 3 days, respectively. The recently applied microwaves irradiation (electromagnetic field assisted) technique, which achieves reaction selectivity and homogeneous heating at low energy within an economic timeframe, is expected to overcome the drawback of conventional heating. Microwaves were first applied to organic synthesis in 1986. Since then, they have been widely applied in inorganic nanomaterial preparations. As shown in Figure 5A, microwave irradiation heats all substances rapidly and simultaneously, thus shortening the heat transfer time and guaranteeing sufficient thermal energy supply. However, conventional heating initially heats the tube wall, then gradually transfers the thermal energy to the tube interior (Figure 5B). Kappe et al. discovered that the microwave absorption ability is determined by the dielectric loss coefficient of a substance. As the loss coefficients differ among materials, the molecules in the metastable state might rotate and vibrate at high speed after the electromagnetic wave irradiation. If the molecule is further ionized, the activation energy of the reaction reduces and the collision probability of the molecule increases. These processes effectively change the reaction kinetics and selectivity.

Pascu and co-workers compared the effectiveness of preparing MNPs by microwave irradiation and conventional thermal decomposition. They found that microwave irradiation eliminates the wall effect, and both the energy consumption and preparation cost are lower than in conventional heating. Although the microwave preparation of nanomaterials has been extensively reported, the microwaves are merely applied as a heating tool, and their main advantages are considered to derive from the critical conditions created by the black-box irradiation and selected solvent. A polar solvent or ionic liquid reportedly contributes the highest thermal resource during the reaction. Strouse et al. systematically studied microwave-enhanced reaction rates for quantum dot synthesis, and Kappe’s group investigated the thermal and non-thermal effects of microwave heating in Pyrex and SiC vials. They demonstrated for the first time that electromagnetic waves allow rapid thermal exchange with the reaction media. Just recently, Gu’s group developed a practical electromagnetic field-assisted technique that combines the advantages of microwave irradiation (Monowave300, Anton Paar) and thermal decomposition for the preparation of monodispersed Fe₃O₄ nanoparticles with excellent performance (6 nm-sized, specific absorption rate (SAR) = 158 W g⁻¹; saturation magnetization (Mₛ) = 76 emu g⁻¹; transverse relaxation rate r₂ = 172 mM⁻¹s⁻¹). These performance were superior to those of polydispersed nanoparticles formed by
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FIGURE 5 The comparison of heating effect by microwave irradiation (A) and oil-bath (B) under the same treatment. Microwave irradiation could realize the simultaneous heating; however, the vessel wall is heated first while reaction mixture in oil heated tube. Adapted with permission. [104] Copyright © 2003, Kluwer Academic Publishers

thermal decomposition under identical reaction conditions. More importantly, the authors proved that the magnetic nanomaterials (not the other additives) are responsible for converting the electromagnetic energy to heat during the nucleation and growth stages. Obviously, the selective heating of nanoparticles under an electromagnetic field can alter the synthesis from a thermodynamic to a kinetic process, thereby accelerating the reaction rate despite the harsh conditions of the aging stage (250° C for only 5 minutes; see Figure 6).

Later, Gu et al. [110] constructed a hydrocooling–magnetically internal heating device assisted by a co-precipitation strategy for Ferumoxylt preparation (an FDA-approved iron oxide nano-drug for iron deficiency therapy). Their method significantly improved the crystallization and magnetism of the as-prepared Ferumoxylt, because the alternating magnetic field (AMF) increased the thermal energy within the system, thereby increasing the Brownian motion of the nanoparticles, boosting the crystallization, and preventing aggregation of the nanoparticles. In particular, the electromagnetic field may induce a spin-coupling state change of the magnetic moment, leading to higher magnetism and higher stability than in nanoparticles fabricated under ambient heating (see Figure 7). Undoubtedly, the electromagnetic field-assisted technique confers obvious advantages and is very conducive to stable, large-scale production.

4 | PROPERTY OPTIMIZATION OF FMIONS

The intriguing properties of FMIONs are closely related to factors such as geometric shape, size, and surface modification, which can be improved by adjusting the reactant concentrations, the types and proportions of surfactants, and the temperature-rise rate and time during the preparation process. In addition, as-prepared FMIONs can be further surface-modified by surfactant and polymer coatings, ligand chelation, and covering with precious metals or inorganic materials. All of these modifications improve the biocompatibility and effectively reduce the toxicity of FMIONs in vivo. [40,62] Albanese et al. [111] summarized the most prevalent parameters of biological systems and bio-responses, along with potential applications of functional nanoparticles. Their summary provides the criteria for designing nanoparticle-based strategies in vivo. Currently, efforts have been devoted for finding correlations between FMION designs and specific biological behaviors, which can deepen our understanding of the interaction between property-regulated FMIONs and biological systems. The correlations between specific biological responses and designed nanoparticles are typically assessed by exposing the biological system to FMIONs with different sizes, shapes, or surface modifications. After systematically measuring the FMION parameters, the behaviors of the nanoparticles in a biological
FIGURE 6 (A) Microwave synthetic mechanism of Fe₃O₄ NPs. The microwave reactor (Monowave 300) with IR infrared detector and ruby optic fiber probe is responsible for real-time recording of the reaction parameters. The reflection loss (RL) value of (B) microwave synthesized Fe₃O₄ NPs, and (C) precursor wax composite in difference frequency and thickness. Reproduced with permission. Copyright © 2016 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

system can be determined from the obtained data (see Figure 8).

4.1 Shape effects of FMIONs

Typically, the shape of nanoparticles can be classified through the related dimensionality ranges from zero-dimensional (0-D) to three-dimensional (3D) simply. Unlike 0-D or 1-D nanostructures, 3D magnetic nanostructures are mainly fabricated as clusters or assembled from units. Directional attachment during synthesis can controllably increase the magnetization of magnetic nanostructures without disturbing their superparamagnetic property. Similarly, the coercivity and saturation magnetization of FMIONs can be tuned for specific applications by manipulating the morphology of the FMIONs. Tanaka et al. summarized the synthetic strategies of FMIONs from 0-D to 3-D. Results showed that the blocking temperature ($T_B$, defined as the temperature below which the superparamagnetic relaxation becomes slower than the time scale of the magnetic-characterization experiments of a nanostructure) is much higher in cubic FMIONs than in spherical FMIONs, and that the saturation magnetization is stronger in pseudo-cubic FMIONs than in pseudo-spherical ones.

The shape of FMIONs is generally optimized by modulating the growth rate on different crystal facets. Such modulated growth behavior is closely related to the monomer concentration and surface free energy in the system.
FIGURE 7  (A) Schematic illustration of mechanism for magneto-enhancing Ferumoxytol preparation used the hydrocooling and magnetically internal heating co-precipitation (HMIHC) approach; (B) the growth and ripening mechanism of classical coprecipitation formed Ferumoxytol; (C) growth and ripening mechanism of magneto-enhancing Ferumoxytol based on HMIHC. Adapted with permission.[110] Copyright © 2018, Royal Society of Chemistry

FIGURE 8  Schematic illustration of most prevalent parameters, bio-interactions and biological responses of FMIONs while applied for biomedical application. Notably, the correlations between nanoparticle design and specific biological response can be accessed by exposure of FMIONs to the biological system. Such investigation is beneficial to populate the next generation of engineering nanoscale devices.
A suitable surfactant favors the selective binding of the units to specific crystal surfaces with low energy, thus boosting the growth rates. Recently, Xie et al.\textsuperscript{[114]} systematically investigated the shape evolution mechanism of high-performance Mn–Zn ferrite nanostructures from 0-D to 3-D (Figure 9). The selective adsorption of OA to some crystal facets (e.g.,\{100\},\{110\},\{111\}) was considered as the main driver of nanocrystals with various morphologies. Notably, the surface energy is lowest at the \{100\} plane and highest at the \{111\} plane. Hence, by regulating the molar ratio of OAm to OA at specific crystal facets, Xie et al.\textsuperscript{[114]} changed the crystal growth rates and formed spherical (ca. 8-10 nm), cubic (ca. 11 nm), and star-like (ca. 16 nm) Mn–Zn ferrite nanostructures. Meanwhile, if the nucleation time is short, nucleation of crystals is insufficient, and a high monomer concentration remains for the growth stage. In fact, the morphological evolution is controlled by growth kinetics and thermodynamics.
Large-scale star-like nanocrystals possess high magnetic dipole interactions, which encourage their orientated aggregation into nanoclusters. Low surface magnetostatic energies induce the secondary growth of nanoclusters, which evolve into unique nanoclusters with sharp edges. Surface defects on these nanoclusters will gradually passivate the sharp edges, merging them into more stable obtuse edges during the aging period. \cite{113,114}

As a typical coating surfactant, OA can stabilize the formation of magnetic nanocrystals during thermal decomposition via the strong coordination effect of its carboxyl group. When present at sufficient amounts, OA covers and stabilizes the crystal facets, and a spheroidal configuration is formed. When OA is insufficient, it preferentially covers and stabilizes the low-surface-energy \{100\} facets, yielding anisotropic nanocrystals. Gao’s group \cite{115} reported that the exposed facets \{111\} of superparamagnetic \(\text{Fe}_3\text{O}_4\) nanoplates could cause the \(T_1\) and \(T_2\) interactional contrast effects. Especially, the \(T_1\) contrast in magnetic nanoplates is dominated by chemical exchange on the exposed facet.

**FIGURE 10** (A) Utilizing different surfactants could induce the various shapes of \(\text{Fe}_3\text{O}_4\) NPs formation through isotropic/anisotropic growth process; (B) the \{111\}, \{100\} and \{110\} facets models of \(\text{Fe}_3\text{O}_4\). Different \(r_1\) values (C) and \(r_2\) values (D) of as-formed NPs with different morphologies. (E) The protons chemical exchange phenomenon for \{100\} and \{111\} facets of metal-rich \(\text{Fe}_3\text{O}_4\) surfaces. (F) The summary of synthetic reaction conditions of as-formed NPs with different morphologies. Reproduced with permission. \cite{116} Copyright © 2015, American Chemical Society

| Solvent | FeOL (mg) | NaOL (mg) | NaOL/FeOL | OA (μL) | Temperature (°C) | Products |
|---------|-----------|-----------|-----------|---------|------------------|----------|
| ODE     | 900       | 30        | 1:10      | 160     | Reflux           |          |
| ODE     | 900       | 60        | 2:10      | 160     | Reflux           |          |
| ODE     | 900       | 180       | 6:10      | 160     | Reflux           |          |
| TOA     | 900       | 0-30      | 0-1:10    | 160     | 340              | Reflux   |
| TOA     | 900       | 60        | 2:10      | 160     | 340              | Reflux   |
| TOA     | 900       | 150       | 5:10      | 160     | Reflux           |          |
| TOA     | 450       | 15        | 1:10      | 80      | Reflux           |          |
FIGURE 11 The nanorods (NRs) and spherical Fe₃O₄ NPs with equivalent volumes under a 3T magnetic field. The induced magnetic field distribution of (A) 30 nm, (B) 40 nm and (C) 60 nm NRs and (D) 9 nm, (E) 12 nm and (F) 16 nm NPs with equivalent material volume, respectively. The T₂-weighted MR images with different concentrations of iron utilizing 3 T magnetic field for (G) Fe₃O₄ NRs and (H) NPs. Plots of r² values with different sizes Fe₃O₄ NRs (I) and Fe₃O₄ NPs (J); (K) Schematic illustration of the Fe₃O₄ NPs and NRs quantum mechanical outer sphere models with same material volume. H means the outside NRs and NPs induced local magnetic field, where d stands for the effective diameter, and r presents the distance between nanostructure and proton spin. Reproduced with permission. [117] Copyright © 2015, Royal Society of Chemistry

large-area, iron-rich [111] facets; however, the T₂ relaxation behavior is controlled by morphological anisotropy of as-prepared nanoplates with an efficient enlarged radius. Gao and co-workers[116] developed an efficient control strategy that synthesizes variously shaped FMIONs via thermal decomposition of iron oleate (FeOL). The reaction temperature and NaOL/FeOL molar ratio critically determine the shape evolution in this method. Interestingly, the obtained FMIONs with their metal-exposed surface structures and effective radii shortened the structure-dependent T₁ and T₂ relaxation times of protons. This work clarified that the anisotropy morphology of FMIONs can enhance imaging behavior, thereby promising high-performance MRI contrast agents in future (Figure 10).

Nanocrystal morphology is affected not only by the surfactant-mediated surface energy, but also by the monomer concentration. During the classical growth stage, most of the nanocrystals continue growing until the excess monomer in solution is nearly exhausted. Growth gradually decreases as the nuclei concentration and surface energy change at each crystal facet. The subsequent shape evolution of the nanocrystals is related to the temperature, heating rate, and aging time. Mohapatra et al.[117] prepared rod-like and spherical-like FMIONs with different diameters by a facile amine-mediated reduction reaction. Owing to the higher surface area, as-prepared nanorods induced a stronger magnetic-field perturbation on the outer-sphere protons of relaxation model than the spheroidal particles, thereby enhancing the MRI contrast effect by ca. 1.5-2 times from that of conventional spherical nanoparticles under identical conditions. Under the magnetic field, the nanorods induced a stronger magnetic field than spherical nanoparticles, and consequently improved the R₂ relaxivity (Figure 11). The shape of a magnetic nanostructure plays important role in physicochemical properties then affects its biological function. For instance, a vincristine-loaded superparamagnetic anisotropic nanoassembly exhibited higher anti-leukemia activity than the isotropic nano-assembly prepared by Xiong et al.[118] Moreover, the authors prepared a worm-like manganese–zinc–ferrite nanochain that loads the chemotherapy drug paclitaxel. The nanochains achieved a better imaging effect
and tumor-targeting effect than monodispersed spherical nanoparticles and clusters, indicating that the 1D anisotropic nanostructure prolonged the blood circulation time and promised an ideal drug-carrier candidate.\cite{119}

## 4.2 Size effects of FMIONs

Size is an important indicator of high-performance FMIONs, as it directly affects both $M_s$ and $T_B$, and identifies the class of the magnetism (ferromagnetic or superparamagnetic).\cite{112,113} In general, the FMIONs ($<20$ nm) are superparamagnetic and they usually behave as single domains due to their negligible coercivity and remanence. Whereas larger FMIONs (size 80-100 nm) may develop a multi-domain structure, and the magnetism typically transitions from superparamagnetic to ferromagnetic.\cite{120} Cheon et al.\cite{121} synthesized spherical Fe$_3$O$_4$ nanoparticles with different sizes (i.e., 4, 6, 9, and 12 nm) and found that the magnetization values showed a size-dependent behavior. In addition, the size of FMIONs can be precisely controlled by adjusting the synthetic parameters (precursor concentration, surfactant ratio, and reaction heating rate during thermal decomposition), or by altering the seed-mediated growth pattern, respectively. Just recently, Hyeon and colleagues\cite{122,123} developed a synthetic strategy for monodispersed nanoparticles preparation over a continuous size spectrum (6-13 nm), and the synthesis process which is highly reproducible and requires without size selection. Such a continuous growth technique without additional nucleation is suitable for repetitive manufacturing and can potentially realize 1-nm precision control of monodispersed MNPs preparation.

Although the size-dependent magnetic properties of FMIONs have been reported, the induced metabolic activities and toxicity of FMIONs in vivo remain poorly understood. Yang and co-workers\cite{124} systematically investigated the in vivo biological behaviors and biosafety of carboxyl coated FMIONs with different sizes (10, 20, 30, and 40 nm). The bio-distribution and transport results were size-dependent; specifically, the smallest FMIONs were most commonly uptake and cleared by the liver and spleen, whereas the largest were primarily eliminated and accumulated in the spleen. Interestingly, the brain–blood barrier was more easily penetrated by the small FMIONs than by the larger FMIONs. Results also demonstrated that the smaller FMIONs effectively boosted the expression level of the Pcsk9 and Hmox1 genes. Consequently, expression of these genes was found to be closely related to oxidative stress and metabolic processes (see Figure 12). Furthermore, as mentioned by Xie et al.,\cite{125} size plays a decisive role in the biological defense response and vascular barrier to FMIONs. Large (>100 nm) FMIONs are easily uptake by the mononuclear phagocyte system (MPS). In contrast, FMIONs smaller than 10 nm can easily pass through the larger fenestrations of the blood vessels, and accumulate at the target tissues, owing to their enhanced permeability and retention effects.

Numerous studies have identified FMIONs as a promising heating medium, and the size of FMIONs also modulates their magnetism, and hence affects their magnetothermal performance while applied with AMF. Moreover, good magnetic loss characteristics of FMIONs are guaranteed by high saturation-magnetization and permeability, along with wide magnetic anisotropy. Especially, the relatively low dielectric constant and the absorption matrix of FMIONs cooperate to ensure good impedance matching. Through their magnetic and dielectric losses, FMIONs absorb electromagnetic waves for energy conversion.\cite{126,127} Just recently, Liang and co-workers\cite{128} investigated the magnetothermal effects of microwave-formed FMIONs (Monowave300, Anton Paar) with different sizes (i.e., 4, 20, 50, and 200 nm). Noted that as the particle size increased, the SAR value and thermal losses decreased, reducing the generated heat and temperature rise rate of the as-prepared FMIONs. In particular, the 20 nm-sized FMION yielded the highest electromagnetic wave absorption (SAR = 398.4 and 1457.2 w g$^{-1}$ at magnetic induction hyperthermia frequencies of 390 and 780 kHz, respectively), which is six-fold higher than those of 200 nm NPs (SAR = 62.5 and 261.9 w g$^{-1}$ at 390 and 780 kHz, respectively) under identical conditions. However, increasing the particle size also increased the reflection loss. As suggested in micromagnetic simulations, coupling interactions and magnetic dipole–dipole interactions in the presence of electromagnetic field might synergistically affect the energy conversion and absorption behavior.\cite{126,128} (see Figure 13).

## 4.3 Surface modification of FMIONs

Recently, many surface modification strategies have been proposed, including polymer coatings (such as polyethylene glycol (PEG), chitosan, and polyaniline), ligand chelators (such as amine-carboxylic acid, sodium oleate, and carbonyl-hydrazine), and surface coatings containing precious metals (such as gold and silver) or inorganic materials (such as carbon and silica).\cite{129,130} The modular design of surface ligands could enhance the biocompatibility of FMIONs. For example, by exploiting the hydrophobic interaction between FMIONs and native ligands, the anchor linked to the capping molecules can be directly bound to the nanocrystal surface. In addition, zwittrionic chains or PEG molecules can effectively prevent nonspecific adsorption, as they strongly bind to water.
molecules. Xie et al. prepared a core–shell structure of PEGylated magnetic nanoparticles by surface-modifying the Mn–Zn ferrite nanoparticle surface with DSPE-PEG2000 lipid, which hydrophobic interacts with OA. The amphiphilic ligand effectively prevents binding between the carboxyl groups of OA and specific proteins, thus extending the blood circulation half-life and inhibiting phagocytosis of the reticuloendothelial system. Moreover, due to FMIONs with large volumes or surface areas, so the atoms on particle surfaces are typically unsaturated, providing suspended bonds and highly active reaction sites to remarkably improve their surface-dominated physical and chemical processes, which are extremely important for enhancing their biofunctions. By modifying the surface functional groups, FMIONs are available for capture, separation and detection in medicine, biotechnology and environmental chemistry. For instance, amine linkers can help with conjugating drug's acid...
(A) Heating curves of 4 sizes of Fe$_3$O$_4$ NPs formed by microwave in isooctane (1 mg/mL) used AMF (390 kHz, 12 A) and (B) 780 kHz, 12 A. (C) Size-dependent magnetization status of single particle under OOMMF simulation; (D) The reflection loss (RL) value of 4 nm, (E) 20 nm, (F) 50 nm and (G) 200 nm microwave synthesized Fe$_3$O$_4$ NPs with wax composite in difference frequency and thickness. Reproduced with permission. [128] Copyright © 2017 Elsevier B.V
groups and DNA molecule, as well as the amide bonds formed via carboxylic acid linkers with the amine groups in proteins.\textsuperscript{125} Surface functionalization also avoids off-target tissue toxicity.\textsuperscript{129} Such surface functionalization by various biomolecules, which can tune pharmacokinetics, drug release, and endosomal release, effectively improves the treatment efficiency and significantly decreases the risk of DNA damage\textsuperscript{132} (see Figure 14).

Moreover, the core–shell FMION structures can be additionally formed from precious metals and inorganic materials. A precious metal (Au/Ag) coating is typically obtained by reducing H\textsubscript{2}AuCl\textsubscript{4}, sputtering the pure Au/Ag target onto the nanoparticle surface, or electroplating on nanoscale templates. However, Sun et al.\textsuperscript{133} developed a facile seed growth strategy for a core–shell structural Fe\textsubscript{3}O\textsubscript{4} NP preparation coated with Au and Ag. The seed growth formed a thick Au shell and the F\textsubscript{3}O\textsubscript{4}/Au/Ag nanostructure was generated by adding AgNO\textsubscript{3} following a simple strategy. The close relationship between the shell thickness and plasmonic properties may be exploitable for disease diagnose (Figure 15A-E). Another effective modification is the encapsulation of FMIONs in inorganic oxides. For instance, silica-related (SiO\textsubscript{2} and mesoporous SiO\textsubscript{2}) coating materials can effectively protect the magnetic core from unwanted interactions. In fact, silica-coated magnetic microspheres are highly promising for many applications, just as efficient adsorbents could remove large microcystins (1000 Da) from solution in such a short time. The construction process of silica-coated magnetic microspheres is shown in Figure 15F-G.\textsuperscript{134} First, uniform magnetite microspheres are cover by a thin silica layer, forming nonporous silica–Fe\textsubscript{3}O\textsubscript{4} composites. Mesostructured CTAB/SiO\textsubscript{2} composites are then constructed by a surfactant-templating strategy using a CTAB
Finally, the CTAB templates are removed by the acetone extraction technique, obtaining high-quality Fe₃O₄@nSiO₂@mSiO₂ microspheres (ca. 500 nm) with well dispersion. Consequently, surface modification guarantees the long-term stabilization of the magnetic core and nanoparticle functionalization, which boosts the thermal stability, biocompatibility, and other advantages of the nanostructures.

5 | THE APPLICATIONS OF FMIONS IN CANCER THERANOSTICS

Recently, FMIONS have been continuously investigated for their potential biomedical applications, especially for cancer diagnose and therapy. For instance, the iron-based nanodrug Feraheme, approved by the FDA for iron deficiency treatment, has recently exhibited an anti-leukemia effect. The free ferrous ions generate reactive oxygen species (ROS) that increase the oxidative stress, leading to cell death. This mechanism of FMIONS is highly promising for leukemia treatment with low ferroportin levels. Besides, the FMIONS possess an intrinsic enzyme activity that mimics natural peroxidases. With the inherent biological effects of nanozymes, such FMIONS are expected to provide new ideas for cancer theranostics. Therefore, Yan and co-workers developed these concepts into a new subject called “nanoenzymology.”

Except for the biomedical applications mentioned above, FMIONS also can be used for gene carries due to they can utilize magnetic force to accumulate the target genes on cell membrane in a short time. Specifically, FMIONS can alter the intracellular distribution of genophore and facilitate the target gene escape from lysosome. As long as the target genes into the cell with FMIONS that can promote the target genes entry into cell nucleus through dose-dependent passive transfer and infiltration in a highly efficient way. Zhu et al. prepared the complex of iron oxide and recombinant baculoviral vector to activate the in vivo CRISPR-Cas9-mediated genome editing, and found that the cellular entry ability could be enhanced via magnetic field. Accordantly, the genome editing in terms of the vector is tissue specific, which could cause a transient transgene expression then to avoid the vector inactivation. Moreover, Xie and co-workers developed a high-performance cancer vaccine which employed the Fe₃O₄ nanoclusters as core and coated with the anti-CD205 modified cancer cell membrane as the cloak. These unique properties enable the vaccine to achieve a large number of T cell proliferation with superior clonal diversity and cytotoxicity. Very recently, the ultra-small Fe₃O₄ NPs are found as nano-immunopotentiators via combining oval-bumin (OVA) to possess the ability to stimulate the maturation dendritic cells and induce T cells to secrete the cytokine interferon IFN-γ, as well as potentially-activate macrophages to secrete large amounts of tumor.
necrosis factor TNF-α. These optimized FMIONs with high-performance could be an effective nanoscale tool to broaden the nanobiotechnology applications, such as micro-nanobots, magnetic hyperthermia, MR imaging and even participated in drug delivery system.

5.1 Micro-nanobots based on FMIONs

In addition, medical micro-nanobots based on FMIONs can be remotely magnetically controlled for in vivo cancer research. Vermesh et al. reported their flexible magnetic wire that retrieves rare biomarkers from blood with high performance. When a standard intravenous catheter is inserted, the as-prepared wire immediately captures the biomarkers which attached to previously injected FMIONs labeled with purified anti-human CD326 antibody (Figure 16A). The capture efficiencies of this system were demonstrated in a live porcine model that equal to enrichments of 10-80 times of circulating tumor cells (CTCs) in a 5 mL blood draw while 500-5000 times higher than the commercially available Gilupi CellCollector. Notably, the labeling and single-pass captured in vivo completes within 10 seconds. Therefore, the technique is a promising replacement of the current methods, which isolate CTCs or nucleic acids for early cancer detection. Inspired by magnetotactic bacteria, Cheng’s group reported a biomimetic magnetic microrobot (BMM) with accurate positioning and a speedy motion response for targeted thrombolysis. As shown in Figure 16B, a static magnetic field induces linear-chain formations of BMMs through interparticle dipolar interactions among the FMIONs. Such an assemblage could deliver and release the clinical thrombolytic drugs in terms of magneto-collective control, then to realize the ultra-minimally invasive. Furthermore, bio-hybrid nanoswimmers have been fabricated by integrating Streptomyces platensis with low-cytotoxicity FMIONs. The as-prepared nanoswimmers enabled tri-modality (fluorescence/photoacoustic/magnetic resonance) imaging and accumulated a high potency against tumors via magnetic actuation. Therefore, the nanoswimmers can feasibly modulate the tumor microenvironment. Especially, they are available as photosynthetic oxygenerators for in situ O₂ generation in hypoxic solid tumors, which can significantly improve the effectiveness of radiotherapy. The chlorophyll in the microorganisms is released as a photosensitizer. Under laser irradiation, the photosensitizer induces cytotoxic ROS that enhance the therapeutic effect (see Figure 16C). Just recently, a soft magnetic micro-swimmer that constructed by nonmotile sperm cells and FMIONs was designed by Magdanz et al. and used to emulate the motile sperm cells motion for in vivo targeted therapy (speed exceeds 0.2 body length/s, i.e., 6.8 ± 4.1 μm s⁻¹). In particular, the FMIONs coating significantly increase the acoustic impedance and localization of as-formed microrobot while using ultrasound feedback. In fact, many functional advantages could be achieved by incorporating the biological entity into FMIONs based micro-nanobots to promise as an innovative technology for in vivo targeted therapy, just like drug loading ability enhanced, targeted drug delivery, surgical navigation and detectable bio-tools. Therefore, these FMIONs based micro-nanobots can enter a small space via non-invasive manner by reducing the intervention level and risk of side effects.

5.2 FMIONs for tumor hyperthermia

In recent decades, FMIONs have been widely explored for cancer therapy. One such therapy is magnetic hyperthermia, which applied under an AMF. The magnetofluid is delivered to the lesion by intratumoral or intravenous injection, generating heat through Brownian relaxation and Neel relaxation, respectively. When the temperature in the tumor area rises to 43°C or higher, the cells are damaged. Based on the tumor position and type of cancer, FMION-mediated magnetic hyperthermia can be divided into two modes (hypothermia and ablation) and three types: (i) external local hyperthermia, (ii) intraluminal hyperthermia, and (iii) interstitial hyperthermia. In hyperthermia mode, the temperature in the tumor tissue reaches 42-46°C and is maintained at that temperature for at least 30 minutes. In ablation mode, the temperature exceeds 50°C and is maintained at that temperature for 10-30 minutes. Ideally, a cancer tissue under mild hyperthermia reaches its threshold thermal exposure, which triggers cellular apoptosis or the anti-angiogenesis effect. Eventually, the tumor vasculature is damaged. In contrast, thermal ablation is intended to kill the tumor cells and induce coagulative necrosis of the tumor tissue. This method alters the microenvironment and immunological function of the tumor.

Magnetic hyperthermia is more environmentally friendly and less invasive than conventional clinical treatments such as surgical resection, radiotherapy, and chemotherapy, and exerts an efficient therapeutic effect with few side effects. Recently, Hayashi’s group prepared superparamagnetic Fe₃O₄ clusters, and enhanced their targeting ability by surface modifications with folic acid and PEG. After 24 hours of enrichment, the tail vein-injected clusters induced hyperthermia under a field intensity of 8 kA m⁻¹ applied at 230 kHz (Ht = 1.8 × 10⁹ A m⁻¹ s⁻¹) for 20 minutes. Accordingly, the intratumor monitoring temperature was raised to 65°C. Hayashi et al. then encapsulated doxorubicin hydrochloride (DOX) in
FIGURE 16  (A) Schematic presentation of the MagWIRE. (i) MPs coated with Antibody (Ab) are injected in the blood to immuno-magnetically label the rare analytes (CTCs). (ii) The as-prepared MagWIRE is used to collect the MP-bound cells through an intravenous catheter during the entire blood circulates. (iii) Post-processing of the MagWIRE after capturing cells. Adapted with permission. [142] Copyright
Fe₃O₄ clusters with a polypyrrole coating, and performed thermos-chemotherapy under the above-mentioned conditions. The therapeutic effects were improved from those of the earlier attempts, as shown in Figure 17.

The thermal efficiency of magnetic hyperthermia agents can be quantified by the SAR value or the inherent loss power (ILP) value. As the SAR does not consider the influence of magnetic field on the parameters, one cannot compare the experimental results collected under different magnetic fields. In contrast, the magnetic field parameters are independent of ILP, so the magneto-thermal conversion performance of FMIONs can be compared under different field conditions. In order to provide valuable reference for the clinical transformation of magnetic hyperemia in the future, Liang and co-workers developed an innovative therapeutic strategy combining trans-catheter arterial embolization and magnetic hyperthermia (TAEMH) for hepatocellular carcinoma (HCC) therapy by using the poly(lactide-co-glycolide) (PLGA)–magnetite microspheres, which are biologically safe and magnetically induced to generate high heat. The TAEMH treatment delivered a good therapeutic outcome with few side effects in orthotopic liver tumor models of rabbits. PLGA underwent a phase transformation that aggregated the microspheres, lengthening the embolization effect in tumor regions. The TAEMH strategy promises to play a third role in HCC therapy, supplementing trans-catheter arterial chemoembolization and radio-embolization (see Figure 18).

Recently, magnetic hyperthermia is thought to directly destroy tumors by macroscopic thermal heating; however, the therapeutic effect has instead been attributed to microscopic thermal effects by FMIONs-mediated thermostherapeutic research. ROS are effective inhibitors of tumor growth and metastases. On the nanoscale, thermal effects not only regulate protease activity, but also significantly enhance the ROS in tumor hypoxic microenvironments. Very recently, Fan et al. reported the as-prepared ferrimagnetic vortex-domain iron oxide nanoring and graphene oxide (FVIOs–GO) hybrid nanoparticles amplify the ROS generation, achieving a high SAR value for active-targeted magneto-thermodynamic (MTD) therapy. By combining the magnetothermal and ROS-related immunologic effects, the as-prepared FVIOs-GO mediated MTD efficiently eliminates tumors at physiological tolerable temperatures, unlike conventional magnetic hyperthermia that exclusively depends on thermal interaction (Figure 19A). Cheng et al. demonstrated that assembled magnetic nanocubes could treat cancer cells by synergistically combining their magneto-based mechanical and thermal stimuli. This combined activity disrupts lysosomes. While applied an AMF may increases the lysosomal membrane permeabilization and simultaneously decreases the mitochondrial membrane potential, then producing a large number of programmed ROS that destruct the subcellular structures. Mechanical-thermal induction therapy has also proven effective against glioma and breast cancer in vivo, indicating that the fate of cancer cells can be manipulated in a programmable way (Figure 19B). Wu and co-workers fabricated a smart injectable magnetic hydrogel nanzyme (MHZ) for sequential tumor therapy. MHZ imparts a low-temperature hyperthermia that minimizes the side effects on normal tissues. The authors listed three possible reasons for the therapeutic effect of MHZ: (i) the ROS induced by the nanzyme-catalyzed reactions during hyperthermia enhance the oxidative stress levels in tumor cells; (ii) the acidic environment of the tumor accelerates the generation of hydroxyl radicals (⋅OH) by the Fe₃O₄ nanozymes, which occurs through the Fenton reaction; (iii) the thermal effect promotes the -OH generation by the nanozymes, accelerating the destruction of heat shock protein 70 (see Figure 19C).

In most of the previous studies, temperatures above 43°C have become the watershed for evaluating tumor-associated angiogenesis and necrosis. However, the pursuit of more safer and efficient hyperthermia remains a major challenge. Espinosa et al. discovered that the iron oxide nanocubes could act as both magnetic and photothermal agents to perform the dual mode hyperthermia. While irradiated with AMF and near-infrared laser (808 nm) synchronously, the specific loss power was calculated up to 5000 W g⁻¹ along with the heating effect of 2-5-fold while compared with single heating mode, and then yielded the unexpected heat-mediated cell death in the mice tumor models. In order to study the multimodality molecular imaging during intracellular photo-magnetic hyperthermia therapy, Kang’s group developed an all in one FMIONS-mediated diagnosis and treatment platform (i.e., MNP@PES-Cy7/2-DG). Results showed that tumors...
**FIGURE 17**  (A) Presentation of heat production of smart NPs while applied an alternating magnetic field (AMF) and the DOX sequentially release. (B) Using the smart NPs for cancer therapy with the combination of chemotherapy and MHT; (C) Thermal image of mouse hyperthermia (injected with Fe$_3$O$_4$/DOX/PPy-PEG-FANPs for 20 minutes). (D) The tumor temperature change of the mice hyperthermia after injection with different Fe$_3$O$_4$ thermal agents. Data statistics of (E) tumor volume (F) survival rate, and (G), body weight. (H) Photos of different groups of mice after treatment. Reproduced with permission.\[148\] Copyright ©2014 Theranostics

**TAEMA for HCC therapy**

**FIGURE 18**  Schematic illustration of TAEMH strategy for HCC therapy. The high performance PLGA-magnetic microspheres with well-controlled sizes (100-1000 µm) were fabricated via rotating membrane emulsification technique and demonstrated with excellent biosafety and therapeutic efficacy while performed in orthotopic liver tumor models of rabbits. Adapted with permission.\[149\] Copyright © 2017 American Chemical Society
FIGURE 19  (A) Illustration of FVIOs-GO mediated MTD proposed mechanism to eliminate the tumors efficiently with suitable temperature. Adapted with permission. Copyright © 2020, American Chemical Society; (B) Schematic presentation of magneto-mechanical thermal induction therapy (MTIT) mechanism used the RGD functionalized nanocubes (RGD-IONs) nanocubes under AMF. Adapted with permission. Copyright © 2020 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. (C) Schematic presentation of the magnetic hydrogel nanzyme preparation and synergistic mechanism of cancer therapy. Adapted with permission. Copyright © 2019, American Chemical Society. (D) Schematic presentation of FMIONs-mediated diagnosis and treatment platform and dual-mode hyperthermia under the guidance of tri-modality imaging. Adapted with permission. Copyright © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

could be eliminated completely under the high effective and precise dual-mode hyperthermia under the guidance of tri-modality imaging (i.e., photoacoustic/near-infrared fluorescence/magnetic resonance) (see Figure 19D). Kang and co-workers further developed another optical-magnetic nanoplatforms (MNP@PES-dye800/FA) for preoperative nano-photothermal therapy (NPTT) and assist in the follow-up surgery under the tri-modality molecular imaging guidance. Contributed by the adjuvant effect of preoperative NPTT, the tumor boundary is more clear for surgical resection, then leading to a better survival rate of tumor-bearing mice models. All these multifunctional theranostic nanoplatforms may promote a further exploration for clinical hyperthermia based on FMIONs.

5.3  |  FMIONs for MR imaging

FMIONs cause ultra-fast relaxation of the protons in the surrounding medium, resulting in a non-uniform magnetic field. This property of FMIONs has been exploited in MRI contrasting for disease detection. Based on the typical relaxation behavior, MRI contrast agents are divisible into two relaxation modes: longitudinal (T₁) (also known as spin-lattice relaxation), and transverse (T₂) (also known as spin-spin relaxation). The T₁ mode is a longitudinal magnetization mode that recovers slowly as the excited protons relax. The T₂ mode is a transverse magnetization mode that decays as the nuclear spins of the protons de-phase. Many positive (T₁) contrasting agents are paramagnetic
complexes such as gadolinium and manganese complexes. When the protons in these complexes lose energy, they provide a bright signal in T1-weighted imaging. The time of T1 relaxation is defined as the time needed for longitudinal magnetization of its original state (from 0) to recover to 63%. In contrast, negative (T2) contrast agents contain iron oxide nanoparticles, and provide a dark signal in T2-weighted imaging. The time of T2 relaxation is required for transverse magnetization of its excited-state to fall to 37%. Obviously, this time is typically short.[45,125]

The enhanced relaxation effectiveness of T1 and T2 contrast agents could be determined by their relaxation rates, defined as r1 and r2, respectively. A contrast agent operates in T2 mode if its r2/r1 ratio exceeds 10, meaning that a T2 contrast agent requires a high magnetization to boost its r2. By contrast, a mode-T1 contrast agent requires a larger r1 and smaller r2/r1 ratio. In phantom experiments, increasing the iron concentration increased the imaging contrast of both T1/T2 contrast agents by shortening the relaxation time and accelerating the relaxation process.[156–158] The contrast effect is closely related to the shape, size, magnetism and surface modification of the FMIONs. To overcome the relatively low transverse relaxivity of spherical FMIONs, Gao et al.[159] controlled the morphology of the octapod-like FMIONs by a new strategy that boosts their transverse relaxivity (with edge length of 30 nm and r2 value of 679.3 ± 30 mM−1s−1). Introducing chloride anions as the capping agent yielded octapod NPs with a unique structure, which achieve extremely high T2 relaxivity through the large effective radii of their magnetic cores. As mentioned above, most of the commercially available T1 contrast agents are gadolinium and manganese complexes. Some of these complexes have been recently implicated in serious negative effects, like nephrogenic systemic fibrosis (NSF) and deposition in human tissues, especially in brain and bone tissues. Meanwhile, a T2 contrast agent is more likely affected by low-contrast signal areas such as in vivo bleeding and metal deposits, which may be misdiagnosed during treatment.[159–161] Therefore, the development of iron oxide-based T1 contrast agents is imperative for improving the diagnostic effect of MRI.

Yu and co-workers[162] prepared the core shell-structured Fe3C@Fe3O4 NPs and used as the ROS nanoreactor in terms of Fenton reaction. Results showed that the as-formed Fe3C@Fe3O4 NPs with high magnetization exhibited excellent magnetic targeting and T2-weighted MRI ability. Interestingly, the T2 signal could be decreased and T1 signal enhanced simultaneously in MRI while these NPs were ionized in acidic environments. Such MR signal conversion could guarantee the visualization of pH-responsive Fe2+ release and ROS generation for cancer theranostics (see Figure 20). Meanwhile, Jia et al.[163] reported the first MRI contrast agent with active-target T1 weighting, which images tiny hepatic tumors using c(RGDyK) to conjugate with ultra-small Fe3O4 nanoparticles. The RGD-modified Fe3O4 probes possess specific targeting ability and an excellent T1 contrast effect (r1 = 7.74 mM−1s−1, with an ultralow r2/r1 value of 2.8). Under a clinical 3T magnetic field, the probes detected hepatic tumors just 2.2 mm in size (Figure 21A,B). Notably, the free Fe2+ and Fe3+ paramagnetic ions is regarded to take responsibility for T1 signal enhancement while the proportion of paramagnetic iron ions on the particle surface increases with decreasing FMION size. As FMIONs size less than 5 nm, the quasi-paramagnetic ions predominantly occupy the surface of the amorphous layer, boosting the paramagnetic behavior of the internal ultra-small ferromagnetic crystal nucleus. Such composite nanostructures not only provide a high r1 relaxation rate and low r2/r1 ratio, but also suppress the magnetization of the crystal nucleus and its related T2 relaxation.[45,157] Fan’s group[164] reported a series of highly monodisperse ultra-small (<4 nm) FMIONs, and systematically related their chemical composition to the enhancement of T1 signal. The as-prepared T1 contrast agent achieved a high r1 (8.43 mM−1s−1) and demonstrated high-resolution T1 angiography ability in vivo (almost double that of the gadolinium-based contrast agent used in clinical settings, and superior to those of ferrite nanoparticles with similar sizes).

To our knowledge, the traditional MRI contrast agents based on FMIONs are usually used as single-mode (T1/T2) contrast agents. However, as most of them are sensitive to endogenous factors such as calcification, bleeding, and air, the single-imaging mode easily generates false positive signals of diseased tissue. The development of T1/T2 dual-mode contrast agents would greatly improve the sensitivity, accuracy, and diagnostic-imaging value of FMION-based MRI contrast agents. Bai et al.[165] reported a c(RGDyK)-modified Fe3O4 tumor-targeting nanoprobe with a time-dependent T1−T2 contrast-enhancing imaging effect. Different from previous reports, the as-formed nanoprobe exhibited a strong T1-weighted enhanced contrast with a high r1 value (7.83 mM−1s−1) and large r2/r1 (19.1). The mechanism operated through combination of the inner and outer sphere models (chemical exchange in the sphere regime and strong superparamagnetism, respectively) rather than by traditional single inner-sphere relaxation. In MRI experiments in vivo, the nanoprobes exhibited time-dependent T1−T2 switchable contrast enhancement, contributed by the active targeting ability of the nanoprobes. In particular, the targeted accelerated aggregation at the tumor site shortened the T1−T2 transformation time, unlike other dual-modal nanoprobes that synchronize the T1- and T2-weighted enhancement effects (Figure 21C). Ultra-small FMIONs thus offer a novel
FIGURE 20  (A) Schematic presentation of ferrous ions release from Fe₃C₂@Fe₃O₄ NPs and T₂/T₁ switching process; (B) The ¹T₁ and ¹T₂ (C) relaxation rates and corresponding MR images of as-prepared NPs at acidic environments, respectively. Reproduced with permission. [162] Copyright © 2019, American Chemical Society

T₁–T₂ dynamic transformation mode for MRI. Highly dispersed FMIONs enhance the T₁ contrast and confer unique paramagnetism, whereas the magnetic dipole interactions among agglomerated ultra-small FMIONs enhance the T₂ contrast and confer superparamagnetism. By regulating the tumor microenvironment, the MRI imaging mode might be dynamically transformed from T₂ to T₁ (or T₁ to T₂), thereby switching the dominant state of the FMIONs from agglomeration (dispersion) to dispersion (agglomeration). Recently, Li et al. [166] prepared an interesting distance-dependent plus two-way magnetic resonance tuning (TMRET) nanoprobe, which encapsulates the T₁ and T₂ MRI agents in polymer micelles. The as-formed nanoprobe responds to the tumor microenvironment, and quenches the T₁ and T₂ signals if they interact before reaching the tumor site. However, if the contrast agent is released from the micelles, the dual-mode MR signals activate as soon as they interact with the tumor microenvironment. To improve the sensitivity of TMRET contrast, Li’s group [166] developed a dual-contrast enhanced subtraction imaging (DESI) technique for post-processing and reconstruction of MRI. The DESI technique accurately detected tiny (0.75 mm³) intracranial tumors in mice, and increased the MRI sensitivity by more than 10 times (see Figure 22). The unique T₁–T₂ dual activation and quenching mechanisms ensure the high specificity and selectivity of the TMRET contrast agent; consequently, TMRET nanoprobes are appealing candidates for early and accurate diagnosis of cancer and many other diseases.

5.4  |  FMIONs in drug delivery system

Tumor cells are highly similar to normal cells and possess superior vitality. To further inhibit the development and metastasis of tumors, the specificity, effectiveness and safety of the drug delivery system must be improved. Among which, drug delivery systems based on FMIONs are attracting increasing attention owing to their excellent biocompatibility and extraordinary loading capability. Although the research of FMIONs-based delivery systems have made encouraging progress, the bottleneck such as easy to be cleared by the immune system, poor targeting specificity, uncontrollable degradation and metabolism of the carriers in vivo remain need to be solved. [167,168]
FIGURE 21  (A) Utilizing the traditional $T_2$ Fe$_3$O$_4$ presents the pseudo-positive contrast effects and (B) $T_1$-Fe$_3$O$_4$ modified by RGD exhibits the positive contrast enhancement behavior. Adapted with permission.$^{[163]}$ Copyright ©2016, Theranostics. (C) Schematic illustration of time-dependent $T_1$-$T_2$ weighted contrast enhancement switched mechanism. Adapted with permission.$^{[165]}$ Copyright © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim
Therefore, how to maximize the effect of drug delivery system has become the primary problem for achieving precise treatment of cancer. Trabolsi et al.\textsuperscript{[169]} developed an anticancer drug DOX loaded covalent organic frameworks (nCOFs) composite which coated with $\gamma$-Fe$_2$O$_3$ NPs and stabilized with poly(L-lysine) cationic polymer for the first time. Unlike other drug carriers, the as-prepared composite acts as the pH-responsive agent to guarantee DOX selectively releasing at late endosomes and lysosomes of cancer cells within acidic microenvironment (pH 5.4) only, rather than pH 7.4 (physiological conditions). In particular, along with the synergistic excellent thermo-chemotherapy and MR imaging capacity, massive potential has been shown by this composite as promising drug delivery candidate for cancer theranostics (see Figure 23). The fact that used FMIONs as drug carriers or construct delivery system all due to their larger surface area, high magnetism and capacity to monitor drug distribution in vivo dynamically. For instance, Xiong and co-workers\textsuperscript{[170]} fabricated a Rubik’s cube-like Fe$_3$O$_4$-paclitaxel (PTX) nano-assemblies. The as-formed assembly Fe$_3$O$_4$ carrier exhibited rapid and prolonged release behavior, led to greater antitumor
Apart from directly use FMIONs as drug carriers, researchers have reported utilizing highly biocompatible liposomes\textsuperscript{[172]} or hydrogels\textsuperscript{[173]} to encapsulate FMIONs as the effective delivery platforms. These novel delivery systems own favorable biocompatibility, high therapeutic agents encapsulation efficacy, and controllable release characteristics. Such variety of excellent properties could prevent the therapeutic agents leaking before reaching the targeted area to minimize their side effects. For example, Liu et al.\textsuperscript{[174]} developed an stimuli-responsive
nanoliposome delivery systems that consists of anethole dithiolethione, H₂S and γ-Fe₂O₃ NPs. Importantly, this system can be navigated by an external magnetic field to targeting the tumor tissue, then continually producing micro-sized H₂S bubbles to bomb the cancer tissue and guided by real-time ultrasound imaging simultaneously. The in vivo observations demonstrated that tumor growth and metastasis have been effectively inhibited (see Figure 24). Similarly, a multifunctional hydrogel contained extremely low concentration (0.6 mg mL⁻¹) of ferromagnetic vortex iron oxide (FVIOs) constructed by Fan et al. and exhibited efficient sustainable therapeutic ability for significant suppression of breast cancer recurrence. Remarkably, these FVIOs possess unique magnetic properties that can guarantee the DOX loaded hydrogel will not exhibit burst release behavior under magnetothermal conditions, thereby can achieve long-term sustained drug release administration. Very recently, Zhang and co-workers developed the concept of magnetocaloric-enzymatic tandem therapy and verified via a novel class of enzyme-laden magnetic-nanogels. The encapsulated FMINs in nanogel can promote the cellular H₂O₂ level under programmed AMF, which is then catalyzed by protective peptides loaded chloroperoxidase to produce the more toxic singlet oxygen (¹O₂) to achieve optimal effect for neutrophils-inspired tumor therapy. Therefore, it can be note that these unique magnetic hydrogels or liposomes delivery systems with optimally functions show strong stimuli-responsive abilities of pH, enzymes, light, and temperature, which are highly promising for cancer therapy and preventing relapses.

Moreover, utilizing biomimetic technology to construct the novel magnetic bio-membrane drug delivery systems also attracts extensive attention. For instance, Zhu et al. developed a compact cancer cell membrane-cloaked DNP@CCCM delivery platform (FMIONs loaded with DOX and coating with cancer cell membranes) by a “top-down” approach, and demonstrated the multifunctions within the biomembrane-camouflaged platform such as biocompatibility, magnetic contribution, targeting effect and cytotoxicity also being remarkably improved. It should be noted that the heterogeneous distribution in tumor tissue and limited penetration depth are important factors that makes it difficult for drug-loaded NPs to maximize their efficacy. To this end, the exploration of circulating cell-mediated drug delivery using host cells with specific tumor tropism will provide new ideas for drug delivery. Lee et al. designed a click reaction-assisted immune cell targeting strategy and then constructed autologous myeloid cells mediated magnetic drug delivery
system as “Trojan horses” to enhance the efficacy of DOX for cancer therapy. Compared with free DOX or DOX-loaded mesoporous silica NPs, the “Trojan horse” strategy could significantly reduce the tumor burden then to achieve enhanced therapeutic efficacy in orthotopic breast tumor models. Just recently, high praise was given to the view that “the function of biological cells is still the holy grail of current materials science” by Jeffrey Brinker et al.\textsuperscript{[179]} After four successive processing steps, Jeffrey Brinker and co-workers rebuilt the artificial red blood cells (RBCs) that are fully mimicked to native RBCs, and endow them additional non-native functionalities, such as therapeutic agent delivery, magnetic manipulation, oxygen delivery, and biodetection (see Figure 25). In our view, the biomimetic rebuilding red blood cells (RRBCs) is expected to be used for toxin sensor in vivo and cancer theranostics due to their remarkably cellular biocompatibility, excellent target ability and vivo circulation duration, so as to the risk of macrophage phagocytosis and hemolysis risk could be effectively reduced.

### 6 SUMMARY AND OUTLOOK

During the past decades, FMIONs have aroused great interest owing to their low toxicity, excellent biocompatibility, and manipulability under the applied magnetic field. These features would facilitate their biomedical applications and provide promising strategies to achieve better clinical effect in cancer theranostics. Since the clinical demand for FMIONs is increasing, various advanced technology to pursue high-quality FMIONs have emerged for realizing the controllable preparation. Thus far, FMION synthesis has concentrated on classical nucleation and growth theories, which have expanded into the Finke–Watzky two step mechanism, orientated attachment and coalescence theory, digestive ripening, and intraparticle growth theory. These studies have provided valuable theoretical guidance for the preparation of FMIONs, such as mimicked the natural mineralization behavior of magnetotactic bacteria, and developed the microwave technique to achieve rapid preparation with low energy.
consumption. However, the manufacture of stably controlled, industrial-scale preparation that fulfill clinical needs at low cost remains a major challenge in the foreseeable future. Herein, we hold the opinion that the electromagnetically assisted chemical methods (especially use the multi-mode microwave reactor) could satisfy the batch-to-batch reproducibility, which would be very promising for achieving scale-up preparation of high-performance FMIONs.

At present, researchers are investigating the correlations between nanoparticle design and specific biological reactions that may affect the performance of FMIONs, such as shape, size, surface modification, biodynamics, and biocompatibility. By adjusting the type of surfactant, monomer concentration, nucleation and growth temperature, and other relevant parameters, the physical and chemical properties of FMIONs can be optimized for their intended applications. To deepen our understanding of the interaction between nanoparticles and biological systems, classical theories with computational simulations should be combined to precise control of active ingredients or nanostructures. In addition, naked FMIONs are highly reactive and toxic that make them inapplicable to biomedical applications, especially, they are aggregated by their intrinsic dipole interactions, and assemble into various nanostructures under an external magnetic field. Therefore, the long-term stability is considered to dictate the dynamics, bio-distribution and biocompatibility of FMIONs. To combat the surrounding environment in biological systems, such as at high temperatures, in the bloodstream, and in vivo microenvironments, the FMIONs must be surface-modified with highly biocompatible materials. According to most of the existing studies, polymers, inorganic materials, polypeptide, precious metals and even biomimetic membrane are favorable candidates that have been validated on the basis for significantly improving the magnetic property, hemolysis risk, biocompatibility, target ability and circulation duration. However, such modifications should also clarify the metabolism, excretion, and long-term toxicity in vivo. We believe that surface functionalization and modification of FMIONs will attract an increasing share of attention in the near future.

Finally, the review discussed the potential applications of FMIONs for cancer theranostics, presented the latest advances of applied FMIONs in cancer vaccine, genetic engineering and cell-fate regulation. In particular, enthusiasm has been bolstered by the recent discovery that the iron oxide nanodrug Ferumoxytol (Feraheme), which has been FDA-approved for iron deficiency treatment, is potentially effective against leukemia. Such optimized FMIONs with high-performance could be an effective nanoscale tool utilized in micro-nanobots, magnetic hyperthermia, MR imaging and even participated in drug delivery system. In fact, FMIONs are promising agents for magnetically induced tumor-hyperthermia treatment. However, FMION-based treatments are currently limited to local tumor or intravenous injection. To widen the clinical validity of hypothermia, major challenges must be overcome: (i) few of the intravenously injected FMIONs can target and accumulate at the tumor site; (ii) magnetofluids are easily leaked and diffused, thereby heating adjacent tissues; (iii) FMIONs uptake and trapped in the liver and spleen can heat those important organs; (iv) the lack of image guidance severely compromises the accuracy of hyperthermia. Besides, a standard therapeutic protocol for hyperthermia induction by an AMF is currently lacking, but solving the above problems would realize a safe and efficient targeted hyperthermia. Moreover, magnetic nanoparticles also used as MRI contrast agents in disease diagnosis. In current clinical practice, gadolinium-based T1 contrast agents are more popular than FMION-based T2 contrast agents due to bright signals in T1-weighted MR images are helpful for observation and diagnosis. However, the commercially available gadolinium-based contrast agents cause undesirable side effects such as NSF, and tend to deposit in human tissues. Accordingly, the T1 or dual-modal T1–T2 contrast agents based on FMIONs with high r1 values will become more promising to provide detailed information about the size, location and boundary of the tumor, thereby resulting in better cancer imaging. The imaging sensitivity and resolution of the T1 or dual-modal contrast agents can be further optimized by tuning the synthetic strategy and surface functionalization, as well as by innovative the scan sequences and image-assistance technology. We believe that with the continuous in-depth investigate on FMIONs-based contrast agents, including long-term stability, toxicity, and solid preclinical or clinical evaluations, the ethical and regulatory approvals will be available soon. Currently, various FMIONs-based drug delivery systems with high-performance were widely explored due to the effective, precise drug delivery and release is the ultimate goal for cancer therapy. Although their potential for increasing the drug loading capacity, target ability and circulation duration, as well as the reducing of side effects have been greatly improved, the development of magnetic drug-loaded nanocarriers remain encounter some concerns. In particular, the heterogeneous distribution of drug-loaded NPs and limited penetration depth in tumor tissue that makes the translation of as-formed delivery system from basic research into clinical practice has not been realized yet. In this regard, the attention should be specific focused on the design and construction of intelligent stimuli-responsive carriers to break through the limitations of biological barriers. Thus, major problems that may affect the endogenous factors, like pH, hypoxia,
intratumoral pressure, lysosomal escape and MPS should be overcome. Based on the above considerations, we found that the biomimetic delivery system constructed by integrating the FMIONs with specific cell membrane or exosome would be more available. They can not only accurately penetrate the therapeutic agents into the avascular area of the tumor, but also can monitor the transportation and metabolic process through real-time imaging, consequently, as expected to make a big difference in clinical transformation. In conclusion, we hope that the current circumstances of FMIONs and the challenges of clinical transformation will draw many researchers into basic FMION research, and the exploitation of FMIONs in future clinical applications.

ACKNOWLEDGMENTS

The research was supported by the National Natural Science Foundation of China (No. 81901881, 81701821), the National Key Research and Development Program of China (No. 2018YFA0902702), the Science and Technology Project for Social Development of Guangdong Province (No. 0903129), Natural Science Foundation of Zhejiang Province (No. LY20E020017), and Fundamental Research Funds for the Provincial Universities of Zhejiang (No. RF-A2019004).

CONFLICT OF INTEREST

All authors declare no competing financial interest.

AUTHOR CONTRIBUTIONS

Yi-Jun Liang: conceived the central idea and manuscript written; Jun Xie and Jing Yu: manuscript revised and participated in cases discussion; Zhaoguang Zheng, Fang Liu and Anping Yang: investigated the relate work. All authors have given approval to the final version of the manuscript.

ORCID

Yi-Jun Liang @ https://orcid.org/0000-0002-1633-5492

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**How to cite this article**: Liang Y-J, Xie J, Yu J, Zheng Z, Liu F, Yang A. Recent advances of high performance magnetic iron oxide nanoparticles: Controlled synthesis, properties tuning and cancer theranostics. *Nano Select*. 2021;2:216–250. https://doi.org/10.1002/nano.202000169