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Magnetic nanoparticles and possible synergies with cold atmospheric plasma for cancer treatment

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The biomedical applications of magnetic nanoparticles (MNPs) have gained increasing attention due to their unique biological, chemical, and magnetic properties such as biocompatibility, chemical stability, and high magnetic susceptibility. However, several critical issues still remain that have significantly halted the clinical translation of these nanomaterials such as the relatively low therapeutic efficacy, hyperthermia resistance, and biosafety concerns. To identify innovative approaches possibly creating synergies with MNPs to resolve or mitigate these problems, we delineated the anti-cancer properties of MNPs and their existing onco-therapeutic portfolios, based on which we proposed cold atmospheric plasma (CAP) to be a possible synergizer of MNPs by enhancing free radical generation, reducing hyperthermia resistance, preventing MNP aggregation, and functioning as an innovative magnetic and light source for magnetothermal- and photo-therapies. Our insights on the possible facilitating role of CAP in translating MNPs for biomedical use may inspire fresh research directions that, once actualized, gain mutual benefits from both.

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1. Introduction

Nanoparticles (NPs), defined as solid colloidal particles ranging in size from 10 to 1000 nm, have been extensively used in biomedical applications due to the many benefits they have demonstrated as compared with larger materials such as increased magnetic properties and surface-to-volume ratio.¹ NPs can be roughly grouped into four nanosystems, *i.e.*, metallic NPs such as gold (Au) and silver (Ag), bimetallic or alloy NPs, including iron-platinum (FePt) and iron-cobalt (FeCo), metal oxide NPs such as titanium dioxide (TiO₂), cerium dioxide (CeO₂), silica (SiO₂) and zinc oxide (ZnO), and magnetic NPs (MNPs) such as magnetite (Fe₃O₄) and cobalt ferrite (CoFe₂O₄).²

Due to unique biological, chemical, and magnetic properties of MNPs such as biocompatibility, chemical stability, and high magnetic susceptibility, MNPs have gained the most popularity among NPs in biomedical applications.³ MNPs can act as diagnostic probes for multimodal tracking and contrast agents for magnetic resonance imaging by virtue of their unique physical properties, which have been extensively reviewed.⁴ In addition, MNPs provide high magnetic moments that render them attractive for hyperthermia onco-therapy. For instance, the feasibilities of using CoFe₂O₄ NPs and MnFe₂O₄ NPs for magnetic hyperthermia have been proposed due to their high chemical stability, favorable biocompatibility, high

magnetization and good magnetic susceptibility.^{5–13} Despite advances on the medical applications of MNPs, several obstacles still remain such as the relatively low treatment efficacy and therapeutic resistance that mitigate the clinical outcome of MNP-based modalities, and the biosafety issue that may impose environmental hazards and health risks.

By introducing the material basics and anti-cancer properties of MNPs, as well as their existing onco-therapeutic modalities and remaining issues, we explored possible synergies of MNPs with cold atmospheric plasma (CAP), an emerging redox modulatory tool for cancer treatment, towards improved therapeutic outcome of MNP-based treatment regimens with mitigated remaining issues, with the aim of truly translating MNPs into clinical use and gaining mutual benefits from both therapeutics.

2. Basics of MNPs

Iron oxide has different oxidation states including iron(II) oxide (FeO), iron(III) oxide (Fe₂O₃) and iron(II,III) oxide (Fe₃O₄), where Fe₂O₃ has different crystalline polymorphs, *i.e.*, α -Fe₂O₃, β -Fe₂O₃, γ -Fe₂O₃ and ϵ -Fe₂O₃ (Fig. 1). Among these MNPs, γ -Fe₂O₃ (also named maghemite) and Fe₃O₄ have the highest biocompatibility, with Fe₃O₄ being widely investigated for biomedical use¹⁴ such as magnetic hyperthermal therapy¹⁵ and photo-therapy.^{16,17} However, Fe₃O₄ is easily oxidized and thus requires coating with a biocompatible shell that may prevent MNP agglomeration and enable additional functionalization or conjugation. Example coatings include polymers,^{18–21} ceramics^{22,23} and metals.²⁴ While the internal inorganic metal core takes on the primary functions under the magnetic field, the coated shell prevents the MNPs from agglomeration

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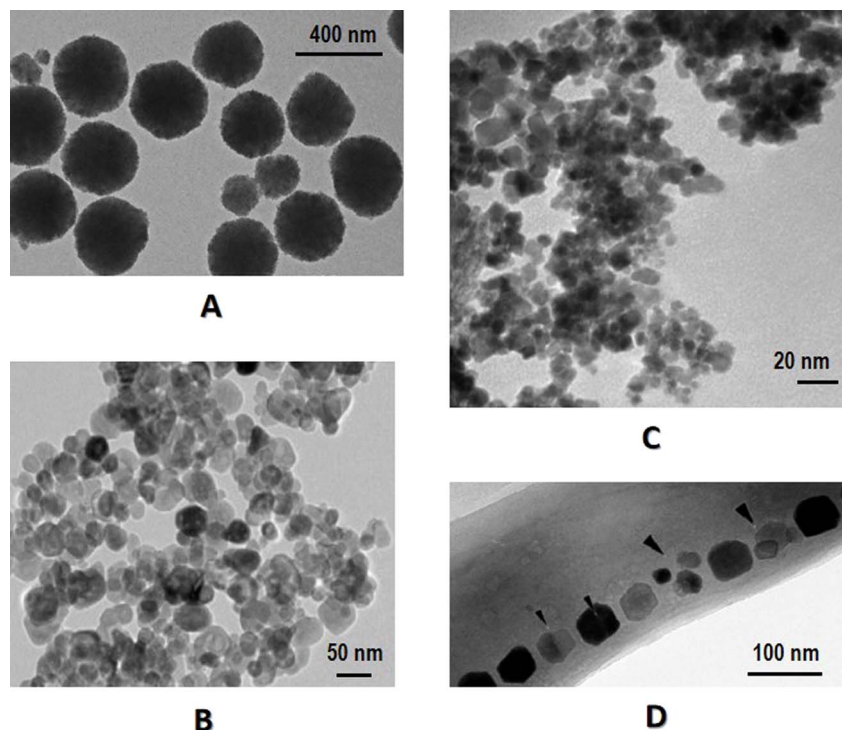


Fig. 1 Transmission electron microscopy images of different types of MNPs. (A) Fe_3O_4 , (B) Fe_2O_3 , (C) FeO . (D) magnetosome. Panel A was reproduced from Fig. 3A of ref. 108 with permission. Panel B was reproduced from Fig. 2A of ref. 109 under Creative Commons Attribution License (CC 4.0). Panel C was reproduced from Fig. 1A of ref. 110 under Creative Commons Attribution 3.0 Unported License. Panel D was reproduced from Fig. 2A of ref. 111 with permission.

through forming a homogeneous and dimensionally stable hydrocolloid dispersion system and attenuating immune reactions.²⁵ The magnetic properties of iron oxide NPs can be further improved by doping with magnetically susceptible elements such as manganese (Mn), cobalt (Co) and nickel (Ni),⁹ with Mn and Co doped ferrites conferring the most promise for biomedical applications.

The presence of magnetosomes, unique organelles composed of membrane-enveloped crystals of a magnetic iron mineral, makes genetically magnetizing non-magnetotactic cells possible and opens an avenue for exciting innovative biotechnological applications. For instance, one would be able to design MNPs harboring tailored magnetic properties *via* combining genes from cells producing diverse magnetosomes for numerous nanotechnological and biomedical applications. This, however, may require adaptations of both the implanted gene cassettes and the host to achieve successful transfer of magnetosome biogenesis pathways into distant non-magnetotactic cells and optimized functionalities²⁶ such as gene-based contrast for magnetic resonance imaging.²⁷

2.1 Characteristics

MNPs have superparamagnetism and magneto-thermal effect, and are featured with large surface area and high adsorption capacity. Superparamagnetism refers to a form of magnetism appearing in small MNPs (typically with a diameter of less than 50 nanometers) that can randomly flip directions under the influence of temperature. MNPs are considered to be at the

superparamagnetic state when the time used to measure NP magnetization is much longer than the Néel relaxation time (defined as the typical time between two flips) in the absence of an external magnetic field. MNPs can be magnetized by an external magnetic field when being attracted at the superparamagnetic state.²⁸ MNPs show the magneto-thermal effect in high-frequency magnetic fields that can be used to kill tumor cells. This is known to rely on two heating mechanisms, *i.e.*, relaxation loss and hysteresis loss for alternating magnetic field (AMF)-induced MNPs.²⁹ Besides the therapeutic role of MNPs, they can also act as the delivery vehicles due to their large surface area that allows them to carry a wide variety of small molecules such as proteins and RNAs³⁰ and high adsorption capacity that enables them for effective enrichment, separation, and directional movement.³¹

2.2 Preparation

The inorganic metal core of MNPs can be prepared by coprecipitation,³² pyrolysis,³³ and microemulsions.³⁴ Coprecipitation is one of the most commonly used and simplest approaches for MNP core preparation that is based on the simultaneous precipitation of Fe^{2+} and Fe^{3+} saline solutions by adding weak or strong bases. Using this method, spherical MNP cores can be achieved in the range of 30–100 nm; yet strong MNP aggregation may be caused due to the large specific surface area of the prepared MNP cores. Many methods have been proposed to resolve this issue, such as modifying the surface of MNP cores with a bioactive coating, preparing them



under a magnetic field, or using alkanolamines as the bases. Pyrolysis refers to the preparation of MNP cores *via* thermal decomposition of organo-iron precursors in high boiling point organic solvents in the presence of stabilizing surfactants.³³ MNP cores obtained using this approach can have a relatively high crystallinity. The microemulsion method refers to the mutual dispersion of two incompatible liquids that are thermodynamically stable. The main advantage of this approach lies in the controllability of the size and morphology of MNP cores it forms that is actualized by varying the size of micelles.³⁴

The organic shells can be coated on MNP cores by organic or inorganic modifications. Commonly used organic materials for MNP encapsulation include, *e.g.*, polymers,³⁵ fatty acids (*e.g.*, oleic acid,³⁶ citric acid,³⁶ lauric acid), and amino acids, among which polymers are the most widely used coating materials. Out of the diversified types of polymer coatings, polyethylene glycol (PEG) and dextran share the highest popularity, where polyethylene glycolized coatings such as PEG ester (C₂H₄O)_n and polyethylene glycolized starch are known to be highly biocompatible. For example, Wang *et al.* utilized PEG to modify Mn-ZnFe₂O₄ that was further linked with hyaluronic acid (HA) for targeting tumors over-expressing CD44. The final nanocomposite, namely HA-modified Mn-ZnFe₂O₄ magnetic NPs (MZF-HA), was shown capable of being enriched in human lung adenocarcinoma cells A549 that expressed high levels of CD44.³⁷ In addition, many stimuli-responsive polymer coatings responsive to, *e.g.*, temperature, pH, light, enzyme, ionic strength, electric and magnetic fields, have emerged. Among these stimuli, temperature and pH are the most widely used in MNP shell design as MNPs swell under acidic environment and temperature control that enable their rapid release at, typically, the target site. Specifically, MNP release is more rapid in the acidic TME as compared with physiological conditions, and the rate of MNP swelling is affected by the temperature. For example, Marziyeh Fathi *et al.* fabricated pH- and temperature-responsive MNPs by conjugating methotrexate to chitosan (CS) for targeting ovarian cancers; attributing to the protonation of the NH₂ functional group of the CS chain that increased MNP swelling and was under pH and temperature control, these MNPs were released faster under cancer-mimicking conditions (40 °C, pH = 5.5) and slower under physiological conditions (37 °C, pH = 7.4), enabling drug release at the target location.³⁸

Inorganic materials such as metals and SiO₂ are also prevalent modifiers of MNP cores.³⁹ For instance, Tao *et al.* utilized Au for MNP surface amendment (Fe₃O₄-Au) that significantly enhanced the biocompatibility and aqueous stability of MNPs.⁴⁰ SiO₂ is prized for its hydrophilicity, biocompatibility, and non-toxicity that render it more amenable for biomedical applications. For example, Lin *et al.* synthesized Fe₃O₄@SiO₂ core-shell structured NPs with an appropriate silica layer thickness that achieved good saturation magnetization; by modifying this silica surface with L-selenocystine and folate, they obtained a novel nanoradiosensitizer with enhanced radiotherapeutic effects against HeLa cervical carcinoma and MDA-MB-231 breast cancer cells and little cytotoxicity.⁴¹

2.3 Physicochemical characterization

Characterization of MNPs can be classified into physicochemical *in vitro* and biological *in vivo* methods. While physicochemical approaches help achieve the best formulation for desired *in vivo* performance, biological *in vivo* methods evaluate the longevity, targetability, cellular uptake, pharmacokinetics, safety, efficacy, and toxicity, among others. Here we focus on some commonly considered physicochemical *in vitro* characterization methodologies.

The size of a MNP can influence cellular uptake and tumor permeability, among others, due to their impact on the enthalpic and entropic properties that govern the adhesion strength between MNPs and cellular receptors.⁴² The size of the magnetic core of a MNP can be determined using transmission electron microscopy (TEM) (Fig. 1), X-ray diffraction (XRD), small angle X-ray scattering (SAXS), and small angle neutron scattering (SANS), among which TEM is the most commonly used that provides information on the internal microstructure or ultrastructure of core besides the external structure.²⁵ Although TEM measures the actual radius of samples, it suffers from the small sample size due to the time-consuming sample preparation process that may also involve many subjective factors. One way to reduce such subjective influences is to take many images at sufficient magnification.

The local concentration of MNPs exposed to each cell is deterministic on the amount of nanoparticles taken up by a cell despite the stochastic nature of the uptake process itself that some cells will take up more nanoparticles than others purely by chance.⁴³ Techniques determining the concentration of particles in suspension are also available. For example, the concentration of iron oxide materials can be determined using UV-vis spectroscopy. If the iron to be detected was at a low concentration or multiple components need to be measured, inductively coupled plasma (ICP) equipped with either a mass spectrometer (MS) or atomic emission spectroscopy (OES) can be adopted.⁴⁴

The surface chemistry of MNPs has been shown to affect their cytotoxic effects by altering the amount of serum proteins adsorbed on the surface, where more serum protein absorption is typically associated with lower cell uptake and higher possibility of causing protein conformational changes and cellular immune responses.⁴⁵ To measure the amount of organic materials on the surface of MNPs, thermal gravimetric analysis is most commonly used. Using this approach, one could gain knowledge on the weight loss and char yield of the organic layer, together with the size distribution of the inorganic core typically obtained from TEM, the number of chains per nm² on the surface of MNPs can be determined.^{46,47} Yet, it requires a special caution that this approach assumes only one organic species on the surface of MNPs. To characterize surface modifications of MNPs, nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy are commonly used approaches. While NMR may produce undiscernible images due to peak broadening as a result of large field inhomogeneity created by magnetic MNPs, FTIR offers a better option that measures the vibrational energy of the bonds. In



addition, Raman spectroscopy (RS) and X-ray photoelectron spectroscopy (XPS) are useful in determining the crystal phase of MNPs, where RS is particularly useful for chemically characterizing ferrite particles.²⁵

The electrostatic potential at the electrical double layer surrounding a MNP in solution is measured by the zeta potential. While MNPs with a zeta potential fell into the range of -10 to $+10$ mV are considered neutral, those with zeta potentials < -30 or $> +30$ mV are highly anionic and cationic, respectively. The zeta potential can affect the tendency of a MNP to permeate membranes, with cationic MNPs being more easily to disrupt negatively charged cell membranes and thus showing higher toxicities.⁴⁸

The colloidal stability and aggregation of MNPs in suspension can be evaluated using the hydrodynamic size distribution of stabilized MNPs, which can be measured using dynamic light scattering (DLS), SAXS and SANS. DLS gains popularity due to its

noninvasive and simple nature that measures the fluctuations of the light scattered by dilute colloidal suspensions.⁴⁹

The saturation magnetization and any presence of hysteresis of MNPs, often reported as the specific absorption rate (SAR, defined as the magnetic power absorbed per unit mass), can be performed *via* traditional approaches such as superconducting quantum interference devices (SQUID) and vibrating sample magnetometers (VSM).²⁵

3. Therapeutic efficacy of MNPs for cancer treatment

3.1 Inducing cancer cell death

Programmed cell death (PCD) is a form of cell death that can be regulated by a variety of biomolecules and manifested in diversified forms such as apoptosis, autophagic cell death and ferroptosis. Given the crucial roles it played in maintaining

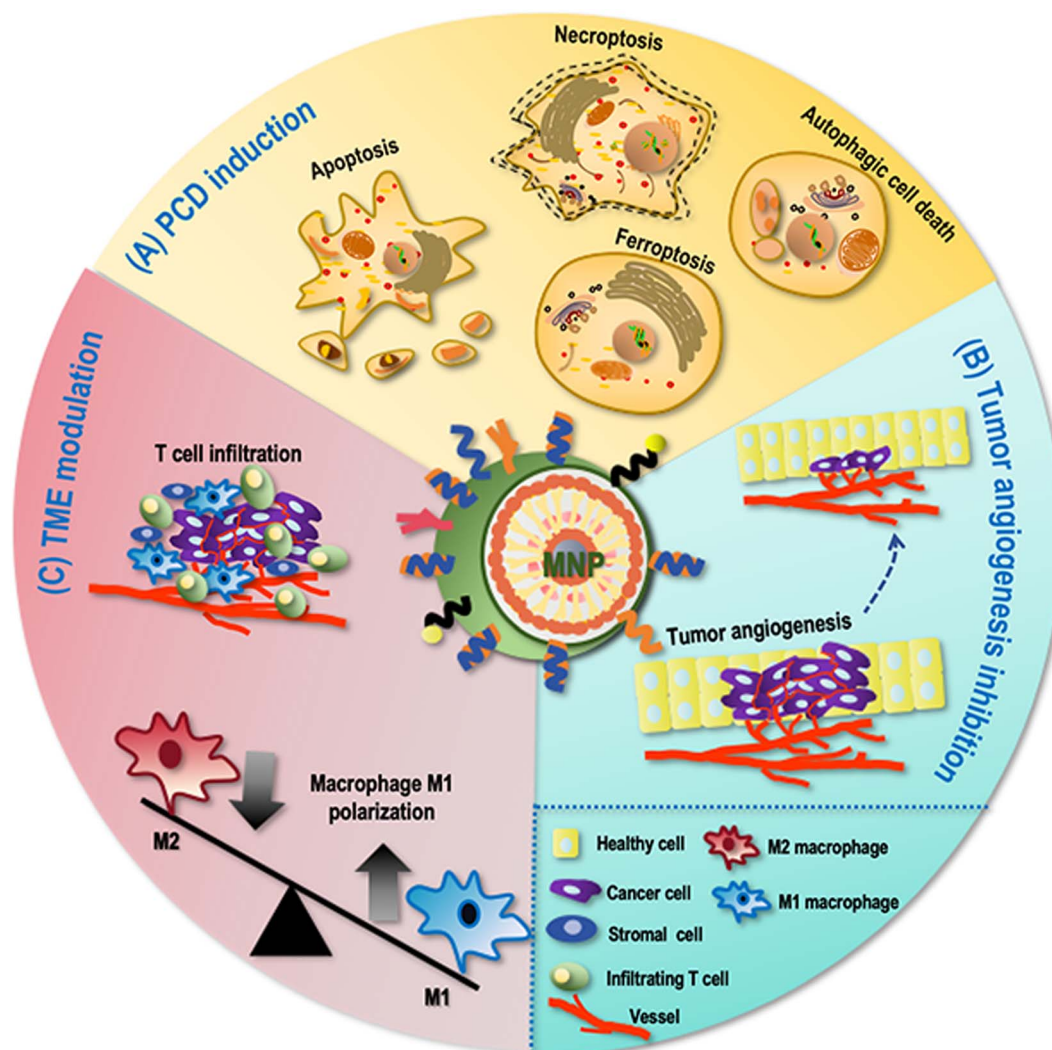


Fig. 2 Onco-therapeutic features of MNPs and possible synergies with CAP. Through inducing magnetic hyperthermia and generating free radicals, MNPs can (A) induce various programmed death of cancer cells such as apoptosis, autophagic cell death and ferroptosis, (B) inhibit cancer cell angiogenesis, (C) and modulate the TME by polarizing macrophage towards the M1 state and enhancing cytotoxic T cell infiltration. CAP can selectively induce various PCDs, reverse the EMT program, and prime macrophages to the M1 state in the TME, and thus may synergize with MNPs due to enhanced ROS generation.



cellular homeostasis by removing damaged and senescent cells, escaping PCD becomes a fundamental hallmark of cancer.⁵⁰

As MNPs can induce magnetic hyperthermia and generate free radicals through, *e.g.*, Fenton reaction, they are able to induce various cell death programs such as cancer cell apoptosis and autophagic cell death (Fig. 2). For example, Sulaiman *et al.* prepared MNPs that induced apoptosis among human breast cancer cells AMJ13 and MCF7 by causing DNA breaks.⁵¹ Using MNPs as drug carriers, Wang *et al.* loaded paclitaxel (PTX) into fluorescent magnetic polyethyleneimine (PEI)-poly(lactic-co-glycolic acid) (PLGA) NPs for simultaneous cell imaging and drug delivery, which triggered autophagic cell death of human glioblastoma U251 cells by initiating autophagosome formation.⁵²

Ferroptosis is iron-dependent and featured by lipid peroxidation.⁵³ As iron is commonly used as the magnetic core of MNPs, ferroptosis is a typical PCD induced by MNPs. For instance, Zhang *et al.* proposed that superparamagnetic iron oxide (SPIO-serum) could induce ferroptosis among ovarian cancer cells by interfering with mitochondrial redox homeostasis; specifically, SPIO-serum led to the accumulation of intracellular irons that triggered oxidative stress within the mitochondria, and decreased expression of glutathione peroxidase 4 (GPX4) that was associated with increased lipid peroxidation.⁵⁴

3.2 Attenuating cancer cell angiogenesis and migration

Tumor angiogenesis is usually produced in tumor cells in response to various stimuli such as hypoxia, ischemia, and acidosis, abnormal regulation of which plays a crucial role in tumor invasion and metastasis, which is another important cancer hallmark.⁵⁰

MNPs can inhibit cancer cell angiogenesis through generating the magnetic hyperthermia effect (Fig. 2). Salimi *et al.* explored the effect of magnetic hyperthermia using fourth-generation polyamidoamine (PAMAM) dendrimer (G4)-functionalized iron oxide NPs (IONPs) in treating breast cancer cells, and found that G4@IONPs were able to reduce tumor angiogenesis *via* magnetic hyperthermia both *in vitro* and *in vivo*.⁵⁵ Mulens-Arias *et al.* investigated the effects of polyethyleneimine-SPIO NPs (PEI-SPIO NPs) on endothelial cells and macrophages, and found that PEI-SPIO NPs reduced the number of blood vessels and promoted macrophage infiltration towards reduced endothelial cell migration and angiogenesis both *in vitro* and *in vivo*.⁵⁶

3.3 Modulating the tumor microenvironment

The tumor mass is composed of not only a heterogeneous group of cancer cells but also the tumor microenvironment (TME). The composition of TME, though varying among tumor types and evolving with tumor progression, typically includes immune cells, stromal cells, infiltrating host cells, blood vessels, and extracellular matrix. The TME can not only determine whether the primary tumor is eradicated, metastasizes or establishes dormant micrometastases, but also shape the therapeutic response and immune sensitivity of tumors.

As an important part of the innate immune system and a professional tumor antigen presenting cell, macrophages are affected by iron oxide NPs which have two functionally distinct states, *i.e.*, the M1 pro-inflammatory and anti-tumorigenic state and the M2 anti-inflammatory and pro-tumorigenic state. Specifically, iron oxide NPs were reported capable of shifting macrophages towards the M1 state due to increased reactive oxygen species (ROS) accumulation.⁵⁷ These M1 macrophages produce and release, *e.g.*, interleukin-1 (IL-1) and vascular endothelial growth factor (VEGF), to support tumor progression by inducing angiogenesis⁵⁸ (Fig. 2). Zanganeh *et al.* showed that ferumoxytol can induce macrophage M1 polarization and a concomitant increase in ROS production, leading to significantly inhibited growth of subcutaneous adenocarcinomas and liver metastases in mice.⁵⁹ Fang *et al.* emphasized the use of magnetic hyperthermia immunotherapy (through magnetic NPs) in promoting cytotoxic T cell infiltration by altering the phenotype of tumor-associated macrophages and activating dendritic cells.⁶⁰

4. MNP-based therapeutics

4.1 Magnetothermal therapy

Magnetothermal therapy refers to a therapy in which tumor killing is achieved by exposing MNPs to an appropriate AMF taking advantages of the thermomagnetic effect.⁶¹ Cancer cells are more sensitive to hyperthermia than their healthy peers due to the acidic TME. The disorganized vascular network leads to reduced blood flow in cancerous tissues that further decreases the convective cooling rate of the tumor, whereas healthy tissues can dissipate additional heat to adjacent tissues through organized blood flow. Due to the superior stability and safety of metal oxides *in vivo*, various metals that have been used in MNP design for thermotherapy that include, *e.g.*, Fe, Ni, Mn, Mg and their oxides. Superparamagnetism is a unique phenomenon that occurs in ferromagnetic NPs, and superparamagnetic NPs do not have any residual magnetization once AMF is removed, making them suitable for biomedical applications. By injecting MNPs directly into the tissue or locating them to the tumor site through magnetic field manipulation and applying AMF to heat these MNPs, magnetic hyperthermia can be achieved for cancer cell killing (Fig. 3). For instance, de la Encarnación *et al.* introduced a hybrid colloidal nanostructure consisting of plasmonic Au nanorods (AuNR) covered by silica shells that was further modified with iron oxide NPs (IONPs); the resulting hybrid nanocomposite, in response to either external magnetic field or near-infrared radiation, demonstrated photothermal effect in treating human glioblastoma cells.⁶² Similarly, Araya *et al.* significantly reduced the tumor volume of mice carrying A549 non-small cell lung cancer cells using carboxydextran-coated synthetic superparamagnetic IONP coupled with AMF exposure for 20 min.⁶³ Pan *et al.* prepared Zn-CoFe₂O₄@Zn-MnFe₂O₄ superparamagnetic NPs (ZCMFs) that showed good and highly controllable magneto-thermal properties due to the exchange-coupled magnetism between the core-shell and the doping of Zn²⁺; using ZCMFs they almost completely inhibited the growth of *in situ* hepatocellular carcinoma cells.¹⁵



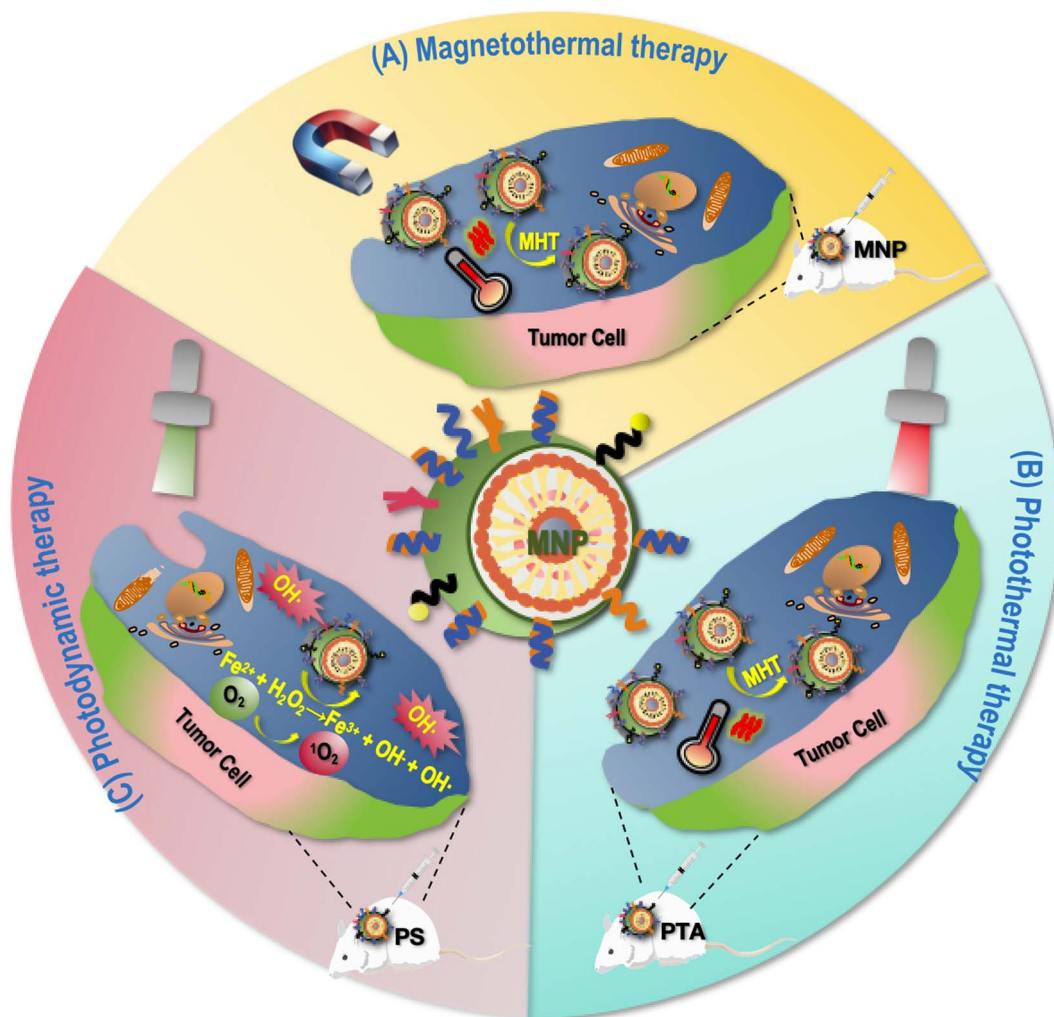


Fig. 3 Onco-therapeutics based on MNPs and possible synergies with CAP. (A) MNPs can be used for magnetothermal therapy where tumor cells are killed by exposing MNPs to an appropriate AMF taking advantages of the thermomagnetic effect. (B) MNPs can be used as PTAs for PTT where MNPs are injected into the body and gathered around tumor cells to convert light energy into heat for cell killing. (C) MNPs can be used as PS for PDT where MNPs are used to absorb and transfer the energy of a specific wavelength of laser to the surrounding oxygen molecules towards the generation of highly cytotoxic ROS for cell killing. CAP can function as a reliable source of light to synergize with MNPs for PTT and PDT, as the spectrum of CAP covers the range of 250–800 nm with a solid peak at 400 nm; and act as a novel source of electromagnetic field for magnetothermal therapy to synergize with MNPs.

4.2 Phototherapy

MNP-mediated phototherapies can be categorized into photothermal therapy (PTT) and photodynamic therapy (PDT). While PTT relies on photo-thermal agents (PTAs) to convert light energy into heat for cell killing that are injected into the body and gathered around tumor cells,¹⁷ PDT takes advantages of photosensitizer (PS) to absorb and transfer the energy of a specific wavelength of laser to the surrounding oxygen molecules towards the generation of highly cytotoxic ROS for cell killing⁶⁴ (Fig. 3).

The PTT effect of Fe_3O_4 magnetic NPs for treating cancers has been demonstrated by many studies. For example, Fe_3O_4 NPs in spherical, hexagonal, and linear shapes were able to be taken up by esophageal cancer cells without significantly affecting the structure and activity of cells and rapidly generate

heat by red and near-infrared laser irradiation (e.g., 808 nm laser irradiation), resulting in significantly damaged cancer cells *in vitro* and arrested tumor growth *in vivo*.¹⁶

As an example of MNP-mediated PDT, Kuo *et al.* prepared methylene blue immobilized copper ferrite NPs (MB-CuFe NPs), where CuFe NPs acted as a Fenton catalyst to convert hydrogen peroxide (H_2O_2) to ROS, and the MB photosensitizer was adsorbed on the surface of this nanocomposite to facilitate the entry of drugs into cells for improved photodynamic therapy under 660 nm laser irradiation and depletion of cervical cancer cells.¹⁷ In addition, the efficacy of PDT can be further enhanced by integrating photosensitizers with metal particles utilizing plasmon resonance effects.^{65,66} For instance, Yao *et al.* established plasmon-enhanced photodynamic therapy for treating gastric cancers *via* integrating photosensitizers with gold nanorods.⁶⁷



5. Issues hindering clinical translation of MNPs

5.1 Low therapeutic efficacy

Although magnetic hyperthermia is non-invasive and the magnetic field makes it possible to target some deep tumors, the limited magneto-thermal efficiency of intravenously injected MNPs and their insufficient accumulation in the tumor site result in a low therapeutic efficiency (Fig. 4). For example, Pan *et al.* fabricated a nanomaterial consisting of zinc-doped cobalt-iron oxides for liver cancer treatment, which exhibited limited magnetothermal efficiency *in vivo* due to the relatively low saturation magnetization strength and specific power of loss as well as insufficient accumulation of intravenously injected

magnetic nanomaterials at the tumor site as a result of mono-nuclear phagocyte system (MPS) clearance.¹⁵ Thus, understanding the working mechanism of magnetic hyperthermia effect against cancers is the key to resolve such a problem.

Eukaryotic cells have evolved complex systems to regulate ROS production and cellular responses that control different aspects of the behavior of a cell, the dysregulation of which is a shared feature of cancer cells.⁶⁸ The basic mechanism of action for MNPs to take on their anti-cancer activities lies in ROS generation. For instance, Maqusood *et al.* prepared copper ferrite NPs and used them to treat MCF7 breast cancer cells, and found that they reduced the activity of cancer cells through ROS production and GSH depletion.³² Hang *et al.* isolated CD44⁺CD133⁺ human ovarian cancer stem cells (HuOCSCs)

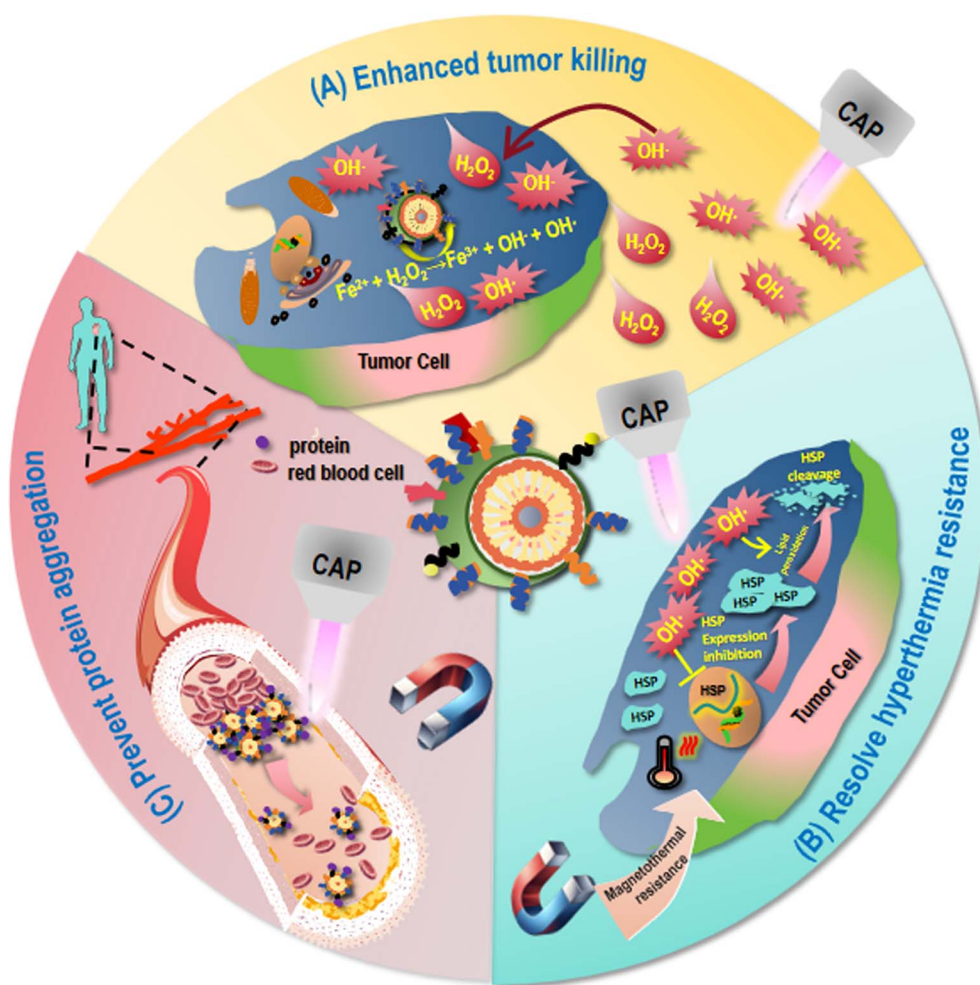


Fig. 4 Issues remained in MNP-based onco-therapeutics and possible solutions brought by CAP. (A) MNPs have relatively low therapeutic efficacy due to the limited magneto-thermal efficiency after intravenous injection and insufficient accumulation in the tumor site. CAP can synergize with MNPs for continuous and enhanced generation of OH[•], and increase H₂O₂ levels within cancer cells that can be further converted into OH[•] through the Fenton reaction with the presence of iron oxide NPs for enhanced tumor-killing. (B) MNPs may lead to hyperthermia resistance as a result of induced synthesis and accumulation of HSPs in tumor cells. CAP may help resolve hyperthermia resistance by reducing HSP expression given its short-lived component OH[•] and cleaving HSPs by causing lipid peroxidation as a result of ROS accumulation. (C) MNPs may not be degradable due to chemical inertness that may block microcirculation and cause cell dysfunction or death. The excretion of MNPs from the body depends on the size, shape, surface properties of these particles as well as their absorption state in the organism. CAP may mitigate the biosafety issue of MNPs by preventing the formation of clusters of proteins adsorbed on the surface of MNPs for reduced aggregation and secretion blockage.



from tumors of clear cell ovarian cancer patients, and reported that SPIO NPs inhibited the proliferation, autophagy, invasion, drug resistance, and tumorigenicity of ovarian cancer cells, as well as activated ferroptosis *via* inducing ROS accumulation and oxidative stress in HuOCSCs.⁶⁹ Thus, therapeutic strategies relying on redox modulation may create synergies with MNPs for enhanced anti-cancer efficacies that hold a great promise in cancer treatment.

5.2 Hyperthermia resistance

Though MNPs have achieved multiple medical miracles, mild hyperthermia treatment by MNPs may lead to the development of therapeutic resistance as a result of induced synthesis and accumulation of heat shock proteins (HSPs) in tumor cells^{35,36} (Fig. 4). For instance, HSP70 is activated in response to temperature rise, leading to thermotherapeutic resistance as a result of reduced protein denaturation and enhanced cell survival.^{70–72} Thus, therapeutic modalities possibly rewiring such a treatment resistance through, *e.g.*, targeting HSPs, shed light on the way of translating MNP-based therapies into clinics.

5.3 Biosafety issue

Another critical issue hindering the translation of MNPs into clinical practice and may be extended to the majority of NPs is the safety issue.⁷³ Degradable NPs can be phagocytosed by cells for degradation into small molecules or dissolved in body fluids on their own after metabolism and excretion. Non-degradable NPs are difficult to be degraded due to chemical inertness and, thus, may block microcirculation and cause cell dysfunction or death.⁷⁴ As most MNPs suffer from low specific absorption rate (SAR) that requires high levels of internal MNP concentrations for desirable magnetic heat efficacy, the toxicity issue is even more severe for MNPs (Fig. 4). For instance, NanoTherm®, an aminosilane-coated Fe₃O₄ MNP approved by the European Union, although has demonstrated satisfactory clinical trial results in treating glioblastoma and prostate cancers, required a very high Fe concentration (112 mg mL⁻¹)³² due to its low magneto-thermal conversion efficiency, *i.e.*, 738–985 pH·m² kg⁻¹. The toxicity of NPs is affected by a variety of factors such as size, shape, and surface features.⁷⁵ The larger the surface area of the NP is and more chemical interactions are involved, the more difficult it is to control the toxicity, as the surface area of a NP is positively proportional to the amount of ions it releases that may induce inflammatory responses.⁷⁶ The aggregation state of NPs in organisms plays a dual role in determining their toxicity. That is, aggregation, on one hand, may decrease the toxicity of NPs due to reduced surface area and, on the other hand, increase the local toxicity by accumulating aggregated NPs in specific tissues such as liver and spleen.⁷⁷ Surface coating or functionalization can be used to reduce the toxicity of NPs. For example, SPIO surface-modified with PEG exhibited improved intracellular distribution, metabolism, biocompatibility, and reduced cytotoxicity.⁷⁸ However, surface modification cannot fundamentally resolve the toxicity issue. Understanding the excretion mechanisms of MNPs *in vivo*, and exploring innovative strategies capable of

improving the biosafety of MNPs through, *e.g.*, synergistic use may be a possible recipe.

The excretion of MNPs from the body depends on the size, shape, surface properties of these particles as well as their absorption state in the organism. In general, MNPs smaller than 5.5 nm can be excreted through the kidneys, and MNPs larger in size may be metabolized by the liver and excreted by the bile.⁷⁹ Certain surface modifications can alter the circulation time of NPs in the bloodstream and affect their biochemical stability *in vivo*, thus affecting their excretion. For example, by absorbing a small amount of proteins on the surface, Au-S strong ligand-bound PEG-modified Au NPs (PEG-GNPs, 6 nm) showed a good dispersion stability under the physiological microenvironment that was conducive to their entry into the liver parenchymal cells for excretion through the bile ducts, and their pass through the filtration barrier of the glomerulus for excretion *via* the urine. However, Au-N weakly ligand-bound polyethyleneimine-modified Au NPs (PEI-GNPs, 6 nm) and electrostatically bound CS-modified Au NPs (CS-GNPs, 6 nm) were unfavorable for liver clearance due to the large amount of serum proteins absorbed on the surface that led to the formation of large particle clusters identified and ingested by hepatic macrophages and hepatic sinusoidal endothelial cells.⁸⁰ In addition, tissue and organ structural characteristics, as well as physiological factors are also crucial factors influencing the clearance profiles of nanomaterials in the liver and kidney that are beyond the scope of discussion here. Therefore, therapeutic strategies blocking the formation of large NP clusters may help enhance the secretion and safety of MNPs for clinical use.

6. CAP: a possible synergizer of MNPs for cancer treatment

CAP, produced by exciting a gas into a plasma state (the fourth state of matter) by applying an electric field under the atmospheric pressure and close to room temperature, is comprised of ROS and reactive nitrogen species (RNS).⁸¹ These reactive species can be grouped into short-lived such as hydroxyl radical (OH[•]), singlet oxygen (O), superoxide (O²⁻), nitric oxide (NO[•]), and long-lived species such as H₂O₂, ozone (O₃), anion (OONO⁻) and protonated form of peroxyxynitrite (ONOOH). While the activity of long-lived species can be relatively easily preserved that can enter cells *via* penetrating through membrane channels such as aquaporins, short-lived species can quench within nanoseconds (*e.g.*, half-life of [•]OH is 10⁻⁹ s *in vitro* and *in vivo*)⁸² and function through generating secondary short-lived species during interactions with cell membrane or other CAP components.^{83–86}

CAP has been widely used in a variety of medical fields including, *e.g.*, sterilization,⁸⁷ wound healing,^{88–90} coagulation,^{88,91} and cancer therapy.^{92–95} Ever since the first report on the oncological efficacy of CAP, its therapeutic effects against cancer cells have been demonstrated in various types of cancers, such as triple-negative breast cancer,⁹² pancreatic cancer⁹³ and lung cancer,^{94,96} and melanoma.⁹⁵ The first FDA-approved clinical trial evaluating the efficacy and safety of CAP as an anti-



cancer approach has been initiated in 2019 and completed in 2021, where 17 out of 20 late-stage solid cancer patients remained alive, supporting the use of CAP combined with surgery as a safe approach with selectivity against cancer cells and exceptional control on local regional recurrence.⁹⁷

Yet, the clinical translation of CAP is primarily hindered by several of its unresolved limitations.⁹⁸ First, given the limited penetration in-depth of CAP, it cannot reach deep tumors and has largely been used for treating surficial cancers such as skin malignancies. Second, the short life span of short-lived species and their leading roles in arresting cancer cells⁹² make the current use of CAP for cancer treatment primarily limited to be combined with surgery. These make it necessary to involve other techniques towards extended scenarios feasible for receiving CAP treatment.

One possible synergy may be actualized *via* combining CAP with MNPs given their shared mechanism of action and diversified application forms of MNPs for cancer treatment. The contribution of CAP brought to MNPs for improved treatment efficacy and mitigated side effects can be summarized into 'enhanced ROS generation', 'reduced hyperthermia resistance', and 'increased particle secretion'.

6.1 Enhanced ROS generation

CAP has been shown capable of selectively killing cancer cells without harming their healthy peers by causing the oxidative stress of transformed cells due to their more easily disrupted intracellular redox homeostasis. This may be attributed to the higher expression of aquaporins on cancer cells that promote the transport of long-lived free radicals into cells, and the lower cholesterol levels of cancer cell membrane that make them more susceptible to oxidative stress induced damage. When the intracellular oxidative stress of cancer cells exceeds the capacity of the antioxidant defense system, cell death will be triggered through several signaling cascades such as the NRF2 and the NFκB pathways.

CAP can synergize with MNPs for continuous and enhanced generation of the primary short-lived species OH[•] (Fig. 4). Although tumor cells produce more H₂O₂ than normal cells, these substances are not sufficient to cause effective cancer cell death. CAP can increase H₂O₂ levels within cancer cells that can be further converted into OH[•] through the Fenton reaction with the presence of iron oxide NPs for enhanced tumor-killing. For instance, CAP and MNPs showed synergistic therapeutic effects on A549 lung cancer cells by significantly inhibiting cell growth *via* suppressing ERK and AKT signaling, and arresting cancer cell migration *via* reversing the epithelial-mesenchymal transition (EMT) program.⁹⁹ Besides, CAP has also been reported capable of regulating macrophage polarization,¹⁰⁰ further supporting its possible synergy with MNPs for cancer control (Fig. 2).

6.2 Reduced hyperthermia resistance

It has been found that ROS such as OH[•] can mitigate thermal resistance under hyperthermia by impairing heat shock protein (HSP) expression,^{101,102} and lipid peroxidation as a result of ROS accumulation can cleave HSPs, thereby attenuating high-temperature-induced heat resistance.¹⁰³ Given that CAP is

enriched with ROS and can effectively induce lipid peroxidation,⁵³ it may help reduce thermal resistance of MNPs induced under mild magnetic hyperthermia (Fig. 4).

6.3 Increased particle secretion

CAP generated using helium as the carrying inert gas could induce protein self-assembly with intact secondary structural features, as evidenced by fluorescence lifetime measurement and SEM analysis.¹⁰⁴ These results not only give an outlook for using CAP in achieving protein self-assembly without denaturing the proteins, but also shed light on the possibility of preventing the formation of clusters of proteins adsorbed on the surface of MNPs, mitigating their likelihood of gaining aggregation and secretion blockage (Fig. 4).

6.4 Novel light and electromagnetic field source

CAP may also function as a reliable source of light and electromagnetic field to synergize with MNPs for photo- and magnetothermal therapies. As the spectrum of CAP covers the range of 250–800 nm with a solid peak at 400 nm, it can be used as a novel light source in PDT. For example, protoporphyrin IX (a photosensitizer) encapsulated in polymers was released in response to CAP exposure and resulted in PDT against melanoma cells.¹⁰⁵ Besides photons, electrons, ions and neutral radicals, CAP also includes electromagnetic fields,^{106,107} the possibility of which in synergizing with MNPs for magnetothermal therapy deserves intensive investigations (Fig. 2).

7. Conclusions

Given the roles of MNPs on inducing cancer cell death especially ferroptosis, attenuating cancer cell migration and rewiring the TME, various promising MNP-based therapeutics have been proposed for cancer treatment in the form of, *e.g.*, magnetothermal therapy and phototherapy. However, their limited therapeutic efficacy, hyperthermia treatment resistance and biosafety issue have remained unresolved that largely limited the clinical translation of MNPs for cancer treatment. CAP, an emerging onco-therapeutic modality relying on redox modulation, can possibly synergize with MNPs for enhanced and prolonged ROS generation within cancer cells due to Fenton reactions, and may reduce hyperthermia resistance of MNPs *via* suppressing HSPs, increase MNP secretion *via* reducing their likelihood of aggregation, and act as novel light and magnetic field source of MNPs for synergized photo- or magnetothermal therapies. Though promising, relatively little has been devoted to this field, and long-term effect and safety of synergized CAP and MNP treatment regimen require extensive examinations. This paper aims at unveiling these possibilities that deserve underscoring and may guide the future direction.

Abbreviation

MNPs	Magnetic nanoparticles
CAP	Cold atmospheric plasma



AMF	Alternating magnetic field
TME	Tumor microenvironment
PCD	Programmed cell death
EMT	Epithelial–mesenchymal transition
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
OH	Hydroxyl radical
NO	Nitric oxide
H ₂ O ₂	Hydrogen peroxide
O ₃	Ozone
MPS	Mononuclear phagocyte system
HSPs	Heat shock proteins
PTT	Photothermal therapy
PDT	Photodynamic therapy
PTAs	Photo-thermal agents
PS	Photosensitizer
SPIO	Superparamagnetic iron oxide
IONPs	Iron oxide nanoparticles
PEI	Polyethyleneimine
PLGA	Poly(lactic-co-glycolic acid)
SAR	Specific absorption rate
SQUID	Superconducting quantum interference device
VSM	Vibrating dample magnetometer
NMR	Nuclear magnetic resonance
FTIR	Fourier transform infrared
RS	Raman spectroscopy
XPS	X-ray photoelectron spectroscopy
DLS	Dynamic light scattering
SAXS	Small angle X-ray scattering
SANS	Small angle neutron scattering
PAMAM	Polyamidoamine
G4	Fourth-generation
NRF2	Nuclear factor erythroid 2-related factor 2
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells

Data availability

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

Author contributions

XD conceptualized the insights, and prepared the initial draft. YZ provided the financial support.

Conflicts of interest

All authors have read and agreed the content of the manuscript as well as its submission.

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