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An overview on the multifunctional nanozyme activities of iron oxide magnetic nanoparticles for nanomedicine

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Iron oxide nanoparticles (IONPs) have emerged as a cornerstone in nanomedicine due to their inherent physicochemical properties and multivariant nanozyme activity. IONPs possess enzyme-like inherent activity, *i.e.*, peroxidase, catalase, and superoxide dismutase mimicry, and thus can control reactive oxygen species (ROS) levels in biological organisms. This mini-review comprehensively addresses the dual functionality of IONPs as therapeutic and diagnostic agents, their potential in targeted drug delivery, cancer therapy, and biomedical imaging. The tunable magnetic properties, biocompatibility, and surface chemistry of IONPs further attest to their utility in precision medicine. We further discuss recent advances in the functionalization and design of IONPs for enhanced nanozyme activity and therapeutic value. Challenges such as long-term biocompatibility, toxicity issues, and scalability are also addressed, providing hints for the future development of IONP-based nanomedicine. The review identifies the disruptive potential of IONPs as multi-functional nanozymes to propel next-generation therapeutic and diagnostic platforms.

1. Introduction

Enzymes play a crucial role as biocatalysts in nature; however, their drawbacks, such as high expense, instability, and strict reaction conditions, have led to the pursuit of alternative options. Nanozymes, nanomaterials mimicking enzymatic

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activity, have emerged as transformative alternatives in the field of biomedicine, addressing the constraints associated with natural enzymes. Acknowledged by IUPAC as a leading emerging technology in chemistry (2022), nanozymes provide economical, stable, and adjustable catalytic platforms, applicable in areas such as biosensing and cancer treatment. Among these, magnetic nanozymes, especially IONPs, are notable for their distinctive blend of biocompatibility, magnetic tunability, and the ability to mimic multiple enzymes.[1–5] Nanozymes combine characteristics of both chemical catalysts and biocatalysts. They can be generally divided into two categories. One of them is hybrid enzymes supported by nanomaterials, which obtain stability from their nanostructured bases, and another one is nanomaterials with intrinsic catalytic properties that imitate natural enzymes. Ongoing studies are concentrating on the latter category because of their wide-ranging applications in biomedicine.[6–9] In recent years, the appealing benefits of low cost, robust performance, adaptable design, and multifunctional capabilities have led to an increasing application of nanozymes in biomedical diagnosis. Nanozymes facilitate the detection of biomarkers with exceptional sensitivity by means of catalytic signal amplification. This can occur through the direct oxidation of substrates (such as glucose), the synergistic action with natural enzymes, or their role as catalytic labels in immunoassays and aptasensors. Furthermore, certain biomarkers like uric acid, dopamine, and glutathione can influence nanozyme activity, leading to the development of indirect detection methods for a range of diseases, including metabolic disorders, cardiovascular issues, and cancers.[1] Among nanozymes, magnetic nanomaterials are distinguished by their exceptional characteristics and extensive applications. Magnetic nanomaterials have been widely utilized across various domains that are intimately connected to our everyday experiences, including catalysis,[10] electromagnetic wave absorption,[11] and particularly in the field of biomedicine.[12] Magnetic nanomaterials comprise magnetic elements, and their physical and chemical characteristics are typically enhanced by modifying their sizes, shapes, structures, and chemical compositions to augment their effectiveness in biomedicine. Due to their minimal toxicity and compatibility with biological systems, iron oxide nanoparticles, particularly Fe_3O_4, are prevalent in biomedical applications,[13] playing essential roles in enhancing magnetic resonance imaging (MRI) contrast,[14] biosensing,[15] and targeted drug delivery.[16] In the initial phases, numerous IONPs demonstrated significant potential for use as nanozymes in the diagnosis and treatment of tumors. For instance, Shi *et al.* developed GOx-Fe_3O_4@DMSNs nanocatalysts by encapsulating glucose oxidase (GOx) along with ultrasmall Fe_3O_4 nanoparticles within dendritic mesoporous silica nanoparticles (DMSNs). The careful design of these nanocatalysts was based on the incorporation of GOx, which reduced glucose levels to deprive cancer cells of energy and generated elevated amounts of H_2O_2, thereby continuously supplying substrates for Fe_3O_4 nanoparticle-mediated Fenton-like reactions.[17] Nanomaterials based on iron oxide have garnered considerable interest owing to their unique characteristics, including catalase-like functionality at neutral pH and peroxidase enzyme activity at acidic pH levels, in addition to exhibiting enhanced enzyme-like activities.[18] In the field of biomedical diagnostics, nanozymes enable the sensitive detection of diseases through catalytic signal amplification. For instance, nanozymes based on Fe_3O_4 replicate peroxidase activity, allowing for the quantification of glucose or the detection of cancer biomarkers through immunoassays. Additionally, their multifunctional capabilities are also applicable in antibacterial therapy, where they produce reactive oxygen species (ROS) to combat pathogens such as Salmonella.[19,20] Moreover, specific bacterial cells can be effectively targeted by modifying IONPs with phytocompounds, which enhances the efficacy of antimicrobial agents at the site of infection while minimizing adverse effects.[21] The most recent advancements were demonstrated in the utilization of magnetic nanoparticles as carriers for drug

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delivery and the administration of treatments targeting conditions such as cancer.[22] Iron oxide is regarded as one of the most biocompatible inorganic materials in nanoparticle form, exhibiting exceptional microscopic physical characteristics such as superparamagnetism, minimal susceptibility to oxidation, stability in liquid solutions, prolonged blood half-life, and adaptable surface chemistry.[23–27] Furthermore, from an application perspective, IONPs exhibit remarkable sustainability when compared to natural enzymes, which present challenges such as elevated costs associated with isolation and purification, restricted thermostability, and a narrow pH range that can hinder enzyme activity during handling, storage, and transportation.[28,29] Therefore, Magnetic nanoparticles (MNPs) show great promise as nanozymes. These nanomaterials replicate the catalytic activities of natural enzymes, including peroxidase, oxidase, catalase, and superoxide dismutase. Due to their unique physicochemical properties, they can effectively catalyze biochemical reactions.

This mini review explores the characteristics of nanozymes, especially Fe_3O_4, outlines the key mechanisms of nanozyme activity within biological systems, and describes some significant biomedical applications in nanomedicine, including biosensing, diagnostics, therapeutics, imaging, theranostics, and considerations for biocompatibility and safety. Herein, also prospective developments of magnetic nanozymes are emphasized, emphasizing their transformative potential as well as the critical challenges in biocompatibility and clinical translation.

2. Synthesis strategies of iron oxide nanozymes (Fe_3O_4 and Fe_2O_3)

Given the importance of iron oxide NPs (Fe_3O_4 and Fe_2O_3), various methods are used to synthesize them. In this section, we focus on the most commonly used synthesis methods of Fe_3O_4 and Fe_2O_3, such as co-precipitation, hydrothermal/solvothermal, sol–gel, electrochemical methods, sonochemical method, and microwave-assisted synthesis.[30,31]

2.1. Co-precipitation

Co-precipitation is a wet chemical method that can also be known as the solvent displacement method.[32] This method has been broadly used for fabricating Fe_3O_4 NPs, which have wide applications in biomedical fields owing to their non-toxic properties.[33] The main steps included in co-precipitation are mainly by adding a base compound to Fe^{3+}/Fe^{2+} ions, resulting in a basic medium that favors Fe_3O_4 NPs synthesis. The synthesis of Fe_3O_4 includes two steps, *i.e.*, growth and nucleation. This method provides co-precipitation synthesis with various advantages, such as ease of use, cost-effectiveness, environmental friendliness, and ability to produce NPs with desired size and shape, among others.[34] The co-precipitation technique has wide applications for synthesizing metal oxides. To stop these particles from clumping, the synthesis of paramagnetic NPs with stability is required. To obtain these NPs, it is significant to control the synthesis temperature optimally.[35]

2.2. Hydrothermal/solvothermal

Another synthesis strategy is hydrothermal/solvothermal. The hydrothermal method is employed to generate a diverse array of crystalline iron oxide NPs, including α-Fe_2O_3, Fe_3O_4, and γ-Fe_2O_3. Furthermore, γ-Fe_2O_3 can be synthesized through the controlled oxidation of Fe_3O_4 or the mineralization of Fe^{3+}. Various wet-chemical techniques facilitate the production of these crystalline materials within a sealed vessel, utilizing high temperatures ranging from 130 to 250 °C and high-pressure conditions between 0.3 and 4 MPa, achieved *via* hydrothermal or solvothermal methods.[36] Typically, this technique involves maintaining a mixture of solid metal linoleate, liquid ethanol-linoleic phase, and a water–ethanol solution under hydrothermal conditions, characterized by elevated temperature and pressure. The standard reaction temperature for the hydrothermal process is approximately 220 °C, with pressures exceeding 107 Pa, and the total reaction duration is 72 hours. A temperature gradient is usually established within a Teflon-lined stainless-steel autoclave, where the cooler section facilitates the deposition of the mineral solute, ultimately leading to the growth of the desired crystals. This method results in NPs that exhibit a highly uniform structure and size, with the capability to adjust NP dimensions from a few nanometers to several hundred. Additionally, the hydrothermal process is recognized for its eco-friendliness and versatility, as it does not necessitate the use of organic solvents or post-treatment processes.[31,37]

2.3. Sol–gel

The sol–gel method represents a suitable wet technique for synthesizing metal oxides. It is among the most researched and widely employed methods for generating NPs. During this procedure, a 'sol' of NPs is created when a precursor compound in an aqueous solution experiences hydroxylation and condensation. Subsequent condensation and polymerization lead to the formation of a three-dimensional network of metal oxides (wet gel), which necessitates additional heat treatment to achieve the final crystalline structure. In recent years, the sol–gel technique has been extensively utilized for the synthesis of various nanomaterials. This method facilitates molecular-level mixing of components, resulting in a uniform dispersion of the metal within the solid matrix. Moreover, the sol–gel approach offers several advantages over alternative methods, such as low operational temperatures, high purity, and excellent chemical homogeneity.[30] Consequently, in this technique, iron oxide NPs are produced through at least a two-step phase transformation: $Fe\ (OH)_3 \rightarrow \beta\text{-}FeOOH \rightarrow \gamma\text{-}Fe_2O_3$. These reactions occur at room temperature, with further heat treatment necessary for crystallization. The structure established during the sol stage of the sol–gel process is critical for regulating the properties of iron oxide NPs.[31]

2.4. Electrochemical technique

Another method for the synthesis of magnetic nanoparticles (MNPs) is through electrochemical techniques. This method has successfully produced a range of NPs and porous structures.

The process entails reactions occurring at both the anode and cathode within a designated electrolyte solution. When a capping agent is present, the anode can oxidize to form metal ions, which subsequently reduce to form metal precipitates. Generally, three electrodes-reference, counter, and working electrodes are employed in the electrochemical synthesis of MNPs. This technique allows for the control of NPs size by optimizing the applied current density during electro-oxidation and/or the electrode potential of the reaction solution. Furthermore, the presence of surfactants during the reaction can prevent the aggregation of NPs.[30]

2.5. Sonochemical

In the sonochemical method, which is advantageous for synthesizing iron oxide, organometallic precursors undergo decomposition through sonolysis to produce NPs. The growth of these NPs is regulated by employing polymers, an organic capping agent, and structural hosts. This approach is also referred to as the sonochemical or ultrasound irradiation technique. The application of ultrasonic irradiation induces acoustic cavitation within an aqueous environment, leading to the growth, formation, and disintegration of microbubbles.[38] The sonochemical technique offers several benefits, including uniform blending and a reduction in crystal growth, which can further enhance the dynamics of chemical reactions and increase reaction rates.[39]

2.6. Microwave-assisted technique

The preparation of magnetic iron oxide NPs with optimized size and shape is commonly achieved through the microwave-assisted technique. This method has garnered significant interest from chemists over recent years due to its extensive application in preparative chemistry and materials synthesis.[31] It is a well-established principle that molecules are excited by electromagnetic radiation, which is the basis for this synthesis. Subsequently, the molecules start to align their dipoles in response to the applied external field. A sufficiently strong disruption in the orientation of the molecules within the external electric field leads to substantial internal heating. Consequently, this synthesis method can effectively lower both energy costs and processing times. The core heating of materials represents a systematic and meticulous approach.[39] Most iron oxide NPs produced *via* this technique exhibit an ellipsoidal shape, with an average diameter of less than 10nm. In comparison to alternative methods such as thermal decomposition, the iron oxide NPs synthesized through the microwave-assisted route can be easily dispersed in water without the need for extensive ligand exchange or purification processes. These attributes are particularly appealing for the large-scale production of iron oxide NPs.[31]

Importantly, different synthesis routes strongly influence the nanozyme activity of iron oxide NPs by modulating their size, crystallinity, and surface chemistry. Such control is crucial for tailoring the catalytic performance of Fe_3O_4 and Fe_2O_3 toward biomedical applications.

3. Nanozyme properties of Fe_3O_4 nanoparticles, key enzyme-mimicking activities

Magnetite nanoparticles (MNPs) exhibit intrinsic enzyme-mimicking activities, making them a prominent class of nanozymes. Fe_3O_4 nanoparticles demonstrate inherent enzyme-like catalytic properties, establishing them as a significant nanozyme. These features resemble those of natural enzymes, including peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and oxidase (OXD). The initial enzyme-like properties identified in IONzyme included its peroxidase-like activity, which facilitates the conventional colorimetric reaction utilizing chromogenic reagents and hydrogen peroxide. This property is observed in both Fe_3O_4 and Fe_2O_3 nanomaterials; nevertheless, the former typically exhibits greater activity than the latter. The catalyzed reaction of H_2O_2 generates free radicals as intermediates, which then interact with a hydrogen donor to yield H_2O and an oxidized donor.[40] In biological contexts, peroxidases perform a multitude of critical functions, such as the detoxification of reactive oxygen species (for instance, glutathione peroxidase) and combating infections (such as myeloperoxidase). The enzyme peroxidase is commonly employed in clinical and bioanalytical chemistry, where it is linked to an antibody and used to catalyze colorimetric substrates for enzymatic signaling or imaging. Another important feature is catalase-like activity. IONzyme exhibits activity akin to that of peroxidase and catalase in the breakdown of H_2O_2 into O_2 and H_2O. According to the Gu group, under basic and neutral pH conditions (pH ~ 7–10), dimercaptosuccinic acid (DMSA)-coated Fe_3O_4 and Fe_2O_3 nanoparticles facilitate the conversion of H_2O_2 to O_2. Notably, Fe_3O_4 demonstrates a more pronounced catalase-like activity compared to Fe_2O_3, paralleling its peroxidase-like catalytic behavior. Thus far, IONzyme has successfully performed both aforementioned actions while ensuring proper pH regulation.[41–43]

Iron-based nanozymes, valued for their compatibility with biological systems and their ability to mimic peroxidase (POD) functions, show significant potential as catalysts in cancer treatment. A recent study by Zhang *et al.* developed a hemin–cysteine–Fe (HCFe) nanozyme that exhibits selective catalytic properties and unique POD-like activities. Their method involved modifying the iron center of hemin with sulfhydryl groups to create an additional catalytic site. By employing a supramolecular assembly approach that integrates Fmoc-L-cysteine, heme, and Fe^{2+} coordination, they successfully created the HCFe nanozyme. This new construct exhibited POD-like activity solely in mildly acidic conditions, a feature associated with the electron-donating capacity of sulfur in its coordination with hemin's iron. Importantly, the lack of catalase (CAT)-like activity makes HCFe particularly effective for tumor treatment driven by reactive oxygen species (ROS). Further research investigated the synergistic effects of HCFe in combination with cisplatin (CDDP) to address CDDP resistance in esophageal squamous cell carcinoma (ESCC). Significant mechanisms involved increased ROS production, decreased drug efflux, and the depletion of cellular glutathione. These results underscore HCFe's potential as a crucial element in multifaceted anticancer

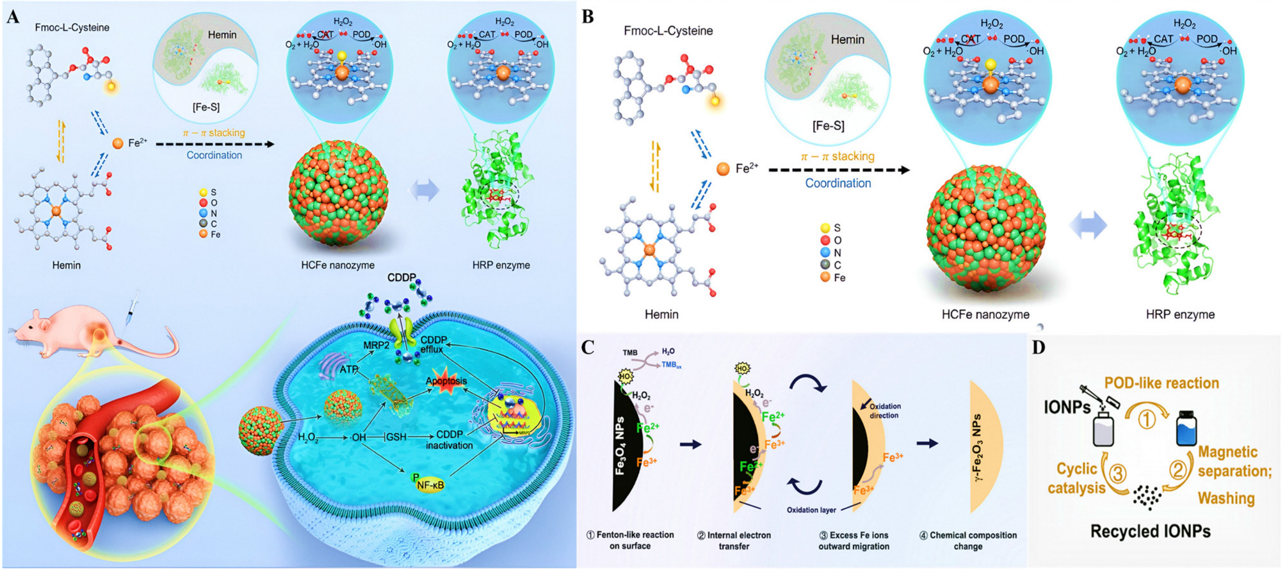

Fig. 1 (A) A diagram illustrating the creation and assessment of HCFe nanozyme, along with its possible uses in combating cisplatin resistance during chemotherapy for esophageal squamous cell carcinoma. (B) A schematic representation illustrating the synthesis process of biomimetic self-assembled iron-based nanozymes.[44] Reproduced from ref. 44 with permission from Springer Nature, Copyright 2024 (C) The catalytic reaction resembling POD involving Fe_3O_4 NPs transpires alongside internal electron transfer and the migration of excess Fe ions. Following extended periods of catalysis, Fe_3O_4 NPs undergo a phase transformation into γ-Fe_2O_3 NPs, which exhibit a diminishing POD-like activity. (D) Illustration of the cyclic catalysis test.[45] Reproduced from ref. 45 with permission from Springer Nature, Copyright 2022.

approaches, particularly in sensitizing tumors that are resistant to chemotherapy (Fig. 1A). To create a nanozyme that incorporates both heme and Fe–S motifs, the researchers employed a biomimetic self-assembly method. This approach utilized π–π stacking interactions between Fmoc-L-cysteine and heme, alongside Fe^{2+} binding with carboxyl groups to maintain structural stability (Fig. 1B). In contrast to typical nanozymes or hemin, which frequently display both POD and CAT activities, HCFe specifically does not exhibit CAT-like functionality, thereby avoiding the breakdown of hydrogen peroxide into water and oxygen. This specificity sets HCFe apart from other catalysts and removes the unwanted production of oxygen, a frequent issue with natural horseradish peroxidase (HRP). Due to its unique POD-like function, the nanozyme is distinguished as a mono-POD nanozyme based on iron, positioning it as an attractive option for improving the effectiveness of chemotherapy in combination cancer treatments.[44]

Recent investigations have highlighted an overlooked dimension of the peroxidase (POD)-like functionality in nanozymes by analyzing the chemical structure and catalytic behavior of recycled Fe_3O_4 nanoparticles during cyclic POD-like reactions. Research indicates that both surface and bulk Fe^{2+} ions play a role in the catalytic characteristics of IONzymes. Internal Fe^{2+} can transfer electrons to surface Fe^{2+}, maintaining catalytic activity by renewing active sites. However, this mechanism is associated with the outward movement of oxidized Fe^{3+}, which may represent a limiting factor in the reaction rate. Over time, extended catalytic activity causes the gradual conversion of Fe_3O_4 into γ-Fe_2O_3, leading to reduced enzymatic efficiency. This deterioration resembles the classic low-temperature oxidation of magnetite, albeit with different electron acceptors. The self-depleting behavior of such nanozymes, where internal atoms participate in the movement of electrons and the migration of ions, is further illustrated by lithium iron phosphate ($LiFePO_4$), a representative material that contains redox-active Fe centers and mobile Li^+ ions within a stable phosphate structure. These findings suggest that internal atoms may also play a role in nanozyme-mediated reactions, despite these reactions primarily occurring on the surfaces of nanoparticles, which raises intriguing considerations for the design, regulation, and application of nanozymes. Notably, Cyclic POD-like catalytic assays (Fig. 1D) were conducted as a form of validation, providing ample substrates for Fe_3O_4 NPs to demonstrate their POD-like capabilities continuously. Therefore, the catalytic mechanism underlying the POD-like activity of Fe_3O_4 nanoparticles can be delineated as follows (Fig. 1C): (1) Fenton-like reaction occurring on the surface. Initially, hydrogen peroxide (H_2O_2) adsorbs onto the particle surface, where it accepts electrons originating from Fe^{2+} on the surface, subsequently dissociating into extremely reactive hydroxyl radicals ($^{\bullet}OH$) that oxidize the substrates. This process results in the oxidation of surface Fe^{2+} to Fe^{3+}. (2) Internal electron transfer occurs next. An adjacent Fe^{2+} found on the surface then donates its electron to the surface Fe^{3+} *via* the Fe^{2+}–O–Fe^{3+} link, which in turn restores the surface Fe^{2+} and supports the processes needed for the continual catalytic reaction. (3) Migration of excess Fe ions outward. As internal Fe^{2+} experiences oxidation in its original location, to preserve electroneutrality, the surplus Fe^{3+} within the lattice must migrate to the surface, creating cation vacancies. (4) Alteration of chemical composition. As the POD-like catalytic reaction persists, Fe_3O_4 NPs are oxidized from the exterior to the interior, ultimately transforming into γ-Fe_2O_3 NPs. This oxidation process, resembling enzymatic activity and initiated by the interaction of Fe_3O_4 nanoparticles, is thought to be similar to conventional low-temperature air oxidation of magnetite, where the movement of iron ions is probably a limiting factor for the reaction rate.[45]

Therefore, Fe_3O_4 nanozymes have a wide range of enzymatic mimicry capabilities, including both POD-like and CAT-like activities, but the stability of their catalytic activities could be reduced by the gradual oxidation process. By contrast, the HCFe system shows higher catalytic selectivity in mildly acidic media, with reduced CAT-like interference. Nevertheless, direct comparison of their activities is difficult due to the differences in experimental conditions. These considerations highlight the importance of achieving a balance between versatility, selectivity, and stability in the design of iron-based nanozymes for biomedical applications.[44,45]

3.1. POD-like activity is governed by structure-based activity relationships

The iron oxide (Fe_3O_4) nanozymes exhibit peroxidase-like catalytic activity through structural determinants such as the Fe^{2+}/Fe^{3+} ratios, protons at crystal defects, or variations in surface coordination geometry. Compared to iron oxide (Fe_2O_3), the enhanced catalytic activity of Fe_3O_4 nanozymes is attributed to the presence of Fe^{2+} are involved in reactions similar to those of Fenton-type catalytic reactions. In addition, the surface defects that exist in the iron oxide structure, and due to the fact that some of the iron atoms are less than 6-coordinated, significantly increase the rate of electron transfer, which enhances the production of ROS. For example, in the nanozymes designed (HCFe), where sulfur coordinated with iron, changing the configuration of electron density around the iron enhances POD-like activity but prevents catalase-type H_2O_2 degradation activity. However, in each Fenton-type reaction to produce H_2O_2 from the catalytic oxidation of Fe (II), the oxidation of Fe(II) to Fe(III) over time during each reaction cycle reduces the availability of active site types of Fe for subsequent catalytic reactions, demonstrating a correlation between the oxidation states of Fe and catalytic stability.[44,45]

3.2. Fabrication strategies of iron oxide nanozymes

Iron oxide nanozymes can be created *via* physical, chemical, or biological methods, each offering distinct advantages and limitations with respect to the size control, crystallinity, scalability, and ability for surface modifications. Examples of physical methods of synthesizing iron oxide nanozymes are *via* laser ablation, thermal decomposition, and physical vapor deposition. These create highly crystalline nanoparticles with controlled shapes and low chemical impurities, providing the highest advantages when exact structural control over the nanozyme is required to modulate its catalytic activity. Chemical synthesis approaches are the most common approaches and include co-precipitation, hydrothermal/solvothermal synthesis, sol–gel processing, and microemulsion. Of these, co-precipitation is the most commonly used strategy because of the ease of use, scalability, and low cost. Additionally, chemical methods provide more precise control over the particle size, surface charge, and doping of particles, which directly affect the efficiency of the catalytic process and enzyme-like selectivity. Green and biological synthesis techniques use either biological materials (*e.g.*, plant extracts) or biological agents (*e.g.*, proteins) to reduce and stabilize the nanoparticles produced during their creation. Some advantages to utilizing green and biological synthesis routes are biocompatibility and environmentally-friendly materials. On the other hand, it may be more difficult to obtain uniform size distributions with biological or green synthesis compared to using chemical methods of manufacturing. Importantly, the selected fabrication strategy determines crystal facet exposure, surface defect density, and functionalization capability, which collectively regulate active site availability, substrate adsorption behavior, and reaction pathway, ultimately governing catalytic efficiency and enzyme-like selectivity.[46–48]

4. Mechanisms of nanozyme activity in biological systems

4.1. ROS generation and Fenton/Fenton-like reactions

Nanozymes mimic the function of natural enzymes and generate reactive oxygen species (ROS) through catalytic actions like peroxidase (POD)-like activity, which breaks down hydrogen peroxide (H_2O_2) into hydroxyl radicals ($^{\bullet}OH$). They also exhibit oxidase (OXD)-like activity, producing superoxide radicals ($O_2^{\bullet-}$) along with H_2O_2. Transition metal-based nanozymes, such as those containing Fe, Cu, or Mn, can drive Fenton or Fenton-like reactions, converting H_2O_2 into harmful $^{\bullet}OH$, particularly in the acidic environments of tumors. This capability opens the door for applications like chemodynamic therapy (CDT) in cancer treatment. Additionally, superoxide dismutase (SOD)-like nanozymes, like CeO_2, turn $O_2^{\bullet-}$ into H_2O_2, while catalase (CAT)-like nanozymes break down H_2O_2 into water and oxygen, offering antioxidant advantages. These processes for regulating ROS allow nanozymes to be used in therapies targeting bacteria, cancer, and inflammation. However, there are still hurdles to overcome in controlling ROS levels to prevent unintended damage and improving catalytic specificity for medical uses.

The dynamic interaction between nanotechnology and the adaptation to microenvironments has become a fundamental aspect of precision medicine, especially in addressing intricate pathological conditions. One notable advancement is the adaptive nanozymes designed to detect and react to disease-specific signals, which are transforming therapeutic approaches by allowing for actions that are controlled both spatially and temporally. In addition to their magnetic characteristics, iron oxide nanoparticles are recognized for their role as an artificial peroxidase and their catalytic function in various reactions. A team of researchers successfully synthesized ultra-small (3.1 ± 1.2 nm) magnetic Fe_3O_4 NPs *in situ* using Polygonum cognatum extract through a green synthesis method. A notable instance of green-synthesized magnetic Fe_3O_4 NPs demonstrating peroxidase-like (POD-like), catalase-like, and reactive oxygen species (ROS) generation activity was showcased for cancer theranostics. The enzyme-like properties and ROS activity of the NPs were further assessed using 3,3′,5,5′-tetramethylbenzidine (TMB) and 1,3-diphenylisobenzofuran

(DPBF) assays, respectively. Research also concentrated on enhancing the peroxidase activity with TMB by optimizing certain parameters to achieve increased enzymatic activity for potential application in cancer cell detection. This system was additionally explored for improved biosensing of H_2O_2. The outcomes of therapeutic applications indicate that enhanced cytotoxicity against cancer cells was attained, with cancer damage occurring through ROS-mediated apoptosis, showing approximately a fourfold increase in the presence of endogenous H_2O_2. Both the diagnostic and therapeutic approaches based on the straightforward Fenton-like function of IONzymes presented in this research are specific to cancer cells and highlight the specificity of green IONzymes for clinical cancer diagnosis and treatment, positioning them as a promising candidate in ROS-related domains.[49]

Overall, infections and oxidative stress hinder the process of wound healing. In recent years, the advent of nanomaterials exhibiting natural enzymatic properties has facilitated the creation of novel antibacterial strategies. Jin *et al.* developed high-performance Cu–Fe_3O_4 nanoclusters that possess multiple enzymatic functions to enhance wound healing in infected tissues (Fig. 2A). Notably, these nanoclusters displayed activity akin to that of horseradish peroxidase (POD) in acidic conditions, while their functions were comparable to those of superoxide dismutase and catalase in neutral or slightly alkaline environments. As illustrated in Fig. 2B, the morphology of the Cu–Fe_3O_4 nanocluster is spherical, as observed through TEM. The diameter distribution of these nanoclusters ranged from approximately 80 to 100 nm. *In vitro* investigations have confirmed the effective free-radical scavenging capability of the Cu–Fe_3O_4 nanozyme in a neutral environment. Under acidic conditions, Cu–Fe_3O_4 nanoclusters exhibit the capability to eliminate MRSA and *E. coli* by producing $^{\bullet}$OH through enhanced POD enzymatic activity, with the bactericidal effect further amplified by the consumption of the reducing agent, GSH. In these conditions, the Cu–Fe_3O_4 nanoclusters facilitate the breakdown of hydrogen peroxide into ROS, which subsequently oxidize TMB to oxTMB; the UV-Vis absorption peak for this reaction is observed at 652 nm. As illustrated in Fig. 2C, the solution turns blue following the interaction of Cu–Fe_3O_4 nanoclusters with TMB under acidic conditions; this color intensifies with the addition of H_2O_2, leading to an increase in the absorption peak at 625 nm. Concurrently, the blue hue of the reaction mixture progressively deepens as the concentration of H_2O_2 rises. In a neutral environment, Cu–Fe_3O_4 nanoclusters serve to shield normal cells from excessive free radicals and damage caused by exogenous hydrogen peroxide, leveraging the combined antioxidant effects of superoxide dismutase and catalase. Furthermore, RNA sequencing (RNA-Seq) was employed to investigate the mechanisms through which Cu–Fe_3O_4 nanoclusters exert their lethal effects on MRSA. RNA-Seq also provided insights into how these nanoclusters influence the transcriptional activities of MRSA bacteria by examining the transcriptome of differentially expressed genes. The sequencing outcomes indicated that Cu–Fe_3O_4 nanoclusters achieved their antibacterial effects by disrupting critical pathways, including energy metabolism, DNA replication, and protein synthesis. Consequently, a variety of enzyme-like Cu–Fe_3O_4 nanoclusters that exhibit responses to different pH levels were successfully synthesized in this research. Cu–Fe_3O_4 nanoclusters demonstrate efficacy in treating full-thickness skin wounds resulting from bacterial infections. These

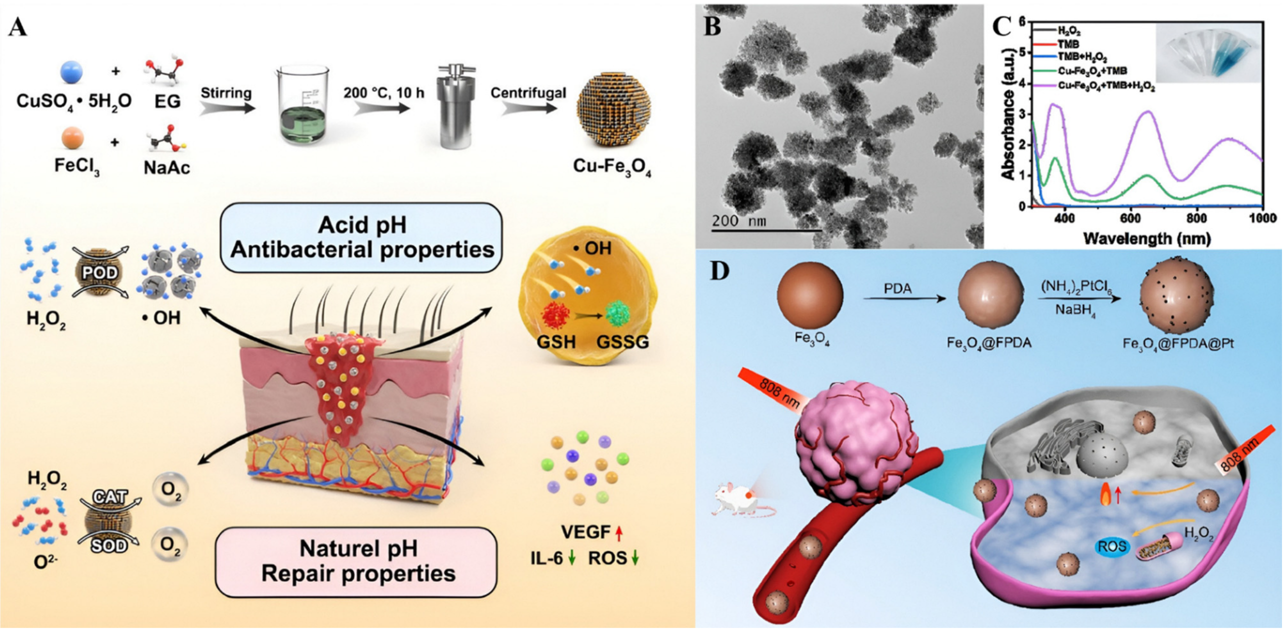

Fig. 2 (A) Schematic representation of the synthesis process for Cu–Fe_3O_4 nanoclusters and the impact of this nanozyme on antibacterial efficacy and wound healing. (B) TEM of Cu–Fe_3O_4 (C) The multienzyme activity exhibited by Cu–Fe_3O_4 nanoclusters, along with the UV-Vis spectra of the solutions.[50] Reproduced from ref. 50 with permission from Elsevier, Copyright 2024. (D) Schematic illustration of the manufacturing procedure for the Fe_3O_4@FPDA@Pt.[51] Reproduced from ref. 51 with permission from Elsevier, Copyright 2025.

nanoclusters possess notable multienzyme activity and peroxidase-like properties when combined with hydrogen peroxide. Thus, they serve as effective antibacterial agents against MRSA and *E. coli*, while also safeguarding normal tissue from $^{\bullet}OH$ and exogenous hydrogen peroxide through the synergistic effects of superoxide dismutase and catalase. As a novel category of antimicrobial agents, Cu–Fe_3O_4 nanoclusters exhibit significant promise in combating drug-resistant pathogens and enhancing wound healing.[50]

As mentioned, Fe_3O_4 nanoparticles, recognized for their magnetic targeting abilities and biocompatibility, have been thoroughly investigated for a range of therapeutic uses. Nevertheless, Fe_3O_4 nanoparticles may agglomerate within the body, potentially compromising their therapeutic effectiveness. To mitigate this concern, Xu *et al.* developed various surface modifications and structural designs aimed at minimizing agglomeration and enhancing activity. They engineered a Fe_3O_4@FPDA@Pt nanozyme, which consists of an Fe_3O_4 core enveloped in flower-like polydopamine (FPDA) and affixed with platinum, intended for photothermal catalytic synergistic cancer treatment. The enzyme-like activity, photothermal capabilities, and both *in vitro* and *in vivo* anticancer effects of the nanozyme were examined. Notably, Fe_3O_4@FPDA@Pt demonstrated significant enzyme-like activity and photothermal performance. Furthermore, the flower-like architecture was readily internalized by tumor cells, facilitating the swift generation of harmful ROS and increased heat at the tumor location. The combination of these two mechanisms can effectively trigger tumor cell apoptosis. As illustrated in Fig. 2D, Fe_3O_4 was initially synthesized using the solvothermal method. By manipulating the mass ratio of P123 and F127, the surfactant's packing parameter can be accurately modified, which in turn affects the self-assembly behavior of flower-like PDA and the resultant mesoporous structure. This system enveloped an FPDA layer with a microporous structure on the Fe_3O_4 surface through self-assembly. Following this, $(NH_4)_2PtCl_6$ was reduced with $NaBH_4$, promoting the *in situ* growth of platinum nanoparticles on the FPDA surface. This sequence of events ultimately resulted in the creation of Fe_3O_4@FPDA@Pt. Therefore, this research presented a novel and straightforward synthesis method for the nanozyme and underscored its distinctive and beneficial structural features for effective cancer treatment.[51]

Comparative studies of these systems show that structural engineering plays a significant role in optimizing ROS amplification efficiency and therapeutic effects. Ultra-small Fe_3O_4 green-synthesized nanoparticles improve surface-dependent Fenton reactions because of the enhanced surface area-to-volume ratio, while Cu-doped Fe_3O_4 nanoclusters demonstrate enhanced catalytic activity based on the synergistic redox cycle between Cu^{2+}/Cu^{+} and Fe^{3+}/Fe^{2+}. In contrast, hybrid Fe_3O_4@FPDA@Pt nanozymes combine both catalytic and photothermal effects, which can improve ROS amplification but with increased structural complexity. These results imply that different catalytic activity improvement methods, including size reduction, heteroatom doping, or multifunctional hybridization, have their own advantages and disadvantages in biomedical applications. Significantly, a direct comparison among these nanozyme systems is difficult due to differences in the synthesis pathways, particle size distribution, surface chemistry, and analytical methods. There can be variations in the methods of ROS detection (such as TMB oxidation or DPBF degradation), buffer composition, and the concentration of endogenous H_2O_2, which can affect the results of catalytic activity. In addition, the green synthesis method may cause variations in each batch, which can affect scalability.[49–51]

In general, the interaction between Fe^{2+} and H_2O_2 produces extremely reactive $^{\bullet}OH$, which is effective in decomposing contaminants in water. Nonetheless, this reaction is rather intricate and encompasses a sequence of processes, mainly involving the initiation, propagation, and termination of the reaction elements.[52] Initially, hydroxyl radicals are formed from the interaction of Fe^{2+} and H_2O_2 (eqn (1)). Subsequently, the resulting Fe^{3+} is reduced back to Fe^{2+} by H_2O_2 (eqn (2)), thereby creating a cyclical process. Nevertheless, several critical factors may hinder the reaction's efficiency, including: (1) the requirement for a substantial quantity of H_2O_2; (2) the presence of a specific narrow pH range ideal for the reaction; and (3) the formation of ferric hydroxide sediment.[53] When it comes to using H_2O_2, a considerable quantity of H_2O_2 is required to achieve acceptable outcomes in industrial applications. Concerning pH levels, Fe^{2+} produces $Fe\ (OH)_2$ when the pH exceeds 3, with the maximum formation occurring at pH 4, where its reactivity significantly surpasses that of Fe^{2+}. In contrast, Fe^{3+} leads to the formation of inactive hydrous oxyhydroxides when the pH is above 3, which considerably inhibits subsequent reactions.[54] Considering these circumstances, heterogeneous Fenton reactions and Fenton-like reactions have been proposed. In the heterogeneous Fenton reaction, iron is kept within the structural framework of the material and produces $^{\bullet}OH$ without the formation of iron hydroxide precipitation.[55,56] Furthermore, various reactions between heterogeneous Fenton catalysts and H_2O_2, including the leaching of Fe ions from the catalysts that react with H_2O_2 (eqn (1) and (2)) and the interaction of surface Fe (in +3 valence state) with H_2O_2 (eqn (3) and (4)).[57,58] Consequently, the redox cycle that includes the various valence states of iron is the key element for the effectiveness of Fenton reactions.[59]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^{-} \quad (1)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\bullet}HO_2 + H^{+} \quad (2)$$

$$\equiv Fe(\text{III}) + H_2O_2 \rightarrow \equiv Fe(\text{II}) + {}^{\bullet}HO_2 + H^{+} \quad (3)$$

$$\equiv Fe(\text{II}) + H_2O_2 \rightarrow \equiv Fe(\text{III}) + {}^{\bullet}OH + OH^{-} \quad (4)$$

The convergence of nanotechnology and robotics has created remarkable possibilities for precision medicine, especially through the development of intelligent systems that replicate biological processes. Among these innovations, nano- and micro-robots that mimic enzymes are particularly noteworthy, as they integrate catalytic functions with the ability for remote-controlled movement aimed at targeted treatments. A noteworthy instance is the research conducted by Murugan *et al.*, who designed robots with a CuS core and a Fe_3O_4 shell capable of performing dual-mode photothermal therapy (PTT) while utilizing magnetic guidance. The Fe_3O_4 shell provides biocompatibility and enables magnetic control, and the CuS core enhances near-infrared (NIR) hyperthermia, facilitating precise tumor destruction. In addition to PTT, these robots display enzyme-like catalytic capabilities, allowing them to modify the tumor microenvironment collaboratively. Therefore, robotic nanozymes present an intriguing blend of accuracy, multifunctional capabilities, and flexibility, positioning them as a valuable asset in cancer treatment. They aim to improve efficacy and address the drawbacks of traditional therapies. Using PVP (C_6H_9NO) with different molecular weights, a flexible polymer renowned for its remarkable properties, serves as a shape-controlling agent at a particular concentration during the production of different CuS core formations. The therapeutic effectiveness, characteristics, and form of these structures depend on the molecular weight of PVP. For instance, PVP (Mw = 10k) produced hollow spherical CuS_{10k} nanostructures, while PVP (Mw = 40k) led to rod-like CuS_{40k} microstructures, and for Mw = 360k produced extended, branched CuS_{360k} superstructure morphologies. These formations occurred when the copper ion was reduced from the precursor ($Cu(NO_3)_2{\cdot}2H_2O$) using citric acid ($C_6H_8O_7$), which also interacted with the sulfur atom (CH_4N_2S), leading to the generation of CuS nuclei. Additionally, PVP showed strong interactions with CuS, significantly influencing its structure at elevated temperatures (200 °C), as illustrated in Fig. 3A–C. A diagram illustrates how core–shell $CuS_{10k}@Fe_3O_4$ and $CuS_{40k}@Fe_3O_4$ are created and used, with the molecular weights of PVP (Mw = 10k, 40k, and 360k) being adjusted for manipulation. (A) It details the chemical makeup and reaction conditions of the $CuS@Fe_3O_4$ core–shell structure, which is created through a simple hydrothermal process using designated precursors. (B) It explains the suggested formation process for hollow spherical and rod-shaped substances, as well as elongated superstructures that result from varying PVP. (C) It outlines how FA-$CuS_{10k}@Fe_3O_4$ nanorobots and FA-$CuS_{40k}@Fe_3O_4$ microrobots operate inside cells. These robotic nanozymes selectively accumulate in MDA-MB-231 cancer cells by utilizing the enhanced permeability and retention (EPR) effect, functioning as Fenton-like catalysts that convert H_2O_2 into OH in the acidic conditions inside the cells. During photothermal therapy (PTT) at 808 nm, a localized temperature spike (>45 °C) occurs in the cancer cells. By generating ROS and utilizing PTT, these nanorobots and microrobots induce DNA damage, interfere with subcellular organelles, and oxidize vital macromolecules, ultimately resulting in the elimination of cancer cells. As a result, the uniquely shaped CuS combined with Fe_3O_4 in these hybrid nano- and microrobots creates a powerful dual-action platform for cancer treatment that merges PTT and ROS generation. This study also presents an innovative approach to enhance the photothermal conversion efficiency (PCE) of different CuS structures, demonstrating significant promise for cancer therapy.[60]

Continuing the discussion of Fenton reactions, a different research team has developed heparin-conjugated $Fe@Fe_3O_4$ nanoparticles (MNPs) that mimic peroxidase activity (Fig. 3D). These nanoparticles facilitate the production of ROS to treat tumors. They achieved this by combining an ultrasound-activated Fenton reaction with the increased levels of H_2O_2 triggered by β-lapachone (La) in the tumor. To start, La was given to mice through an injection in the abdomen, leading to a notable increase in H_2O_2 production from a specific reaction in the tumor. The MNPs then catalyzed this reaction, creating highly reactive hydroxyl radicals ($^{\bullet}OH$). Additionally, using ultrasound significantly boosted the treatment's effectiveness against malignant tumors. The increased levels of H_2O_2 produced by La within the tumor, along with the improved effectiveness of MNPs through ultrasound, demonstrated remarkable therapeutic outcomes in a 4T1 xenograft model, opening up a new avenue for nanozyme-assisted tumor treatment. As a result of being absorbed by tumor cells, MNPs functioned as a peroxidase, facilitating the generation of $^{\bullet}OH$ from H_2O_2 within the cancer cells for therapeutic purposes. The

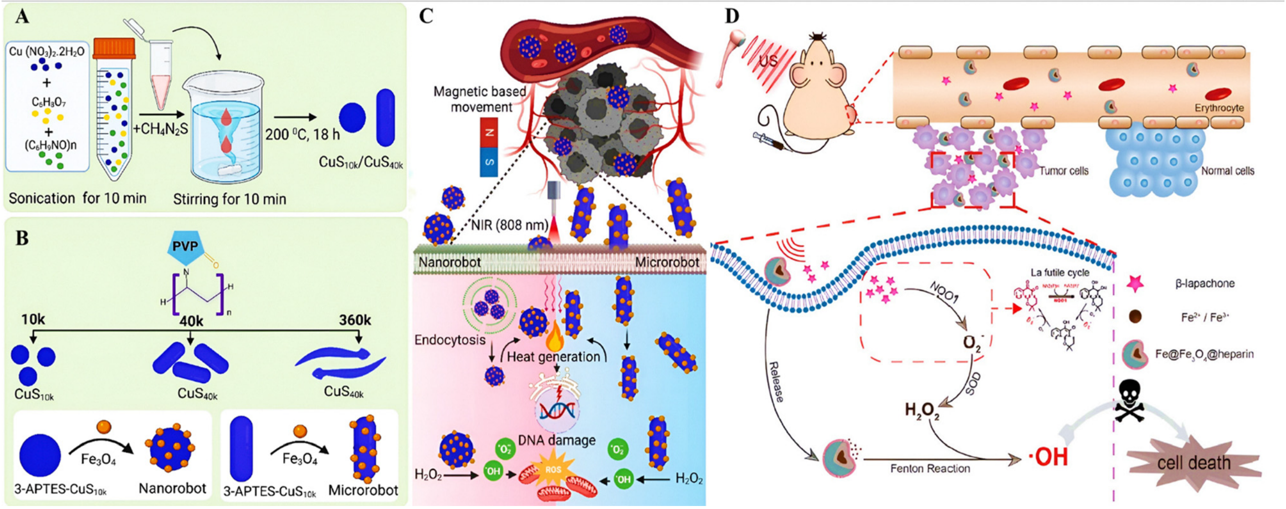

Fig. 3 (A)–(C) A diagram depicts the synthesis and utilization of core–shell $CuS_{10k}@Fe_3O_4$ and $CuS_{40k}@Fe_3O_4$, which are modified by altering the molecular weights of PVP.[60] Reproduced from ref. 60 with permission from Elsevier, Copyright 2025. (D) Visual depiction of the catalytic process assisted by ultrasound and La-enhanced magnetic nanoparticles in cancer treatment.[61] Reproduced from ref. 61 with permission from ACS Publications, Copyright 2019.

addition of La as an anti-drug, in combination with ultrasound application, greatly improved therapeutic effectiveness by boosting the generation of H_2O_2 within the tumor and speeding up the transformation of H_2O_2 to $^{\bullet}OH$.[61]

The evidence provided by iron-based nanomaterials shows us how both structure and method of activation affect the effectiveness of these systems at inducing therapeutic effects. The $CuS@Fe_3O_4$ nanozyme has both photothermal and redox accelerated ability in synergy with each other to create a potent therapeutic agent; whereas $Fe@Fe_3O_4$ has sustained release of Fe^{2+} ions coupled to an external source of hydrogen peroxide. To increase Fenton-like reactions through multimodal activation, the additional complexity of the resultant structures, and the need for an external source of activation can compromise both reproducibility and potential for translation. Therefore, the design of the nanomaterial must provide both catalytic amplification and controlled activation, as well as functionality.[60,61]

4.2. Kinetics and comparison with natural enzymes

Natural enzymes demonstrate remarkable catalytic efficiency and selectivity for their substrates; nonetheless, their restricted stability in different conditions and elevated production costs have prompted scientists to seek out other molecules. The creation of 'Artificial Enzymes' as synthetic substitutes for natural enzymes is one approach to tackle these issues. Among the various options, nanozymes, nanomaterials capable of catalyzing biological reactions, offer numerous benefits compared to their natural equivalents, including enhanced environmental stability, increased durability, and cost-effectiveness. Notably, Magnetic nanozymes, which are nanoscale materials that exhibit properties similar to enzymes, display reaction kinetics that can be adjusted based on parameters such as magnetic fields, substrate levels, and the structure of the nanozyme. Grasping this kinetics is essential for enhancing their effectiveness in a range of uses, including biosensing and biomedicine. The creation of nanozymes, especially IONzymes, includes various metallic and non-metallic substances, many of which have redox properties that enable them to mimic the role of oxidoreductases.[62]

Fe_3O_4 NPs have some fascinating enzyme-like qualities, mimicking the roles of natural peroxidases, catalases, and superoxide dismutase. Their catalytic action revolves around the redox cycling of surface Fe^{2+}/Fe^{3+}, which facilitates the transfer of electrons during various reactions. For instance, regarding peroxidase-like activity, Fe_3O_4 NPs facilitate the decomposition of H_2O_2 into $^{\bullet}OH$ through Fenton mechanisms, thereby enhancing the oxidation of various substrates. The kinetics of these reactions typically exhibit Michaelis–Menten characteristics; however, they demonstrate a reduced affinity for substrates and exhibit varying turnover rates (k_{at}) in comparison to natural enzymes. Factors like particle size, surface modifications, and pH levels influence these variations. IONzymes offer numerous benefits, including cost-effectiveness, stability, and multifunctionality. However, they lack the evolutionary precision inherent in natural enzymes. Scientists can enhance the kinetics of these nanozymes by using surface modification techniques, such as incorporating graphene oxide composites, which significantly facilitate electron transfer. While natural enzymes are unmatched in biological systems, nanozymes present a solid alternative for situations where durability and scalability are more important than having the highest catalytic efficiency. Moreover, natural enzymes function as exceptionally selective catalysts. Although the advancement of nanozymes that can engage with various substrates might lead to lower dosages and fewer side effects, the selectivity of the nanozyme continues to be a vital consideration in many cases. Consequently, significant efforts must be directed towards the creation of nanozymes that exhibit substrate selectivity alongside their catalytic functions. In the future, researchers are keen to enhance the design of nanozymes to mimic enzyme-like kinetics while simultaneously maintaining their synthetic flexibility.[62,63]

The K_m values of Fe_3O_4 nanozymes for H_2O_2 typically range from 0.5 to 5 mM, while for horseradish peroxidase (HRP), it is approximately 0.01–0.05 mM, suggesting a lower affinity for the substrates. The catalytic efficiency (k_{cat}/K_m) of Fe_3O_4 nanozymes is roughly 10–100 times lower than that of natural peroxidases. But nanozymes can work in a wider range of pH, like 4–10, and temperatures approximately 20–60 °C, range compared to HRP.[62,63]

4.3. Environmental influence: pH, temperature, cellular environment

The catalytic efficiency, size, and shape of IONzymes are heavily influenced by pH, which affects their surface charge and redox properties. When the pH is around 4 to 5, Fe_3O_4 shows strong peroxidase-like activity, effectively converting H_2O_2 into ROS through Fenton reactions. Nevertheless, this activity diminishes at neutral or alkaline pH levels because of the decreased availability of Fe^{2+} and the aggregation of nanoparticles. Conversely, catalase-like activity is more pronounced at neutral pH, rendering IONzymes adaptable for various biomedical and environmental applications. Thus, by adjusting the pH, we can optimize their enzymatic properties, facilitating targeted ROS production for therapeutic or antibacterial purposes. Consequently, pH is a vital factor influencing both the synthesis and functionality of IONzymes. Understanding and controlling the pH is crucial for achieving the desired properties and improving the effectiveness of these nanomaterials in various applications.[64,65]

In contrast to natural enzymes that diminish in effectiveness with increasing temperatures, IONzymes are notable for their remarkable catalytic performance over a broad temperature spectrum. Their inorganic composition allows them to retain thermal stability, and their peroxidase-like activity enhances as temperatures rise (up to 60–80 °C) due to improved reaction kinetics, similar to the bimetallic nanozyme $Fe_3O_4@Co_3O_4$/CN. Nevertheless, when temperatures exceed 100 °C, substances may begin to aggregate or experience phase transitions, leading to a decrease in their surface reactivity. Conversely, natural enzymes such as HRP typically cease functioning when temperatures surpass 40 °C. This remarkable heat resistance makes IONzymes perfect for industrial uses, biosensing in extreme conditions, and for long-term storage without much risk of degradation.[64]

In cellular environments, IONzymes engage with biomolecules, potentially altering their catalytic efficiency in reactions. For instance, Fe_3O_4/MnO_2@DOX hybrid nanozymes (FMDHN) exhibit dual functionality, serving both as a means for drug delivery and as a catalyst to inhibit MCF-7 cells. The findings suggest that the utilization of FMDHN, which exhibits catalytic properties and facilitates targeted drug delivery, could offer a new therapeutic pathway for tackling drug-resistant cells. Furthermore, proteins and lipids might stick to the surface of these nanoparticles, potentially blocking active sites or enhancing their compatibility with biological systems. The slightly acidic conditions found in tumors (pH ~6.5–6.9) can specifically activate their peroxidase-like activity, resulting in the generation of harmful ROS for cancer treatment. However, an excessive amount of ROS can adversely affect healthy cells, making it essential to regulate the dosage appropriately. Grasping these interactions is critical for enhancing nanozyme-based treatments while reducing undesirable effects within biological systems.[66]

Therefore, IONzymes show a flexible enzyme-like activity that can change based on different environmental factors like pH, temperature, and cellular conditions. This adaptability brings several advantages, such as being durable, cost-effective, and multifunctional when compared to natural enzymes. While their catalytic efficiency (k_{at}/K_m) usually doesn't quite match that of natural enzymes, their strength in harsh conditions and their capacity to adapt through surface modifications make them very promising for use in biomedical, environmental, and industrial applications. By diving deeper into optimizing their design and understanding their biological interactions, we can enhance their specificity and performance.

5. Biomedical application

5.1. Therapeutics: chemodynamic therapy, synergistic treatments

In general, the increase in bacterial resistance and the rapid development of biofilms pose considerable obstacles to infections linked to these biofilms. Micro and nanorobots that are magnetically actuated have demonstrated considerable potential in a range of medical uses. Ma *et al.* developed an electromagnetically actuated magneto-nanozyme-mediated synergistic therapy (EMST), which has been shown to possess substantial capability in eliminating biofilm. Furthermore, the mesoporous iron oxide nanoparticles (MIONPs) developed in this research generate three types of ROS, specifically hydroxyl radical, singlet oxygen, and superoxide anion, when accompanied by H_2O_2, promoting both the killing of bacteria and the breakdown of the extracellular polymeric substance (EPS) present in biofilm. EMS was employed to mechanically eliminate biofilm, chemically eradicate bacteria, and extract biofilm remnants, while activated microflora (AMF) was utilized to enhance the generation of ROS (Fig. 4A). Motivated by the impressive antibacterial effectiveness of MNP/H_2O_2/EMS/AMF, an *in vitro* mouse skin infection model was utilized to evaluate the effects of MNPs on biofilm *in vivo*. As illustrated in Fig. 4B, each mouse received a subcutaneous injection of 100 μL containing 10^6 CFU mL^{-1} MRSA. Following 24 hours, 100 μL of MNPs in 1% H_2O_2 was applied to the wound, after which, the mice were sedated and situated at the center of an EMS. Following 45 minutes of rotational motion, the gathered MNPs triggered an increase in ROS generation and showed improved synergistic antibacterial properties due to the effect of AMF. To assess the bactericidal efficacy, wound tissues were excised after 10 days of treatment, and internal organs, along with blood samples, were gathered for quantitative analysis of histopathology and biochemistry. The findings indicated that mice treated with the MNP/H_2O_2/EMS/AMF regimen demonstrated superior wound healing compared to other groups, implying that EMST possesses enhanced biofilm eradication capabilities (Fig. 4C). Additionally, the therapeutic effects of these MNPs on wound tissues were assessed using H&E staining, showing a notable decrease in the quantity of inflammatory cells in wounds treated with MNP/H_2O_2/EMS/AMF (Fig. 4D). This suggests the remarkable antibacterial and therapeutic capabilities of the MNP/H_2O_2 system in facilitating the wound healing process. As a result, engineered MNPs can physically disrupt the EPS of biofilm, chemically eliminate the bacteria contained within, and work in synergy to eradicate biofilm through the influence of EMS and AMF. Therefore, Magnetic nanoparticles function as nanozymes by utilizing their redox-active surfaces and adjustable physicochemical characteristics. Their enzyme-mimicking properties, along with the ability to be controlled magnetically, render them highly effective instruments in the fields of biomedicine and biosensing.[67]

As mentioned previously, Magnetite nanoparticles have shown significant promise as nanozymes in chemodynamic therapy (CDT) for cancer treatment. Nevertheless, their effectiveness *in vivo* is still constrained. A different research team created magnetite nanozymes (MNZs) that demonstrate dual enzyme functions and highlighted how their effectiveness in eliminating tumors varies with their shape. Three different types of MNZs, including flaky (FMNZ), elliptic (EMNZ), and spherical (SMNZ) shapes, were developed to investigate the impact of geometric shape on therapeutic effectiveness and to clarify the mechanisms involved in both *in vitro* and *in vivo* environments. These MNZs demonstrate two distinct enzymatic activities: (1) the glutathione (GSH) oxidase-like (GSH-OXD-like) function facilitates the oxidation of GSH while simultaneously minimizing the consumption of intracellular $^\bullet$OH, and (2) the peroxidase (POD)-like function promotes the breakdown of H_2O_2, resulting in the generation of $^\bullet$OH and the subsequent reduction of GSH. Research has indicated that these self-cascading MNZs demonstrate efficacy in CDT that depends on their shape at both the cellular and tissue levels. Notably, FMNZ exhibits an exceptional capacity to reduce tumor size in a challenging representation of triple-negative breast cancer (Fig. 4E). FMNZ was synthesized through a three-step procedure. Initially, ferric chloride interacts with ammonia to generate ferric hydroxide, which subsequently decomposes into iron oxide nanocrystals *via* dehydration at elevated temperatures. In the second step, the surplus ammonia and water molecules present in the autoclave are subjected to heating at 200 °C, resulting in the generation of an extremely high level of pressure inside the reactor. The resulting iron oxide nanocrystals

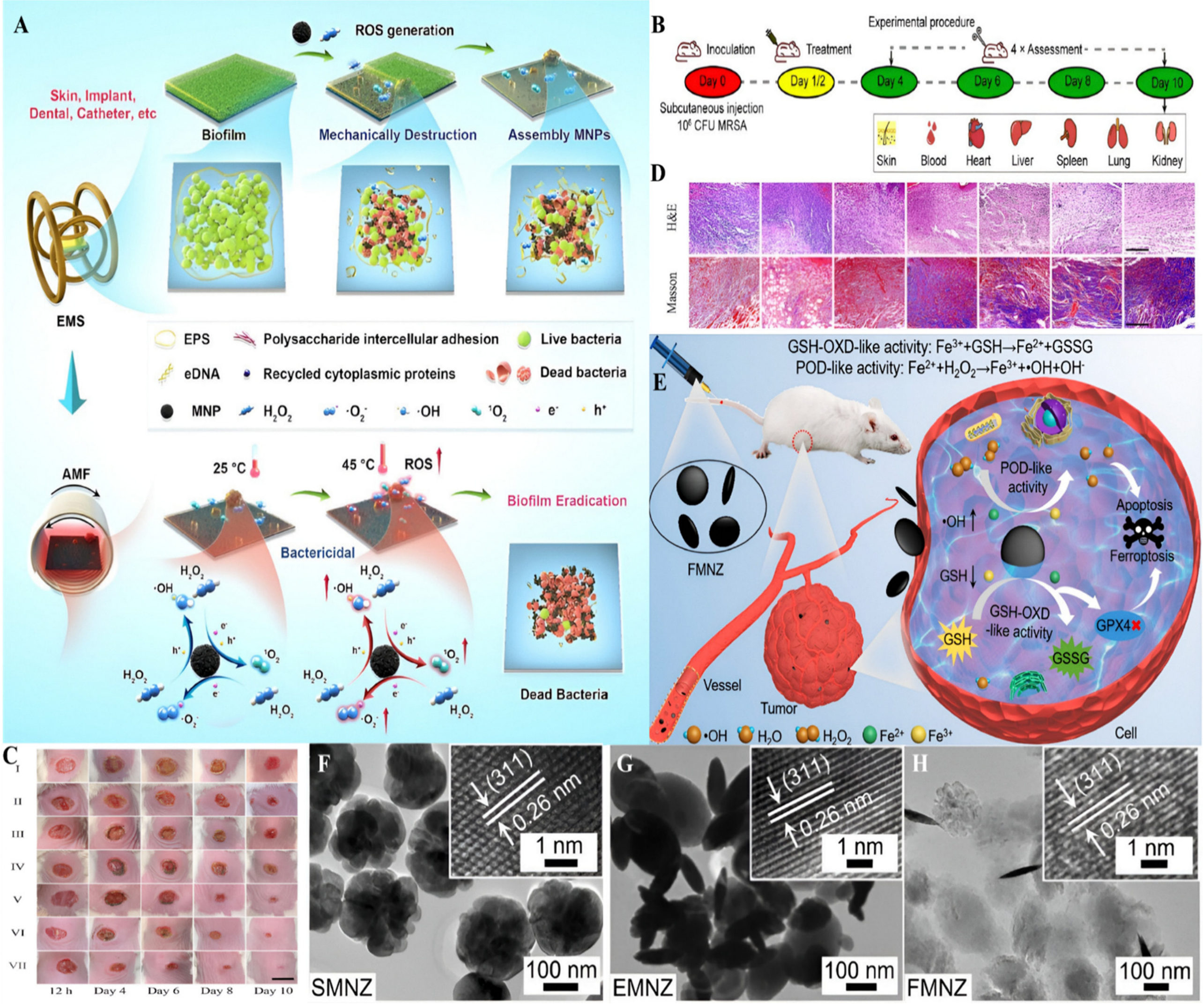

Fig. 4 (A) Schematic representation of the effective elimination of biofilm by MNPs *via* EMST. (B) Antimicrobial protocol for different therapies in a mouse model of skin infection. (C) Images depicting injuries managed with (I) PBS, (II) H_2O_2, (III) MNP, (IV) MNP/H_2O_2, (V) MNP/H_2O_2/AMF, (VI) MNP/H_2O_2/EMS, (VII) MNP/H_2O_2/EMS/AMF. (D) H&E and Masson staining of the tissues from these wounds that have been treated differently.[67] Reproduced from ref. 67 with permission from Elsevier, Copyright 2022 (E) Schematic representation of self-cascade MNZs exhibiting dual enzymatic activities akin to GSH-OXD and POD for tumor-targeted CDT. (F)–(H) TEM images.[68] Reproduced from ref. 68 with permission from Elsevier, Copyright 2024.

engage in clisions with one another as well as with NH_3/H_2O molecules, facilitating continuous growth under high pressure and resulting in the development of a disk-shaped formation. This disk-shaped structure is favorable in this particular reaction from a thermodynamic perspective, as it encourages the growth of surfaces with higher energy in comparison to those with lower energy. Finally, the produced iron oxide nano discs, characterized by a reddish-brown hue, undergo further reduction using sodium borohydride as a reducing agent, culminating in the formation of FMNZ. Fig. 4F–H illustrates the TEM images of SMNZ, EMNZ, and FMNZ, respectively. As shown, all variants of MNZs exhibit good dispersion and crystallinity, displaying a discernible lattice structure with the crystal planes characteristic of magnetite. FMNZ demonstrated a more rapid and extensive release of iron ions compared to SMNZ and EMNZ across all tested pH conditions, which can be attributed to the superior surface area of FMNZ relative to SMNZ and EMNZ. This study marks a significant breakthrough in the design of eco-friendly inorganic nanozymes that effectively mimic natural catalytic activity, offering a powerful and innovative approach to advancing tumor therapy. By harnessing their robust and tunable enzymatic properties, these nanozymes present a promising strategy for precise and sustainable cancer treatment, paving the way for next-generation nanomedicine with reduced environmental impact. These findings not only underscore the therapeutic potential of these nanomaterials but also open new avenues for developing biocompatible, high-performance alternatives to conventional therapies.[68]

Overall, the results of comparative analysis suggest that FMNZ has the strongest catalytic and therapeutic activity among the shapes, due to its highest surface area, number of active edges, and Fe^{2+} release rate, which is responsible for the highest ROS production and tumor inhibition. The moderate and lowest activities were shown by EMNZ and SMNZ, respectively, which emphasizes the importance of geometric structure in nanozyme activity. The results of all experiments were consistent and reproducible among several replicates.[67,68]

5.2. Biosensing and diagnostics: colorimetric detection, biomarker sensing

Fe_3O_4 nanozymes exhibit intrinsic activities akin to peroxidase, catalase, oxidase, and superoxide dismutase, making them valuable assets in the fields of biosensing and diagnostics, especially for colorimetric detection and biomarker identification. These magnetic nanoparticles facilitate chromogenic reactions, such as the oxidation of TMB in the presence of H_2O_2, resulting in visible color changes that allow for the detection of glucose, heavy metals, and antioxidants. Furthermore, their surface functionalization enables the specific recognition of biomarkers, including proteins like PSA and nucleic acids. The advantages of these nanozymes, including their high stability, capability for magnetic separation, and cost-effectiveness, make them especially appropriate for point-of-care diagnostic applications.

The integration of nanotechnology with biosensing has marked the beginning of a revolutionary phase in diagnostic tools, where multifunctional nanomaterials connect high sensitivity with practical, real-world applications. Among these innovations, catalytic colorimetric nanoparticles are particularly notable for combining vibrant visual detection with the enhanced signal amplification provided by enzymes, allowing for quick, equipment-free diagnostics while maintaining accuracy. A groundbreaking illustration of this is the three-in-one nanohybrid developed by Chen *et al.* Fe_3O_4@MOF@Pt, which incorporates Fe_3O_4 nanoparticles, MIL-100(Fe), and platinum nanoparticles into a unified entity designed for immunochromatographic assays. Catalytic colorimetric nanoparticles are a type of colored nanomaterial that exhibits intrinsic catalytic characteristics similar to enzymes. Because of their distinctive dual roles in colorimetry and catalysis, these nanoparticles have attracted increasing interest in the development and improvement of extremely sensitive colorimetric biosensors. Fe_3O_4@MOF@Pt exhibits improved brightness in colorimetric signals, swift magnetic reactivity, and notable action that simulates peroxidase. These characteristics significantly improve the responsiveness of lateral flow immunoassays (LFIA) through the integration of magnetic extraction and catalytic enhancement. When used alongside a dual-antibody sandwich LFIA platform, this nanohybrid facilitates an extremely sensitive immunochromatographic assay for procalcitonin. This level of sensitivity is about 2280 times higher than that of conventional gold nanoparticle (AuNP)-based LFIA and exceeds previously documented immunoassays. Consequently, this research indicates that the suggested catalytic colorimetric nanohybrid shows potential as a signal reporter to enhance ultrasensitive immunochromatographic diagnostics for disease identification (Fig. 5A).[69]

Another group of researchers created an innovative magnetic and reusable R-Fe_3O_4/Au nanozyme exhibiting peroxidase-like activity that was developed through surface modification and electrostatic self-assembly (Fig. 5B). This nanozyme displayed enhanced peroxidase-like activity and showed exceptional stability and reusability. Furthermore, a simple and responsive colorimetric-SERS dual sensing platform was developed by employing the R-Fe_3O_4/Au peroxidase to identify GSH and cholesterol (Fig. 5C and D). This approach showed commendable accuracy and practicality when applied to real serum samples. The R-Fe_3O_4/Au nanozymes functioned as mimetic peroxidases, capable of catalyzing the oxidation of the colorless compound 3,3′,5,5′-tetramethylbenzidine using hydrogen peroxide. Simultaneously, they acted as SERS substrates for the detection of Raman signals from the oxidized products, thereby providing a successful approach to investigate the reaction kinetics of enzymes. Through redox reactions, these nanozymes facilitated colorimetric-SERS dual-mode sensing of glutathione (GSH) and cholesterol, achieving detection. Moreover, the nanozymes enabled the swift identification of GSH and cholesterol in serum without requiring complicated sample preparation. The R-Fe_3O_4/Au catalyst maintained remarkable peroxidase activity even after being utilized five times. In conclusion, the suggested colorimetric-SERS dual-mode sensors exhibited impressive accuracy and consistent reproducibility, opening avenues for the creation of multifunctional sensors while highlighting their considerable potential in biosensing applications.[70]

In general, the comparative analysis of these nanozyme-based biosensors shows complementary advantages, where Fe_3O_4@MOF@Pt has the highest sensitivity because of its higher number of catalytic sites and improved adsorption of the substrate, while R-Fe_3O_4/Au has better stability, reusability, and dual-modal detection. The results of both systems were consistently reproducible in multiple replicates, ensuring the reliability of their enzymatic activity and potential application in point-of-care diagnostics.[69,70]

Another notable application of nanozymes is a straightforward colorimetric biosensing approach that has been established for the quantification of ATP, exhibiting significant selectivity and sensitivity through the use of Fe_3O_4 NPs. Li *et al.* discovered that Fe_3O_4 NPs exhibited significantly enhanced catalytic activity when conjugated with ATP-specific aptamers (Apts), achieving a catalysis rate approximately six times greater than that of unmodified Fe_3O_4 NPs. However, In the presence of ATP, the Apts are released from the Fe_3O_4 NPs due to the binding interaction between the Apts and their specific targets, resulting in a logical reduction in catalysis that is associated with varying ATP concentrations. Consequently, a colorimetric method was devised to enable the extremely discerning identification of ATP. The developed ATP sensor was subsequently used to assess blood ATP levels, demonstrating analytical performance that surpasses that of previously reported detection methods, indicating its potential use in clinical laboratories for the detection and diagnosis of diseases associated with ATP levels. Crucially, this detection method, which is based on catalysis, ought to be adapted to various types of nanozymes that exhibit inherent catalytic activity. This adaptation would provide a potentially universal solution for the monitoring of diverse biological entities by utilizing target-specific recognition components, including Apts and antibodies. Fe_3O_4 nanozymes have been shown to provide a versatile biosensing platform with inherent enzyme-like activity, as well

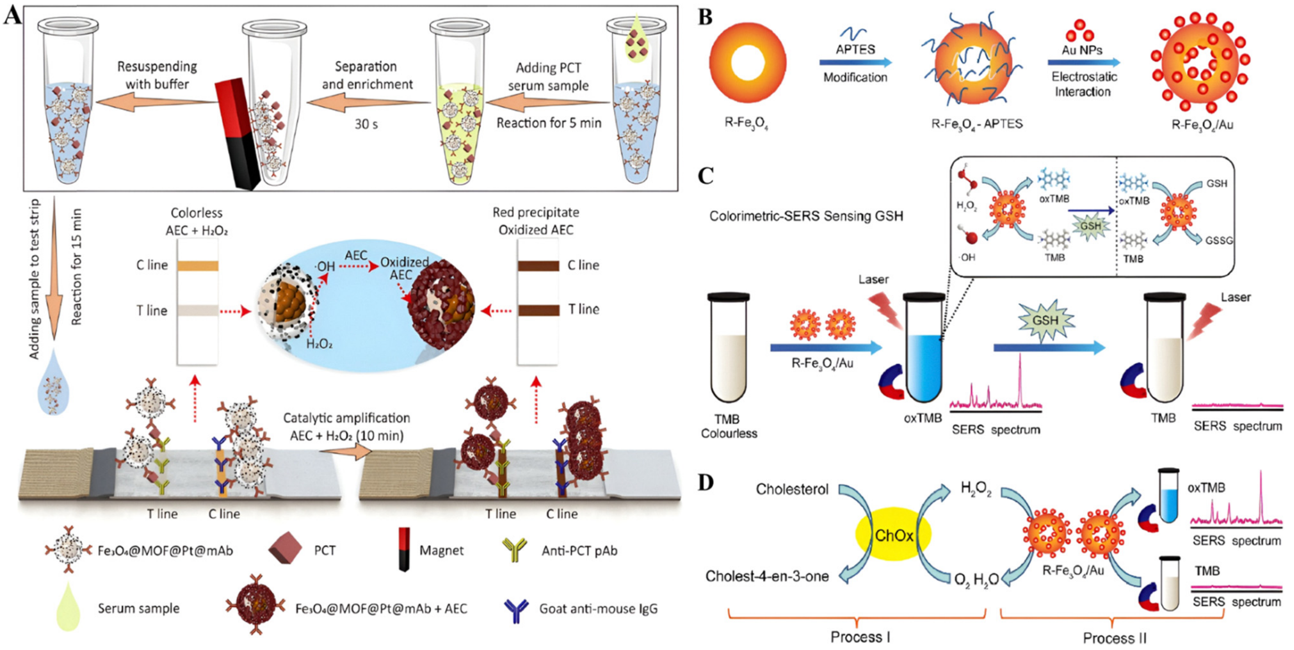

Fig. 5 (A) Schematic Representation of Fe_3O_4@MOF@Pt-Immunolabeled Lateral Flow Immunoassay.[69] Reproduced from ref. 69 with permission from ACS Publications, Copyright 2022. The synthesis process for R-Fe_3O_4/Au (B) and the detection of GSH (C) and cholesterol (D) utilizing the peroxidase-like activity of R-Fe_3O_4/Au.[70] Reproduced from ref. 70 with permission from Elsevier, Copyright 2022.

as the ability to be selectively functionalized. Aptamer modification, for example, has been demonstrated to enhance target recognition and sensitivity. The stability, reusability, and magnetic properties of these nanozymes also make them suitable for use in diagnostic applications.[71]

5.3. Imaging and theranostics: MRI, multimodal imaging platforms

The Fe_3O_4 nanozyme functions as a multifunctional theranostic agent, merging capabilities for magnetic resonance imaging (MRI) with various imaging modalities and therapeutic applications. Its superparamagnetic characteristics facilitate high-contrast T_2-weighted MRI, while its peroxidase- and catalase-like catalytic functions support ROS-mediated therapy and the modulation of the tumor microenvironment. Furthermore, Fe_3O_4 nanoparticles can be tailored into multimodal imaging systems by incorporating fluorescence, photoacoustic, or computed tomography (CT) imaging techniques, which allows for accurate diagnosis and therapeutic outcomes.

The pursuit of accurate cancer theranostics has led to the creation of multifunctional nanoplatforms that effectively combine diagnostics and therapeutic interventions. Within this realm, iron oxide-based systems are particularly notable for their distinct capability to connect high-resolution imaging with targeted treatment strategies, presenting an innovative approach to cancer care. A groundbreaking example is Xie *et al.*'s developed innovative self-assembled ultrasmall Fe_3O_4 nanoclusters designed for tumor-targeted dual-mode T_1/T_2-weighted MRI that facilitates synergetic chemodynamic therapy (CDT) and chemotherapy (Fig. 6A–C). These ultrasmall Fe_3O_4 nanoclusters, which are self-assembled, are produced by simply altering ultrasmall Fe_3O_4 nanoparticles using the molecule 2,3-dimercaptosuccinic acid (DMSA). This modification provides them with enhanced sustained stability and the potential for large-scale production. The resulting ultrasmall Fe_3O_4 nanoclusters exhibit remarkable dual-mode T_1 and T_2 MRI functionalities, alongside a favorable CDT performance attributed to their optimal size effect and the high density of Fe ions present on their surface. After conjugating with the tumor-targeting ligand Arg-Gly-Asp (RGD) and the chemotherapeutic agent doxorubicin (DOX), these functionalized Fe_3O_4 nanoclusters exhibit enhanced tumor accumulation and retention, along with a synergistic effect in both chemotherapy and chemodynamic therapy (CDT), thus creating a strong integrated theranostic platform for cancer treatment. The dual-mode MRI of Fe_3O_4-DMSA and Fe_3O_4-RGD nanoclusters, designated as T_1/T_2, was examined both *in vitro* and *in vivo*. As illustrated in Fig. 6D, the phantom images for T_1-weighted imaging exhibit increased brightness with a rise in Fe_3O_4 concentration. This observation substantiates that Fe_3O_4-DMSA nanoclusters exhibit the characteristic properties of T_1 contrast agents. *Conversely*, for T_2-weighted imaging, the phantom images appear darker as the Fe_3O_4 concentration escalates (Fig. 6E), illustrating the standard characteristics of T_2 contrast agents. MRI signals for both T_1-weighted and T_2-weighted imaging across varying concentrations of Fe_3O_4 nanoclusters suggest that Fe_3O_4-DMSA nanoclusters serve as an exceptional dual-mode T_1 and T_2 MRI contrast agent. The relaxation times T_1 and T_2 of the nanoclusters were concurrently measured and are represented as T_1 and T_2 mappings along the time axis, respectively (Fig. 6F and G). The relaxivity values, denoted as r_1 and r_2, were obtained from the graphs that illustrate the relationship between inverse relaxation time ($1/T_1$ and $1/T_2$) and iron concentration

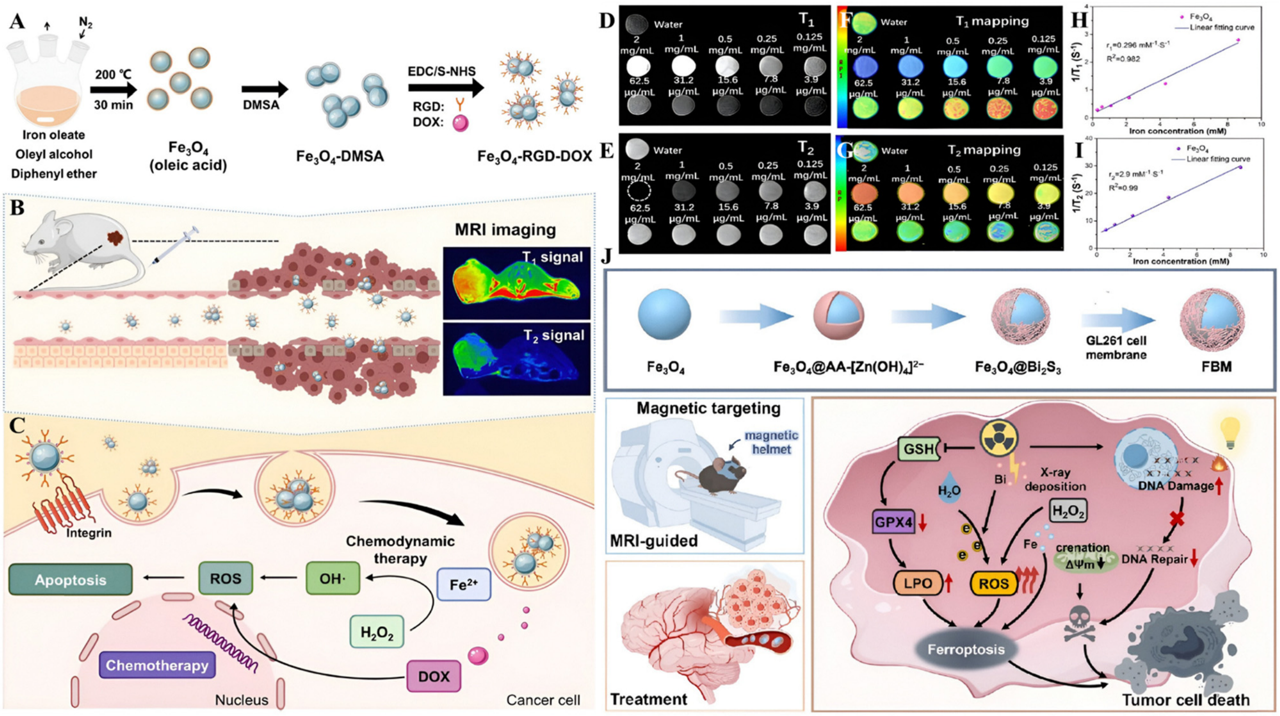

Fig. 6 (A)–(C) Diagram illustrating ultrasmall Fe_3O_4 nanoparticle-derived nanoclusters designed for tumor-targeted dual-mode T_1/T_2-weighted MRI, alongside a synergistic combination of chemotherapy and CDT. (D)–(I) T_1/T_2 dual-mode MRI analysis of Fe_3O_4-DMSA and Fe_3O_4-RGD nanoclusters was conducted both *in vitro* and *in vivo*. (D) T_1-Weighted MR images, (F) T_1 mapping, and (H) T_1 relaxation time of Fe_3O_4-DMSA nanoclusters at different iron concentrations. (E) T_2-Weighted MR images, (G) T_2 mapping, and (I) T_2 relaxation time of Fe_3O_4-DMSA nanoclusters at varying iron concentrations.[72] Reproduced from ref. 72 with permission from Springer Nature, Copyright 2024 (J) Schematic representation of FBM design and its utilization in cancer treatment.[73] Reproduced from ref. 73 with permission from ACS Publications, Copyright 2025.

(Fig. 6H and I). Therefore, a highly efficient approach for the mass production of ultra-small Fe_3O_4 nanoparticles has been introduced. Subsequent surface modification using DMSA imparts hydrophilic characteristics and enhances colloidal stability in suspension, along with promoting self-assembly. The Fe_3O_4 nanoclusters produced demonstrate exceptional T_1/T_2 dual-mode MRI functionalities and considerable promise as contrast agents, which can be ascribed to their ideal size effect. The ultrasmall Fe_3O_4 nanoclusters demonstrate a significant CDT effect, which is linked to the elevated ROS levels in tumor cells. The synergistic application of the chemotherapy drug DOX alongside the Fe_3O_4 nanoclusters in CDT treatment provides a highly effective antitumor response for cancer therapy.[72]

Another group investigated glioblastoma cell membrane-coated $Fe_3O_4@Bi_2S_3$ nanoparticles, referred to as FBM, which were designed for magnetic field-targeted delivery to enhance glioblastoma radiotherapy through significant accumulation of radiosensitizers within tumors and effective induction of ferroptosis (Fig. 6J). Additionally, MRI was employed to accurately locate the tumor and assess the therapeutic response. The FBM would accumulate in the brain's blood vessels under the influence of an external magnetic field, subsequently targeting the tumor through homologous mechanisms. Due to the presence of Bismuth (high-Z), FBM demonstrates a remarkable capacity to deposit radiation energy, thereby facilitating DNA damage and impeding DNA repair processes within the tumor. Moreover, FBM has the potential to stimulate ROS production, accumulate iron species and lipid peroxidation (LPO) products, reduce glutathione (GSH) levels, and downregulate GPX4, thereby triggering ferroptosis and suppressing tumor growth in an *in situ* glioblastoma model. Significantly, the rate of tumor growth inhibition achieved through FBM-mediated radiotherapy utilizing external magnetism rises to around 75% in the orthotopic glioblastoma model. FBM, which demonstrates capabilities in MRI, indicates promising applications in tumor diagnosis and therapeutic monitoring, consequently minimizing harm to surrounding healthy tissues. Therefore, this innovative multifunctional nanoplatform holds the potential for image-guided radiosensitization through the activation of ferroptosis, thereby offering an effective radiotherapeutic strategy for glioblastoma.[73]

Fe_3O_4 nanozymes are theranostics that support T_1/T_2 MRI and ROS-based therapy. The highly paramagnetic Fe_3O_4-DMSA/RGD nanoclusters showed strong relaxivity (r_1/r_2) and synergistically improved CDT by DOX, while the glioblastoma-targeted nanoparticles $Fe_3O_4@Bi_2S_3$ (FBM) inhibited ~75% of the tumor by ferroptosis-assisted radiotherapy. The performance of these nanozymes depends on particle size, surface engineering, and external stimuli, reflecting both their promise and challenges for optimal design.[72,73]

Continuing the discussion of Imaging and theranostics, another important study conducted by researchers involved the creation of a dual-responsive CuMnO@Fe_3O_4 (CMF) core–shell nanozyme designed for MRI-guided tumor theranostics. The surface of CMF (PCMF) was modified with PEGPDGFB chains *via* electrostatic attraction, which facilitated the tumor-targeting ability of PCMF. Once inside cancer cells, elevated levels of GSH and acidic conditions led to the disassembly of the shell, thereby revealing the copper core and subsequently releasing USIO and metal ions, which stimulated T_1 and T_2 differentiation for accurate tumor visualization. The liberated metal ions rapidly depleted cellular GSH and catalyzed Fenton and Fenton-like reactions, considerably increasing ROS levels and aiding in the chemodynamic therapy (CDT) of tumors. Furthermore, these metal ions significantly suppressed the expression of GPX4, thereby inducing ferroptosis in cancer cells. PCMF also exhibits considerable near-infrared absorption at 808 nm, facilitating photothermal laser ablation of tumors. Consequently, the core–shell PCMF presents diverse opportunities for improving tumor theranostics.[74] On the other hand, despite the potential of ferrotherapy in the treatment of cancer, existing ferrous therapeutics are hindered by inadequate antitumor ferroptosis effectiveness and a lack of specificity for tumors. In this research, Qin *et al.* developed a protease-activatable nanozyme (Fe_3O_4@$Cu_{1.77}Se$) designed for photoacoustic imaging and MRI-guided second near-infrared photothermal ferroptosis therapy for cancer. Fe_3O_4@$Cu_{1.77}Se$ exhibits stability under physiological conditions, yet it disintegrates to enhance the reactive ferrous supply within tumors. This process leads to an increase in hydroxyl radical generation *via* the Fenton reaction, while simultaneously reducing GSH levels due to the excessive expression of matrix metalloproteinases within the tumor microenvironment. This procedure leads to an increased occurrence of ferroptosis in tumor cells, as well as enhanced contrast in T_2-weighted MRI scans. The further combination with second near-infrared photoirradiation to generate localized heat not only initiates effective photothermal therapy and photoacoustic imaging but also significantly enhances the Fenton reaction, facilitating ferroptotic death of tumor cells. This synergistic effect promotes the polarization of tumor-associated macrophages from the M2 type, which supports tumor growth, to the M1 type, which is effective in killing tumors and induces immunogenic cell death in tumor cells. Consequently, this process fosters the maturation of dendritic cells and the infiltration of cytotoxic T lymphocytes into the tumor, leading to substantial tumor suppression. Therefore, this research introduces a novel activatable ferrous nanotheranostic approach for the spatial-temporal regulation of antitumor ferroptosis responses.[75]

Therefore, these studies illustrate the importance of activatable dual/multi-functional nanozymes that combine imaging and therapeutic functions in a precise manner for tumor targeting, ferroptosis, and photothermal therapy, and thus provide promising approaches for the development of next-generation theranostic systems with high specificity and efficacy for cancer therapy.[74,75]

5.4. Preclinical evaluation of iron oxide nanozymes

Besides examining the effects of iron oxide nanozymes in an isolated environment, research has also investigated their effectiveness in living organisms, or animal models, which provides insight into the possible medical usefulness of this type of material. For example, multidisciplinary studies showed impressive suppression of neoplasm growth with the use of various iron oxide nanozymes, especially Fe_3O_4, when testing on mice implanted with a neoplasm. Enhanced levels of reactive oxygen species (ROS) present in the surrounding area of the neoplasm were thought to be responsible for this observed effect. Studies conducted to evaluate the pharmacokinetics of these nanozymes indicate that modifying the surface of the nanozymes will improve circulation times and accumulation in diseased tissue through an enhanced permeation and retention effect. Studies that track the distribution of iron oxide nanozymes demonstrate significant levels of accumulation in the liver and spleen, likely attributable to the reticulohistiocytic system (the cells responsible for the formation of blood), all of which point to the need for surface modifications to be made to increase the effectiveness of iron oxide nanozymes. Studies designed to assess the toxicity of iron oxide nanozymes have shown no significant toxic effects when examining blood, histological sections of tissue, or biochemistry. Furthermore, MRI studies that validate the MRI imaging capabilities of iron oxide nanozymes promote their use for the dual functions of treatment and diagnosis. While there have been several encouraging results obtained thus far for iron oxide nanozymes, there are still numerous challenges to overcome before use in humans, including making these materials biodegradable, repeatable, and standardized.[76–80]

5.5. Biocompatibility and safety considerations

Magnetic nanozymes display encouraging biocompatibility and safety considerations, showing low cytotoxicity across different cell lines at suitable concentrations. This is attributed to their limited generation of ROS and their cellular uptake through endocytosis. Being biodegradable materials, IONzymes progressively decompose into iron ions in physiological environments, which are processed through natural iron homeostasis mechanisms, thereby minimizing the risks associated with long-term toxicity.

5.5.1. Summary of cytotoxicity, pharmacokinetics, and biodegradation profiles. The quest for nanozymes that achieve catalytic efficiencies equal to or greater than those of natural enzymes is considered a significant goal in nanomedicine, especially in addressing therapy-resistant cancers. A landmark achievement in this field comes from Meng *et al.*, who engineered a pyrite peroxidase nanozyme that shows an extraordinarily high affinity for H_2O_2, resulting in a 4144-fold increase in catalytic efficiency when compared to the traditional Fe_3O_4 nanozyme and a 3086-fold enhancement over natural horseradish peroxidase. Their study revealed that the pyrite nanozyme exhibits inherent glutathione oxidase-like activity, facilitating the oxidation of reduced glutathione and resulting in the production of H_2O_2. Thus, this dual-function pyrite nanozyme acts as a self-cascade platform that generates a significant quantity of $^{\bullet}OH$ and diminishes glutathione levels, thereby promoting apoptosis and ferroptosis in tumor cells. As a result, it effectively targeted and eradicated apoptosis-resistant tumor cells harboring KRAS mutations by inducing ferroptosis. Furthermore, the pyrite nanozyme displayed advantageous tumor-specific cytotoxic effects and showed biodegradability, thereby guaranteeing its overall biosafety. The results obtained from *in vivo* antitumor studies suggest that the highly effective pyrite nanozyme could serve as a promising therapeutic agent and may contribute to the advancement of nanozyme-based catalytic treatments for tumors. Therefore, researchers express optimism regarding the potential of pyrite nanozyme application in cancer treatment, which may promote the development of therapeutic strategies based on nanozymes.[81]

Due to the potential for bacterial infections to result in serious medical complications, including significant health crises and even mortality, they present a considerable obstacle in the field of clinical trauma repair. The creation of novel antimicrobials that prioritize biocompatibility and safety aspects, such as cytotoxicity and biodegradation characteristics, has emerged as a critical focus for healthcare professionals. In this research, a methodical hydrothermal reaction approach is employed to synthesize Fe_3O_4@MoS_2 core–shell nanozyme, which exhibits photosynthesis-like activity aimed at combating bacterial infections. The nanoparticles reliably promote the production of ROS from hydrogen peroxide through processes akin to photosynthesis, while also converting light energy into thermal energy with a photothermal efficiency that surpasses 30%. Moreover, the ROS produced through photosynthesis, in conjunction with the iron-mediated cell death mechanism associated with the Fe_3O_4@MoS_2 NPs, endows them with remarkable and broad-spectrum antibacterial capabilities. Considering the benefits of this nanozyme, including its outstanding *in vitro* photothermal performance, chemodynamic activity, sterilization effectiveness, cell safety, and positive biocompatibility, it is advantageous for treating chronic wounds in diabetic patients. Consequently, researchers have established a full-thickness diabetic wound model to assess the healing effects of Fe_3O_4@MoS_2 on wounds *in vivo*.

Also, the nanozyme developed is anticipated to find extensive applications in various medical domains, particularly in the eradication of bacteria, due to its straightforward synthesis, remarkable therapeutic effectiveness, and commendable biosafety.[82]

Overall, both studies indicate strong therapeutic potential, with the pyrite peroxidase nanozyme particularly standing out in cancer treatment through self-cascade reactions and the induction of ferroptosis, while the Fe_3O_4@MoS_2 nanozyme demonstrates superior antibacterial properties through photothermal and chemodynamic mechanisms. Regarding biocompatibility and safety aspects, the Fe_3O_4@MoS_2 nanozyme is considered more advantageous due to its established cell safety, biodegradability, and wider therapeutic uses in diabetic wound healing without notable cytotoxic effects. Although the pyrite nanozyme shows high effectiveness in targeting tumors, it may need further investigation on its long-term biosafety because of its intense production of ROS and mechanisms that deplete glutathione. Therefore, for applications that emphasize minimal side effects and improved biocompatibility, the Fe_3O_4@MoS_2 nanozyme is distinguished as the safer and more adaptable choice (Fig. 7).

5.5.2. Stability and reusability of iron oxide nanozymes. Apart from biosafety, other significant factors to be taken into consideration for efficient application of these nanozymes are stability and reusability. Nanozymes have been reported to show better physicochemical stability under a wide range of pH, temperature, and ionic strength conditions compared to natural enzymes. In this context, Fe_3O_4-based nanozymes have been reported to show excellent stability under physiological and harsh conditions.[83] Surface engineering approaches have been reported to show efficient results in improving structural stability and retaining catalytic activity after several reaction cycles. In addition, these nanozymes are highly recoverable due to their magnetic properties, which is another significant advantage of these nanozymes over natural enzymes.[84] In addition to these, nano safety is another significant requirement to be fulfilled before applying these nanozymes. Unlike other nanostructures, nanozymes have to undergo additional evaluation due to their enzyme-mimicking redox activity. In this context, nanotoxicity is reported to occur due to both inherent physicochemical properties and ROS generated from catalytic activity. In addition, nanozymes have been reported to show either antioxidant or oxidizing effects depending on the redox conditions. Therefore, controlled catalytic activity is required to prevent ROS-induced damage.[85,86] Significant improvements in the field of colloidal nanozyme engineering enable the controlled manipulation of size, shape, and surface characteristics using methods such as laser ablation, electrochemical deposition, and biomineralization. These methods improve the solubility, structural stability, biocompatibility, and targeting ability of nanozymes through the incorporation of organic and inorganic surface modifiers (Table 1).[87,88]

6. Patents on the iron oxide magnetic nanoparticles-based nanozymes

Various patents on Fe_3O_4 nanozyme development have been noted, with emphasis on peroxidase-like nanozyme catalysts for biosensor applications. In one instance, the emphasis was on glucose sensing as well as the detection of tumor biomarkers. Other patents have noted the development of multifunctional iron oxide nanozymes for cancer treatment. In theranostics, diagnosis and treatment are merged. These patents point to the commercial fascination with nanozyme technology, mainly based on iron oxide nanostructures.

There are several patents registered regarding the application of iron oxide magnetic nanoparticles in the biomedical field. For example, WO2008140831A3 refers to the functionalized iron/iron oxide nanoparticles intended for the diagnostics and therapies for cancer, which include hyperthermia and imaging methods.[112] US8323618B2 describes ultrasmall superparamagnetic iron oxide particles that have been subjected to various surface modifications and are thus suitable for applications as MRI contrast agents or for drug delivery.[113] In addition, US8945628B2 talks about magnetic nanoparticle platforms that are created specially to meet biomedical needs like MRI, drug delivery systems, and theranostic imaging.[114] Patents like US11305024B2 detail iron oxide nanoparticles intended for

Fig. 7 Diagram illustrating the conceptual framework of Iron oxide Nanozyme research.

the delivery of siRNA and the modulation of therapeutic genes, while US10568970B2 pertains to ultra-small iron oxide nanoparticles utilized for the delivery of nucleic acids within biological systems.[115] Furthermore, the recent publication US20240066154A1 reveals iron oxide magnetic particles that may have applications in the diagnosis and treatment of cancer.[116] These patents actually emphasize the promising translational capabilities of magnetic nanozyme and similar nanoparticle technology within the medical nano field.

7. Challenges and outlook

Currently, most nanozymes investigated replicate the catalytic roles of natural enzymes, yet their structural compositions frequently differ from those of biological enzymes. The lack of complex structures around the active sites results in a shortfall of substrate specificity. Attaining elevated selectivity in measurements remains a significant challenge faced within the bioanalytical field centered on nanozymes. In contrast, nanozymes act as highly efficient catalysts; however, their limited capacity for recycling hinders their potential uses. Improving the recyclability of nanozymes can lower enzyme usage and minimize environmental pollution, thereby promoting green chemistry and sustainable practices. Furthermore, progress in the recyclability of nanozymes may reduce expenses in catalytic processes, enhance production efficiency, and ultimately provide economic benefits. Numerous studies have indicated the inherent toxicity associated with nanoscale materials. As a significant category of nanomaterials, the application of nanozymes in biomedicine, especially for *in vivo* monitoring and bioimaging, requires careful evaluation of their potential toxicity. It is beneficial to choose materials with low toxicity or those that are non-toxic for the fabrication of nanozymes. At the same time, altering their shape, surface chemistry, and size to produce safer nanozymes is a feasible strategy. Improving the retrievability, degradability, and excretion capabilities of nanozymes can help alleviate this concern. To promote the clinical use of nanozymes, it is essential to perform a thorough assessment of their toxicological risks. IONzymes are incredibly promising for future studies due to their remarkable stability, customizable catalytic properties, and adaptability. These attributes can be fine-tuned for a diverse array of applications, such as biosensing, medical therapies, and environmental remediation. In the medical field, scientists are investigating the potential of IONzymes for use in treatments that target inflammation, oxidative stress, and cancer.

Table 1 Typical size of nanomaterials, Catalytic efficiency, Mechanisms, Medical applications, and Functional properties of some significant novel Nanozymes

Nanozyme	Typical size of nanomaterials (nm)	Catalytic efficiency	Mechanisms of nanozyme	Medical application	Functional properties	Ref.
Magnetic nanorobots Fe_3O_4	~50	POD, OXD	Multi-enzymatic cascade	Chemodynamic therapy	Magnetic fields	89
A single-atom nanozyme (Fe-SA) integrated TiO_2 nanoarray	~50	POD, OXD	ROS, kinetic analysis, temperature dependent	Ultrasensitive electrochemical screening, detection, and sensing	Stability, high catalytic efficiency	90
Fe_3O_4@MON-F@Ru	(Overall nanostructure) ~500	POD, CAT	DFT calculations, kinetic	Perfluorooctanesulfonate (PFOS) detection, health safety	Sensitive colorimetric, photothermal, magnetic	91
Fe_3O_4@Au-Pt nanozymes	(Overall nanostructure) ~100	OXD	pH-dependent activity	Sensitive detection of saxitoxin	Magnetic fluorescent	92
Fe-coordinated carbon nanozyme dots (Fe-CDs)	~20	POD, HRP, CAT	Kinetic, temperature-dependent	T_2-Magnetic resonance imaging (MRI), glucose detection	Magnetic nanoparticle, cell cytotoxicity	93
$NaGdF_4$: Yb, Tm@$NaYF_4$: Yb, Nd (DWN nanozyme)	(Core–shell) ~50–20	OXD, POD	ROS generation, multienzyme, pH & temperature dependent	T_2-Weighted MRI/second NIR fluorescence window (NIR-II), tumor diagnosis and therapy guidance	Magnetic field, primarily catalytic	94
MSPN nanozyme	~100	OXD, CAT, HRP	ROS generation, pH-dependent activity	MRI, photoacoustic (PA) imaging, and Cancer therapy of tumors	Photoacoustic, high sensitivity	95
Nanozyme Fe_3O_4@AuNPs	(Overall nanostructure) ~200	OXD, POD, GOD	Enzymatic cascade, Michaelis–Menten kinetics	Novel biosensing platform, accurate detection	Sensing platform, catalytic mechanism, electrochemical	96
Fe_3O_4@Cu-TCPP	(Overall nanostructure) ~100	OXD	Fenton reaction, pH-dependent, ROS generation	T_2-Weighted MRI, chemodynamic, photodynamic, synergetic tumor therapies	Tumor microenvironment (TME), cytotoxicity	97
Fe_3O_4/MoS_2 nanozyme	Nanoscale (particle size not explicitly reported)	CAT	Fenton reaction, synergistic nanozyme adhesion mechanism of the catechol group	Strong interfacial adhesive, toughness, antibacterial ability	Recyclability, stability, magnetic, thermostability	98
Prussian blue nanoparticles (PBNPs)	(Overall nanostructure) ~50	CAT, POD, SOD	ROS production	Overcome oxidative stress, enhance nerve repair, clinical application, biodegradability, MRI, photoacoustic imaging	Photothermal cancer therapy	99
Fe_3O_4 nanoparticles functionalized with chitosan (Fe_3O_4@CS)	(Overall nanostructure) ~100	CAT, Oxidative stress	Catalytic kinetics, cellular environment	Biocompatibility, brain delivery treatment of depression	Catalytic process of hydroxylation, Magnetic	100
NC/Fe_3O_4/Ag peroxidase nanozyme	(Overall nanostructure) ~100	POD, CAT	Temperature & pH-dependent activity, kinetic, chromogenic reactions	Antibacterial & wound healing agent	Stability& recyclability, magnetic	101
MNPs/GOD@CS/IR820 nano system	(Overall nanostructure) ~10	GOD, POD, CAT	Kinetic reaction, ROS	Synergistic chemodynamic & photodynamic cancer therapy, biocompatibility, MRI	Magnetic, catalytic, and enzymatic reactions with hydrogen peroxide	102
$CaCO_3$@Pd@C nano system	Overall: ~250; catalytic domains: ~50	POD	Kinetic, ROS, temperature & pH-dependent	Biocompatibility, biomedical applications, synergistic effects	Photothermal	103
A novel nanozyme Fe/Mn-SNC	(Overall nanostructure) ~200	OXD, SOD, POD	Multienzyme, cascade activities, DFT calculations	Antibacterial, biosafety, applications in medical technology, and consumer care	Catalytic process	104
FPB-Co-Ch NPs	(Overall nanoparticle) ~200–500	OXD, SOD, POD, CAT	Multifunctional cascade nanozyme activities, ROS	Biocompatibility, biosafety, antibacterial, anti-inflammation, MRI	Oxygen-sensing, sensitive, and resistant *H. pylori* infections	105
Au@Fe_3O_4 nanoparticles	~150–330	CAT, OXD	Chromogenic reaction	Colorimetric detection, potential for colorectal cancer (CRC) screening & diagnosis	Magnetic, photothermal, and catalytic activity	106
uBSPIO nanoparticle	(Overall nanoparticle) ~50	OXD	Electron transfer, temperature & pH-dependent	Contrast agent for MRI, biocompatibility	Bio-stability, cytotoxicity	107
N-CDs/Fe_3O_4 nanozyme	(Overall nanostructure) ~100	CAT, POD, GOD	Kinetic, solvothermal, hydrothermal, ROS	Colorimetric detection, biocompatibility	Magnetic, visual glucose detection, reusability	108
Zn, manganese, Cu-doped iron-based magnetic nanocrystals	(Overall nanostructure) ~50	GOD, POD, CAT	DFT calculations, kinetic, temperature & pH-detection	Colorimetric & magnetic resonance signal detection, diagnosis of biomarkers	Magnetic, colorimetric sensing	109
Fe_3O_4 nanozymes synergy with SnF_2	Nanoscale (particle size not explicitly reported)	OXD	ROS, pH-dependent activity	Prevent a widespread oral disease with reduced fluoride exposure, and biocompatibility	Catalytic activation, stability, antibiofilm activity, cytotoxicity	110
Fe_3O_4-rGO nanozyme	(Overall nanostructure) ~200	POD	Michaelis–Menten kinetics, temperature & pH-detection	Biomedical applications, H_2O_2 detection, biocompatibility	Sensing platform, high catalytic activity, magnetic	111

They utilize their functionalities, akin to peroxidase and catalase, to regulate ROS in various disease conditions. Nonetheless, there are several challenges to address, such as enhancing biocompatibility, increasing targeting efficiency, and guaranteeing long-term safety to meet regulatory requirements for human use. Therefore, advancements in surface modification, hybrid nanostructures, and AI-assisted design could accelerate their transition from experimental concepts to real-world medical applications.

8. Conclusion

In this mini review, we have presented a summary of nanozyme materials, with a particular focus on Fe_3O_4 nanozyme, which is utilized in the development of methods and tools for biomedical diagnostics. The swift advancement of nanozymes, along with a variety of effective approaches, has been utilized in recent years to enhance the catalytic characteristics of nanozymes and to address their limitations in comparison to natural enzymes. Primarily functioning as catalytic labels, nanozymes are increasingly being applied in biomarker detection and disease diagnosis, as well as in therapeutics, particularly in chemodynamic therapy and synergistic treatments, imaging (MRI), theranostics, and considerations of biocompatibility and safety, as discussed in this review. While certain challenges persist, new techniques are being investigated to expedite progress within the nanozyme field. Therefore, it is expected that nanozymes will increasingly supplant natural enzymes in various applications and assume a more prominent role in biomedicine and health management. This review presents a scientific understanding by synthesizing the physicochemical properties, catalytic processes, and biomedical applications of Fe_3O_4-based nanozymes into a cohesive framework, thereby bridging the gap between fundamental nanozyme chemistry and practical nanomedicine. The logical design of the next generation of magnetic nanozymes is a reasoned basis *via* critical examination of structure–activity relationships and/or multifunctional capabilities in diagnostics, imaging, and therapy. Such future work should concentrate on the furtherance of catalytic selectivity and activity in physiological media, arriving at a consensus on benchmarking protocols so that consistent comparisons can be made, and performing systematic long-term *in vivo* safety and biodegradation studies. In addition, scalable, reproducible, and regulation-compliant manufacturing approaches will be critical for the translation of iron oxide nanozymes from bench to bedside.

Conflicts of interest

There are no conflicts to declare.

Abbreviations

IONPs	Iron oxide nanoparticles
MRI	Magnetic resonance imaging
Gox	Glucose oxidase
DMSNs	Dendritic mesoporous silica nanoparticles
ROS	Reactive oxygen species
MNPs	Magnetic nanoparticles
POD	Peroxidase
CAT	Catalase
SOD	Superoxide dismutase
OXD	Oxidase
IONzyme	Iron oxide nanozyme
DMSA	Dimercaptosuccinic acid
HCFe	Hemin–cysteine–Fe
CDDP	Cisplatin
ESCC	Esophageal squamous cell carcinoma
HRP	Horseradish peroxidase
$LiFePO_4$	Lithium iron phosphate
H_2O_2	Hydrogen peroxide
$^{\bullet}OH$	Hydroxyl radicals
$O_2^{\bullet -}$	Superoxide radicals
CDT	Chemodynamic therapy
TMB	3,3′,5,5′-tetramethylbenzidine
DPBF	1,3-Diphenylisobenzofuran
MRSA	Methicillin-resistant Staphylococcus aureus
RNA-Seq	RNA sequencing
FPDA	Flower-like polydopamine
PTT	Photothermal therapy
NIR	Near-infrared radiation
EPR	Enhanced permeability and retention
PCE	Photothermal conversion efficiency
MNPs	$Fe@Fe_3O_4$ nanoparticles
La	β-lapachone
FMDHN	Fe_3O_4/MnO_2@DOX hybrid nanozymes
EMST	Electromagnetically actuated magneto-nanozyme-mediated synergistic therapy
MIONPs	Mesoporous iron oxide nanoparticles
EPS	Extracellular polymeric substance
AMF	Activated microflora
MNZs	Magnetite nanozymes
FMNZ	Flaky magnetite nanozymes
EMNZ	Elliptic magnetite nanozymes
SMNZ	Spherical magnetite nanozymes
GSH	Glutathione
LFIA	Lateral flow immunoassays
AuNP	Gold nanoparticle
SERS	Surface-enhanced Raman scattering
ATP	Adenosine triphosphate
Apts	ATP-specific aptamers
CT	Computed tomography
DMSA	2,3-Dimercaptosuccinic acid
RGD	Arg-Gly-Asp
DOX	Doxorubicin
FBM	$Fe_3O_4@Bi_2S_3$ nanoparticles coated with glioblastoma cell membrane
LPO	Lipid peroxidation
CMF	$CuMnO@Fe_3O_4$
PEGPDGFB	Polyethylene glycol–platelet-derived growth factor-B
USIO	Ultrasmall iron oxide

Fe-SA	Fe single-atom nanozyme
PFOS	Perfluoro octane sulfonate
Fe-CDs	Fe-coordinated carbon nanozyme dots
MSPN	Manganese-semiconducting polymer- based nanozyme
PA	Photoacoustic
TME	Tumor microenvironment
PBNPs	Prussian blue nanoparticles
CRC	Colorectal cancer
DFT	Density functional theory

Data availability

No primary research results, software, or code have been included and no new data were generated or analysed as part of this mini review.

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