

Chapter 1

Iron Oxide Magnetic Nanoparticles in Photodynamic Therapy: A Promising Approach Against Tumor Cells

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Abstract Iron oxide magnetic nanoparticles have been extensively employed in biomedical applications due to their biocompatibility, small size, ability to surface functionalization, superparamagnetism behavior, and targeting properties. Upon the application of external magnetic field, iron oxide nanoparticles can be guided to the target site of application, minimizing possible side effects to nontarget tissues. Recently, several papers describe the combination of iron oxide magnetic nanoparticles with photosensitizer (PS) molecules for photodynamic therapy (PDT). PDT is a clinical treatment based on the administration of a photosensitizer to the tumor site, which under the irradiation with visible-near-infrared light generates reactive oxygen species (ROS) able to cause deleterious effects to the treated tumor site. The main disadvantage of PDT is the lack of selectivity; therefore, the combination of magnetic iron oxide nanoparticles with photosensitizer is a new and promising approach in PDT. In this direction, this chapter discusses the recent advantages in the design and applications of magnetic iron oxide nanoparticles in conjugation with photosensitizer in PDT to combat cancer.

Keywords Iron oxide nanoparticles • Photodynamic therapy • Cytotoxicity • Magnetic nanoparticles • Singlet oxygen • Fe₃O₄ nanoparticles

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Nomenclature

$^1\text{O}_2$	Singlet oxygen
$^3\text{O}_2$	Triplet oxygen
AHP	Hyaluronic acid
AIPcS4	Tetrasulfonic phthalocyanine aluminum
Ce6	Chlorin e6
CSQ	Chitosan quaternary ammonium
DOX	Doxorubicin
Eca-109	Esophageal squamous carcinoma
EPR	Enhanced permeability and retention
ESIONs	Extremely small iron oxide nanoparticles
FDA	Food and Drug Administration
Fe_3O_4	Magnetite
HeLa	Human cervical cancer cell line
HMNSs	Hollow magnetic nanospheres
HP	Hematoporphyrin
IC_{50}	Maximal inhibitory concentration
IONCs	Iron oxide nanoclusters
IR820	Indocyanine green
Jurkat	Human T-cell leukemia cells
LED	Light-emitting diode
MCF-7	Human breast cancer cells
MDA-MB-231	Breast cancer
MLs	Magnetoliposomes
MNCs	Magnetic nanoclusters
mSiO_2	Mesoporous silica
NIH3T3	Mouse embryonic fibroblast
NRI	Near infrared
OA	Oleic acid
PC-3	Human prostate cancer
PDT	Photodynamic therapy
PEG	Polyethylene glycol
pheoA	Pheophorbide A
PHPP	2,7,12,18-Tetramethyl-3,8-di(1-propoxyethyl)-12,17-bis-(3-hydroxypropyl) porphyrin
PMNs	Magnetic nanogrenades
PpIX	Protoporphyrin IX
PS	Photosensitizer
RGD	Integrin-binding cell adhesive peptide
rGO	Reduced graphene oxide
ROS	Reactive oxygen species
S-180	Sarcoma cells
SK-OV-3	Ovarian cancer cells
SPIO	Superparamagnetic iron oxide

1.1 Introduction

Photodynamic therapy (PDT) is a clinical treatment that involves the administration of a photosensitizer (e.g., photofrin) in the tumor site (Bechet et al. 2008; Banerjee et al. 2017). Once at the tumor tissue, appropriated light irradiation (visible or near-infrared wavelengths) yields excited photosensitizer. Upon light activation, excited triplet photosensitizer in the presence of molecular oxygen (naturally available triplet-oxygen ($^3\text{O}_2$)) produces highly reactive singlet oxygen ($^1\text{O}_2$), a well-known mediator of cell death (Allison et al. 2008; Chatterjee et al. 2008; Bhattacharyya et al. 2011; Smith et al. 2015). The efficiency of PDT depends on singlet oxygen formation, which is highly reactive and able to readily diffuse through the biological membranes (Bechet et al. 2008). Besides the generation of singlet oxygen, various free radicals are generated. All those reactive species might induce cell death via apoptosis, necrosis, and cell membrane damages with release of contents (Allison et al. 2008; Kim et al. 2016a; Lee et al. 2017). Indeed, singlet oxygen reacts with nucleic acids, lipids, amino acids, and proteins in the tumor tissue preventing angiogenesis in solid tumors (Smith et al. 2015). The generated oxygen-based cytotoxicity is expected to occur upon light irradiation, since in dark conditions, the photosensitizers are harmless. Therefore, in PDT light is used as a tool to trigger and modulate the selective destruction of malignant tissues/organs (Bechet et al. 2008). It should be noted that in PDT, the wavelength of light irradiation is a very important parameter. Irradiation in the near-infrared (NIR) region (700–1,000 nm) is the most appropriated wavelength to be employed. Hemoglobin is the major protein in the blood, which absorbs light up to 600 nm; therefore, tissues/organs must be irradiated at higher wavelengths to allow a suitable light penetration (Bechet et al. 2008; Huang et al. 2012; Chen et al. 2015a).

The clinical uses of PDT started in 1933, although it has been studied for over a century (Smith et al. 2015). Nowadays, there are several photosensitizers approved by the Food and Drug Administration (FDA) (Smith et al. 2015). The major clinical use of PDT is in cancer treatment (Bhattacharyya et al. 2011), although noncancerous applications have also been applied. Against cancer, PDT has been employed in the treatment of superficial tumors, such as melanoma, and tumors in the esophagus and bladder (Bechet et al. 2008). The noncancerous applications of PDT are mainly in the skin to treat diseases such as psoriasis and actinic keratosis, in the eyes in particular to treat macular degeneration, as well as at localized sites of inflammations including rheumatoid arthritis and dental infections (periodontitis) (Chatterjee et al. 2008). For example, a methylene blue (photosensitizer)-containing gel is applied to the infected gum pocket, and upon light irradiation via an optical fiber, singlet oxygen is locally generated in the infected pocket leading to cytotoxic effects (Chatterjee et al. 2008).

The advantages of PDT over traditional therapies are (i) relatively high cure rates, (ii) cost-effectiveness, (iii) localized treatment, (iv) no cumulative toxicity upon repetition of the therapy, and (v) induction of immunity (Chatterjee et al. 2008). In contrast, the disadvantages of PDT are the long in vivo half-life of the photosensitizers, consequently, their nonspecific distribution in vivo, and the limitation to reach deeper tumors in the body of the patient (Smith et al. 2015).

1.2 Why Use Superparamagnetic Iron Oxide Nanoparticles in PDT?

Although there are promising clinical uses of PDT, some drawbacks need to be overcome. For instance, the selective targeting and accumulation of the photosensitizers in the disease organ/tissue are necessary to minimize toxicity to normal cells (Bechet et al. 2008). Moreover, photosensitizers have poor water solubility and tend to aggregate in the biological medium, decreasing their photo-efficiency (Paszko et al. 2011).

The combination of photosensitizer and nanomaterials may overcome these issues. In this context, the combination of nanoparticles as carrier of photosensitizers is a promising approach in PDT (Bechet et al. 2008). The advantages of nanoparticles as nanocarriers of photosensitizers in PDT are:

- (i) Ability to carry and to deliver therapeutic amounts of the photosensitizers in deep-seated tumors
- (ii) Due to the high surface area/volume ratio, the nanoparticle surface can be used as multifunctional vehicle upon its functionalization with other therapeutic molecules, such as chemotherapeutic agents and/or tumor-seeking molecules
- (iii) Enhanced solubility of hydrophobic photosensitizers with proper size to accumulate in the tumor tissues via enhanced permeability and retention effect (EPR effect) (Wang et al. 2004; Paszko et al. 2011; Gangopadhyay et al. 2015)

One of the major advantages of using nanoparticles in PDT is the EPR effect, which is the passive accumulation of nanoparticles in tumor sites (Kim et al. 2016a). This effect can be explained by considering the altered anatomy of tumor tissues characterized by an excessive angiogenesis and leaky blood vessels with impaired lymphatic drainage system. Therefore, nanoparticles can extravasate from blood pool and spontaneously accumulate into tumor sites. Once accumulated, the nanoparticles are retained in tumor tissues due to the compromised lymphatic drainage (Kelkar and Reineke 2011; Kim et al. 2016a, b). Interestingly, therapeutic amounts of photosensitizer-containing nanoparticles are accumulated in solid tumors due to EPR effect (Mbakidi et al. 2013), making nanoparticles promising vehicles for singlet oxygen generators in PDT.

Recently, iron oxide magnetic nanoparticles have been successfully employed as drug carriers for photosensitizers and also for chemotherapeutic agents. Magnetic nanoparticles such as magnetite (Fe_3O_4) have interesting properties that make them suitable nanocarriers in several biomedical applications. These properties can be summarized as: ability to reach the desired site of treatment upon application of an external magnetic field (magnetic targeting), high surface area, small size, biocompatibility, chemical stability, relatively low-cost production, and several different synthetic routes including chemical, physical, and biogenic synthesis (Seabra et al. 2013, 2014; Seabra and Duran 2015, Cheng et al. 2016). Therefore, iron oxide magnetic nanoparticles have been widely employed in several biomedical applications of targeted drug delivery system, magnetic resonance imaging, biosensing, immunoassay, and magnetic hyperthermia (Cheng et al. 2016). Magnetic hyperthermia

can be allied to PDT to significantly enhance the cytotoxicity toward cancer tissues. Upon the appropriate targeting of superparamagnetic iron oxide nanoparticles using a magnetic targeting, an alternative magnetic field is applied for a certain period of time, leading to a local increase in the tissue temperature (42–45 °C), which targets cancer cell death by apoptosis (Kandasamy and Maity 2015). Therefore, hyperthermia therapy can be employed to enhance the efficacy of various cancer treatments.

Magnetite is the mostly used for synthesis of iron oxide magnetic nanoparticles due to its superparamagnetism, which allows rapid nanoparticle magnetization upon the influence of a magnetic field; however, once this field is turned off, there is no permanent magnetization. In particular, superparamagnetic iron oxide nanoparticles have been employed in cancer therapy and diagnosis by magnetic targeting (Kandasamy and Maity 2015).

In this scenario, the use of magnetic iron oxide nanoparticles with PDT is based on the combination of a chromophore that is a photosensitizer and a phototrigger, and the ability to target the photosensitizer to the desired site of application, with minimum side effects to healthy cells/tissues (Kandasamy and Maity 2015). In this direction, the next section presents and discusses the recent advantages on the design and uses of superparamagnetic iron oxide nanoparticles as nanocarrier of photosensitizers in PDT.

1.3 Iron Oxide Magnetic Nanoparticles in PDT Applications

Recently, the photosensitizer protoporphyrin IX (PpIX), a metal-free porphyrin, was loaded in magnetoliposomes (MLs), which are comprised by liposomes and Fe₃O₄ magnetic nanoparticles (Basoglu et al. 2016). PpIX-containing MLs have a hydrodynamic size of 221 nm and displayed phototoxicity against human breast cancer cells (MCF-7). Cell irradiation was performed by using a LED (light-emitting diode) downlight white light (visible light irradiation in the range of 400–700 nm, at 20 W). MCF-7 viability was reduced to 65% upon cell incubation with 350 nM of PpIX-containing MLs under dark condition; however, upon 5 min of light irradiation, a significant cell death was observed at nanoparticle concentration of 250 nM. This result demonstrated the effective phototoxicity of PpIX-loaded magnetoliposomes (MLs) at nanomolar concentrations (Basoglu et al. 2016).

As stated before, near infrared (NIR) is a suitable wavelength range for PDT applications. In this direction, gold nanopopcorns containing self-assembled iron oxide cluster were synthesized for magnetic field targeting in PDT applications (Bhana et al. 2015). The nanoparticle surface was coated with the photosensitizer silicon 2,3-naphthalocyanine dihydroxide, followed by stabilization with polyethylene glycol (PEG) linked with 11-mercaptoundecanoic acid. The hybrid nanomaterial demonstrated superparamagnetic behavior and 61% of photothermal conversion efficiency under NIR irradiation. Upon the application of a gradient magnetic field, the hybrid nanomaterial demonstrated superior release of the photosensitizer and cellular uptake. The nanomaterial showed enhanced ability to carry

therapeutic molecules deep into tumor tissue through magnetic manipulation and combat tumors locally by PDT (NIR light irradiation of 0.55 W/cm^2) and by photothermal effect (Bhana et al. 2015). NIR and photothermal effect are expected to efficiently eradicate tumors, due to the delivery of high and therapeutic concentrations of photosensitizer-containing magnetic nanocarriers in deep tumors via applying a magnetic field on tumor surface.

The design of hybrid nanocarriers in PDT is a promising strategy. In this context, multifunctional silica-based magnetic nanoparticles were prepared (Chen et al. 2009). The nanoparticles are composed by Fe_3O_4 magnetic nanoparticles coated with 2,7,12,18-tetramethyl-3,8-di(1-propoxyethyl)-12,17-bis-(3-hydroxypropyl) porphyrin, shorted as PHPP, employed as photosensitizer (encapsulation efficiency of PHPP, 20.8%). Magnetic Fe_3O_4 nanoparticles and PHPP were incorporated into silica nanoparticles by sol-gel and microemulsion techniques; the final nanoparticles have a spherical shape and a diameter of 20–30 nm. The magnetic nanoparticles demonstrated good compatibility in dark conditions and ability to generate toxic singlet oxygen upon irradiation with $\lambda = 488 \text{ nm}$ (argon-ion laser) with energy density of 4.35 J/cm^2 during 10 min toward SW480 carcinoma cells (Chen et al. 2009). Therefore, the nanoparticles displayed remarkable antitumor effects under irradiation.

A hybrid nanomaterial composed of reduced graphene oxide (rGO), magnetic iron oxide, and gold nanoparticles was designed. rGO- Fe_2O_3 @Au core-shell nanoparticle-graphene nanocomposite was prepared for magnetic targeting in cancer treatment via PDT and photothermal synergist effect and bioimaging agent (Chen et al. 2015b). The presence of Fe_2O_3 is responsible for magnetic field-assisted singlet oxygen generation and chemophotothermal effect direct to the cancer tissue. The hybrid nanomaterial was found to be superparamagnetic at room temperature, demonstrated high capacity to load the chemotherapeutic doxorubicin (DOX, 1.0 mg mg^{-1}), and had considerable photothermal conversion efficiency under 808 nm (NIR) laser irradiation (2 W cm^{-2}). The authors demonstrated that NIR irradiation triggers doxorubicin (DOX) release with an initial burst of the release of the therapeutic molecule. The *in vitro* cytotoxicity of DOX-rGO- Fe_2O_3 @Au NPs (DOX-loaded hybrid nanomaterial) was evaluated against human cervical cancer cell line (HeLa) upon 5 min irradiation with NIR laser. Cancer cells significantly uptake DOX-containing nanoparticles within 4 h of incubation. Moreover, magnetic field-guided chemophotothermal synergistic therapy might find important applications in cancer treatment (Chen et al. 2015b).

In a similar approach, magnetofluorescence nanoparticles composed of Fe_3O_4 @ SiO_2 @APTES@glutaryl-PPa (MF NPs) were synthesized (Cheng et al. 2016), where APTES is (3-aminopropyl)triethoxysilane. The obtained nanoparticles have a mean diameter of 50 nm and can be used for PDT and fluorescence imaging in medical applications. The surface of core-shell Fe_3O_4 @ SiO_2 @APTES NPs was functionalized with the photosensitizer chlorine pyropheophorbide-a (PPa) for PDT application. The cytotoxicity of the nanoparticles was evaluated toward HeLa cells, and the results indicated that the nanoparticles were able to quickly permeate the tumor cells due to their lipo-hydro partition coefficient, leading to cell apoptosis and death. Indeed, due to their small size, the nanoparticles were absorbed on the cancer cell membrane, followed by cell internalization via endocytosis vesicle formation

by deformation of cell membrane, which led to the dispersion of the nanoparticles into the cytoplasm of HeLa cells. In addition, the nanoparticles demonstrated light-dependent cytotoxicity to the cancer cells (irradiation with visible light for 10 min at dosage of 25 Jcm^{-2}). As expected, reactive oxygen species (ROS) generated in HeLa cells (Cheng et al. 2016). Hence, these results highlight the promising therapeutic uses of these nanoparticles in PDT treatment with good magnetic targeting to site-specific toxicity.

In an interesting paper, iron oxide magnetic nanoparticles (Fe_3O_4) were synthesized and coated with carboxyl-terminated poly(ethylene glycol)-phospholipid to increase nanoparticle dispersion in water (Chu et al. 2013). The phototoxicity of the nanoparticles was evaluated toward esophageal cancer cells upon irradiation with NIR ($\lambda = 808 \text{ nm}$, power density of 0.25 W/cm^2 for 20 min) light exposition. Figure 1.1 shows the schematic representation of the light-triggered toxicity of Fe_3O_4 -based nanoparticles toward cancer cell. The application of magnetic nanoparticle and NIR laser irradiation is responsible to decrease esophageal cancer cell viability and to inhibit mouse esophageal tumor growth, leading to tumor reduction (Chu et al. 2013).

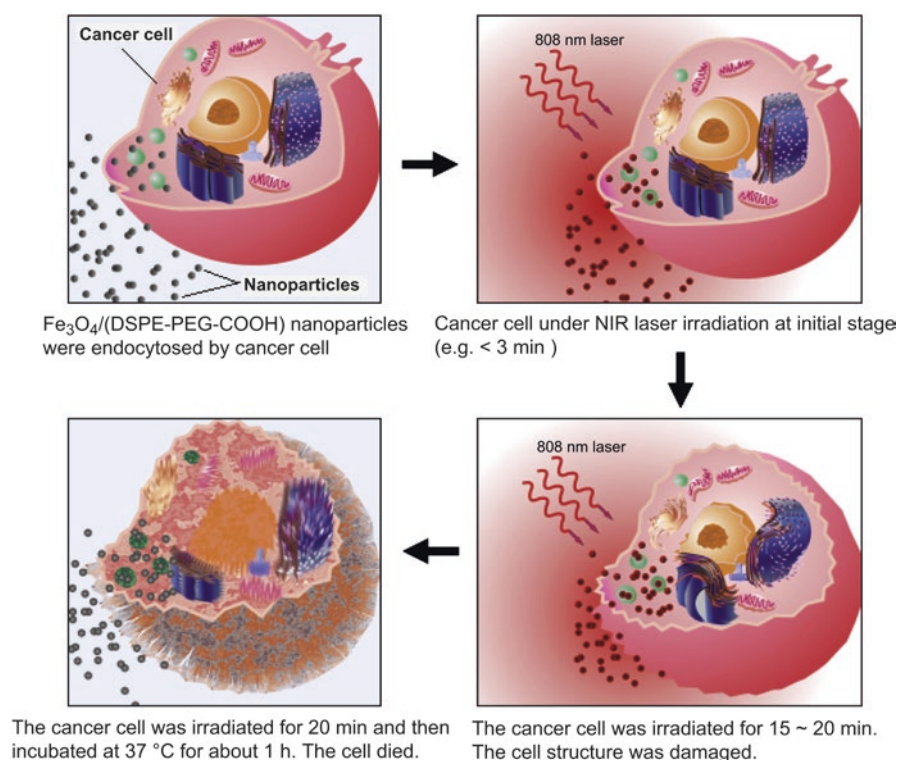


Fig. 1.1 Schematic representation of cancer cell death by treating with Fe_3O_4 -based nanoparticles upon NIR laser light irradiation (Reproduced from reference Chu et al. 2013 with permission from Elsevier)

In a promising strategy, magnetic photosensitive liposomes were synthesized by loading aqueous core iron oxide nanoparticles with lipid bilayer containing therapeutic amounts of photosensitizer payload (Corato et al. 2015). Under laser excitation, the obtained nanoparticles were able to generate toxic singlet oxygen and heat upon alternating magnetic field stimulation, for hyperthermia application. Figure 1.2 shows the schematic representation of the biomedical application of magnetic photosensitive liposome nanoparticles (Corato et al. 2015).

In a further example, magnetic nanoclusters (MNCs) are composed of Fe_3O_4 nanoparticles and poly(acrylic acid-co-propargyl acrylate) on the surface for multi-modal imaging probes (Daniele et al. 2013). The NRI photosensitizer azide-modified indocyanine green was inserted on the surface of the MNCs. In biological medium, the photosensitizer complexed with bovine serum albumin leading to an extended coating of serum on the MNCs, which induce turn-on of NRI emission for PDT applications (Daniele et al. 2013). Recently, $\text{SPIO@SiO}_2\text{-Re@PEG}$ magnetic nanoparticles for optical probes and PDT sensitizers were obtained and characterized (Galli et al. 2016). Firstly, superparamagnetic iron oxide (SPIO) nanoparticles with an average size distribution of 10 nm were synthesized by thermal decomposition of iron oleate, followed by nanoparticle coating with silica shell by reverse microemulsion technique. Upon pyridine ligand via triethoxysilane moiety, the surface

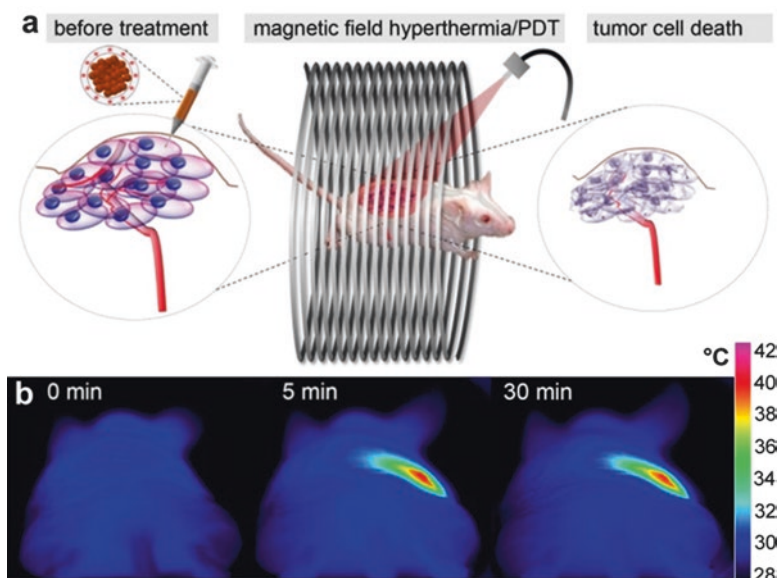


Fig. 1.2 Schematic representation of the treatment of magnetic photosensitive liposome nanoparticles on tumor-bearing mice: (a) therapeutic strategy sketch, liposomes were injected intratumorally, and mice were subsequently subjected to combined treatment with magnetic hyperthermia and laser irradiation. (b) Increased local temperature during magnetic hyperthermia treatment was monitored with an infrared thermocamera. The maximum temperature was reached about 5 min after field application and maintained for the entire treatment cycle (30 min) (Modified from reference Corato et al. 2015 with permission of American Chemical Society)

of the nanoparticles was functionalized with luminescent $[\text{Re}(\text{phen})(\text{CO})_3(\text{py})]\text{CF}_3\text{SO}_3$ complexes anchored to the silica layer. To increase the stability and dispersion of the nanoparticles in physiological conditions, the nanoparticle surface was further covered with PEG layer leading to the formation of SPIO@SiO₂-Re@PEG nanoparticles. PEG also increases the nanoparticle circulation in vivo by avoiding nanoparticle uptake and clearance by macrophage. The final nanocomposite had an average size of 40 nm and demonstrated satisfactory efficiency to generate singlet oxygen. The toxicity of the nanoparticles was evaluated toward human lung adenocarcinoma A549 cells. The confocal microscopy demonstrated that after 4 h of cell incubation with the nanoparticles, the latter were efficiently internalized and accumulated in the perinuclear region of the cancer cells. Moreover, increased cytotoxicity was revealed upon irradiation with visible light (by using a 150 W/NDL lamp, 390 nm cut off filters, 142 mW/cm⁻²). No significant cytotoxicity was observed in the dark condition, indicating the promising uses of this nanoparticle in PDT applications (Galli et al. 2016). Guo et al. (2016) described the preparation of monodisperse Fe₃O₄ nanoparticles with sizes in the range of 60–310 nm. The authors observed that smaller Fe₃O₄ nanoparticles displayed enhanced cell internalization and, hence, deeper penetration into multicellular spheroids, which make them suitable for PDT applications. Larger Fe₃O₄ nanoparticles tended to accumulate in tumors increasing tumor growth inhibition (Guo et al. 2016).

Similarly, multifunctional magnetic nanoparticles with average size of 108 nm were synthesized for tumor treatment and diagnosis (Kim et al. 2016b). The magnetic nanoparticle core (MNP) is composed of Fe₃O₄, and the nanoparticle surface was conjugated with hyaluronic acid (AHP), leading to the formation of AHP@MNPs. The cytotoxicity of AHP@MNPs was evaluated toward mouse embryonic fibroblast (NIH3T3) cells, via heat generation properties of the nanoparticles and generation of singlet oxygen, upon magnetic and laser irradiation conditions. The nanoparticle concentration was fixed at 12 g/mL, and NIH3T3 cells were exposed to PDT (4 Jcm⁻²) and magnetic heat treatment alone or in combination. Cell viability decreased to 30% with PDT treatment. Live/dead staining experiments revealed that the nanoparticles displayed controllable cell death via targeting CD44 receptor-mediated endocytosis (Kim et al. 2016b). Lee et al. (2016) reported the synthesis of caffeic acid-polyethylene glycol-folic acid; FA-PEG-CA, caffeic acid-polyethylene glycol-pheophorbide-a; and pheoA-PEG-CA-coated Fe₃O₄ nanoparticles for PDT targeting. The presence of caffeic acid on the surface of iron oxide nanoparticles increased the nanoparticle dispersion in aqueous environment, while folate is responsible for mediation of tumor targeting. The cytotoxicity of the nanoparticles was evaluated toward human breast carcinoma cell line (MDA-MB-231). The cell was incubated with the nanoparticles, and light irradiation was performed by using 670 nm laser source (5 mW/cm²) for 200 s. The irradiation increased twofold the cytotoxicity. Furthermore, due to the presence of folate on the surface of the nanoparticle, the nanoparticles were internalized into tumor cells, which over express folic acid receptors (Lee et al. 2016).

In another study, a widely employed photosensitizer in PDT applications, i.e., chlorin e6 (Ce6), was loaded on PEG-functionalized iron oxide nanoclusters

(IONCs), leading to the formation of IONC-PEG-Ce6 nanoparticles (Li et al. 2013). The absorbance and excitation peaks of the IONC-PEG-Ce6 were found to be located in the NIR (~700 nm), making the nanoparticles suitable for PDT due to improved tissue penetration. The *in vivo* efficacy of IONC-PEG-Ce6 was performed based on magnetic tumor targeting by using the 4T1 tumor model on Balb/c mice. The results revealed a significant delay in tumor growth upon a single nanoparticle injection and the application of a magnetic field-enhanced PDT treatment. The light source for PDT application was 704 nm light at 5 mW/cm² for 1.5 h (27 J/cm²). Under dark conditions, tumors of mice that received IONC-PEG-Ce6 injection showed the same growth trend compared to the untreated groups. This result suggests that IONC-PEG-Ce6 is not toxic under dark condition. However, significant inhibition of tumor growth was observed upon light irradiation after IONC-PEG-Ce6 injection. Indeed, little tumor growth was observed over a course of 16 days post-PDT application, revealing the post-effect of the treatment in the combat to cancer. These nanoparticles have desirable properties for cancer treatment, such as no cytotoxicity under dark condition, high generation of singlet oxygen upon NIR illumination, tumor targeting upon application of external magnetic field, and high cancer cell uptake (Li et al. 2013).

The same photosensitizer, Ce6, was combined with magnetic nanoparticles for PDT (Ling et al. 2014). The authors reported an elegantly pH-dependent PDT to selectively kill cancer cells. Tumor tissues are acidic with pH range of 6.8 which can also reach lower values (5.0–5.5) in endo-/lysosomes. Therefore, the changes in the pH environment from healthy tissues to tumor sites can be used to trigger the release of therapeutic agents on the surface of nanocarriers. In this context, self-assembled extremely small iron oxide nanoparticles (ESIONs), average size of 3 nm, were prepared and coated with polymeric ligands, leading to the formation of pH-sensitive magnetic nanogrenades (PMNs). PMNs can be activated under low pH values found in tumors for combating resistant heterogeneous tumors *in vivo* and also as bimodal imaging agent, as represented in Fig. 1.3. According to Fig. 1.3a, the ESIONs were functionalized via catechol-anchored ligand A. Figure 1.3b shows the schematic representation of self-assembly ESIONs into colloidal magnetic core-shell nanomaterial. In this structure, the ESIONs are the hydrophobic core, which is coated with hydrophobic blocks of the tumor-sensing polymeric ligands. The obtained nanoparticles had hydrodynamic diameter of 70 nm, which is suitable for tumor treatments due to the EPR effect. Moreover, the nanoparticle surface allows a pH activation resulting in surface charge reversal in tumor periphery that increases cell adsorption and permeation and endo-/lysosomal pH-dependent theranostic effect of the nanoparticles. Interestingly, the PMN photoactivity (generation of oxygen singlet) was quenched at physiological pH (7.4) due to fluorescence resonance energy transfer. In contrast, at lower pH value found in tumor environment (pH <6), the photoactivity of the PMNs significantly increased due to the disassembly, in a similar manner observed for other self-assembled polymeric ligands. Therefore, pH controls the fluorescence intensity and NIR generation of toxic singlet oxygen. The cytotoxicity of PMNs was demonstrated toward human colorectal carcinoma (HCT116) cells under irradiation and compared to dark conditions. As expected,

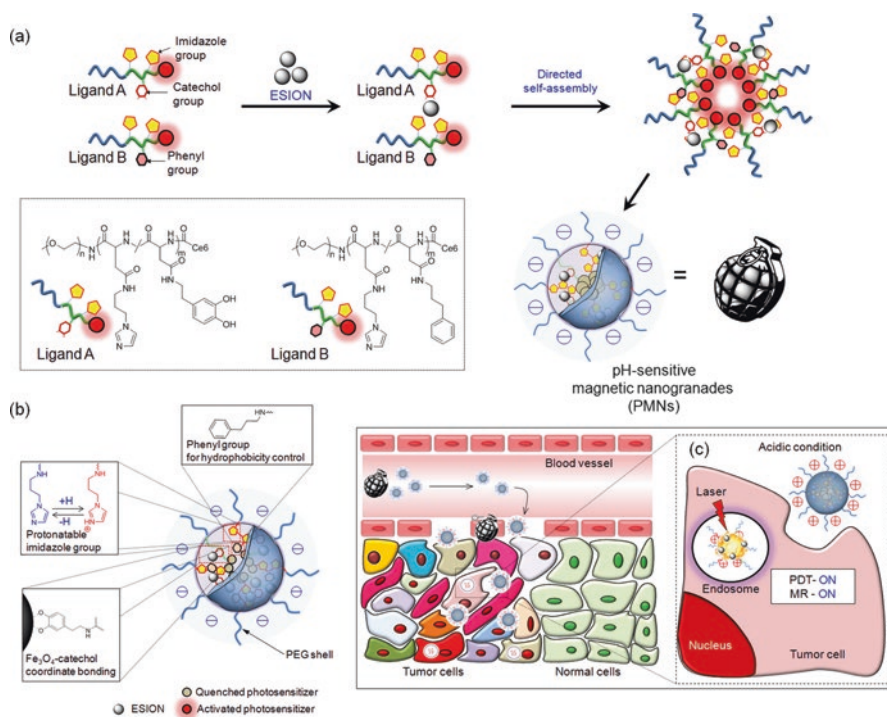


Fig. 1.3 Schematic representation of the design and mechanism of pH-sensitive magnetic nanogranades (PMNs) for tumor pH activation: (a) Schematic representation of pH-responsive, ligand-assisted self-assembly of extremely small iron oxide nanoparticles (ESIONs), (b) schematic representation of tumor pH-recognizable treatment strategy using PMNs (Reproduced from reference Ling et al. 2014 with permission from American Chemical Society)

PMNs had no toxicity in the dark and significant toxicity under irradiation. In vivo studies were also performed by using HCT116 tumor-bearing mice injected with PMNs, followed by irradiation. A significant tumor regression of treated groups was observed, in comparison with control groups. The nanomaterial was designed to be toxic only at the tumor site, since the photosensitizer on the nanoparticle can be self-quenched in the normal tissues, until it reaches the target tumor site. In the tumor, the low pH value suppresses the self-quenched effect, particularly, the intracellular pH stimulus, resulting to a high specific toxic effect to the target region. One week post-PMN injection, the tumors were completely destroyed, and only a remaining scar tissue was observed in the animals (Ling et al. 2014). Taken together, these results demonstrated the efficacy of tumor-targeting iron oxide nanoparticles for PDT and tumor diagnosis.

In a similar interesting strategy, hydrophilic chlorin-conjugated magnetic nanoparticles were designed for PDT in the combat of melanoma (Mbakidi et al. 2013). The nanomaterial is comprised of an iron oxide magnetic core covered with water-stable and biocompatible dextran shell bearing polyaminated chlorin p6 (the

photosensitizer). The toxicity of the nanomaterial was assayed toward variants of B16 mouse melanoma cell line (B16F10 and B16G4F, with and without melanin, respectively). The *in vitro* results demonstrated that the nanoparticles had no cytotoxicity in the dark between 0.1 and 1 μM , while 1 μM of the nanoparticles had strong phototoxicity toward B16F10 cell line (Mbakidi et al. 2013).

Nam et al. (2016) reported the PDT activity of hematoporphyrin-conjugated Fe_3O_4 nanoparticles. The Fe_3O_4 nanoparticles were conjugated with hematoporphyrin (HP) leading to the formation of $\text{Fe}_3\text{O}_4@\text{HPs}$. The phototoxicity of the $\text{Fe}_3\text{O}_4@\text{HPs}$ was evaluated toward human prostate cancer (PC-3) and breast cancer (MDA-MB-231) cell lines. The authors used a LED, with an electrical power of 3 W at 505 nm maximum wavelength and a power density of up to 7 mW/cm^2 . A significant phototoxicity of $\text{Fe}_3\text{O}_4@\text{HPs}$ was found toward both cancer cell lines, which was dependent on the amount of HP on the nanoparticle surface. In addition, the results demonstrated a cell membrane translocation and nuclear fragmentation of cancer cells due to apoptosis (Nam et al. 2016).

The photosensitizer pheophorbide-a (pheoA), which produces fluorescent emission in the range of 660–670 nm, was conjugated with iron oxide nanoparticles for PDT and magnetic resonance imaging (Nafiujjaman et al. 2015). The synthesis of the nanohybrid material was performed by the conjugation of pheoA with heparin (pheoA-Hep), which was further bound with APTES-coated Fe_3O_4 nanoparticles. The final material, spherical pheoA-Hep- Fe_3O_4 nanoparticles (average hydrodynamic size of 90 nm), had improved biocompatibility and can easily penetrate the cell surface via ERP effect. The phototoxicity of pheoA-Hep- Fe_3O_4 nanoparticles was demonstrated toward epithelial cancer (KB) cells, at nanoparticle concentration of 50 $\mu\text{g mL}^{-1}$ and 24 h of incubation. The irradiation was performed by using a laser source 670 nm (4 mW/cm^2) for 10 min. The results showed that pheoA-Hep- Fe_3O_4 nanoparticles caused 80% cell death, under irradiation, while in the dark, no toxicity was observed. Moreover, a high degree of nanoparticle internalization was reported (Nafiujjaman et al. 2015).

Oleic acid (OA)-coated magnetic iron oxide nanoparticles were synthesized by coprecipitation technique, followed by addition of Mn^{2+} -doped $\text{NaYF}_4:\text{Yb}/\text{Er}$ luminescent shell (Qin et al. 2016). The obtained monodispersed $\text{Fe}_3\text{O}_4@\text{Mn}^{2+}$ -doped $\text{NaYF}_4:\text{Yb}/\text{Er}$ core-shell nanoparticles were able to emit luminescence under NIR excitation indicating their potential uses for PDT (Qin et al. 2016). Similarly, core-shell nanoparticles (size of 99 nm) composed by magnetite/silica/titania were obtained by sol-gel technique (Sakr et al. 2016). The photosensitizer ruthenium polypyridyl dye was added to the surface of the synthesized nanoparticles. The cytotoxicity of the nanoparticles was demonstrated toward lung cancer cells (A549) under a laser source at 532 nm (5 mW/cm^2). Indeed, upon irradiation, the ROS generation increased fivefold compared to bare NPs, which caused cell death (the half maximal inhibitory concentration, IC_{50} , was found to be 3 $\mu\text{g}/\text{mL}$). The mechanisms of cell death are under investigation (Sakr et al. 2016).

The photosensitizer porphyrin was added on the surface of superparamagnetic iron oxide nanoparticles (SPION-TPP) through click chemistry (Thandu et al.

2014). Phototoxicity of the obtained nanomaterial was demonstrated toward murine amelanotic melanoma B78-H1 cells, upon cell irradiation at NIR region (14 Jcm^{-2} , 30 min). Cell internalization of the nanoparticles was improved by the conjugation of the nanoparticles with TAT peptide (a cell-penetrating peptide) (Thandu et al. 2014). Unterweger et al. (2015) reported the synthesis of hypericin-linked-containing dextran coating on the surface of superparamagnetic iron oxide nanoparticles with 55–85 nm of hydrodynamic size. Dextran was used as stabilizing agent for the nanoparticles, and hypericin is a PS. The phototoxicity of the obtained nanoparticles was demonstrated toward human T-cell leukemia cells (Jurkat) upon white irradiation (40 Wm^{-2}) up to 15 min. The results showed a time-dependent and concentration-dependent cytotoxicity due to ROS generation (Unterweger et al. 2015).

A versatile nanomaterial was synthesized for multifunctional anticancer treatment and diagnosis (Wan et al. 2015). The nanomaterial, $\text{Fe}_3\text{O}_4\text{-NS-C}_3\text{N}_4\text{@mSiO}_2\text{-PEG-RGD}$, is composed of C_3N_4 (PS, a graphitic carbon nitride), mSiO_2 (mesoporous silica to enhance the stability of the nanomaterial and the ability of drug loading), and RGD (integrin-binding cell adhesive peptide, which is a tumor biomarker). The versatile nanomaterial allied PDT and low pH-triggered anticancer properties. The phototoxicity of the nanomaterial was evaluated toward A549 and HeLa cancer cells, upon visible light irradiation (450 nm, 40 mW/cm^{-2} for 10 min). Figure 1.4 shows images, obtained by confocal laser scanning microscopy, of the cells after incubation with the nanomaterial and PDT treatment, compared with control groups. As can be observed, a significant green fluorescence was emitted from the cells that were irradiated, in comparison with a less intensive green fluorescence light emission from the cells in the dark (no irradiated). This result illustrates the ability of the nanoparticles to generate ROS under irradiation with visible light. Interestingly, the confined beam of light source employed in this experiment caused a desired site-targeted cytotoxic effect on the treated cells, which might be able to decrease potential side effects toward noncancer cells (Fig. 1.4) (Wan et al. 2015).

Core-shell nanomaterial was obtained by coating hollow magnetic nanospheres (HMNSs) with silica (SiO_2) shells followed by conjugation with carboxylated graphene quantum dots (GQDs) leading to $\text{HMNS/SiO}_2\text{/GQDs}$ (Wo et al. 2016). The anticancer drug DOX was added to the nanomaterial, which was further stabilized with liposomes. The phototoxicity of the multimodal nanoparticle was proved toward human esophageal squamous carcinoma (Eca-109) cells upon NIR laser irradiation (671 nm or 808 nm for 20 min). The authors demonstrated that liposome-stabilized nanomaterial was adsorbed by the tested cancer cells, which led to membrane and nuclei damages. As expected, by DOX loading on the nanoparticle, the cytotoxicity toward cancer cells was significantly increased (Wo et al. 2016).

Other multifunctional iron oxide-based nanoparticles were synthesized and applied in PDT. Firstly, $\text{Fe}_3\text{O}_4\text{@NaYF}_4\text{:Yb/Er}$ core-shell nanomaterial was synthesized, followed by addition of tetrasulfonic phthalocyanine aluminum (AIPcS4), as the photosensitizer and PEG coating (Zeng et al. 2013). The phototoxicity of the

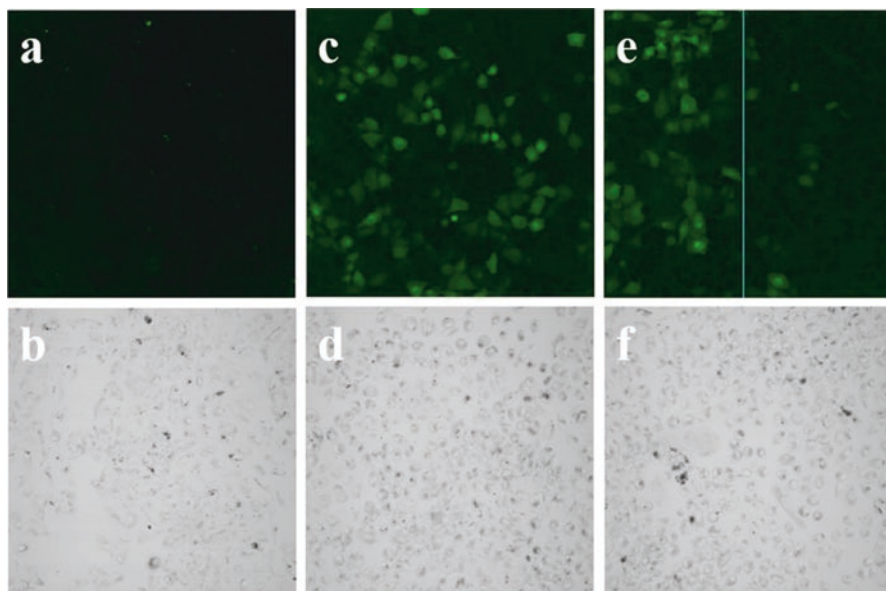


Fig. 1.4 Detection of intracellular ROS in A549 cancer cells treated with $\text{Fe}_3\text{O}_4\text{-NS-C}_3\text{N}_4\text{@mSiO}_2\text{-PEG-RGD}$ either (c) under visible light irradiation or (a) not; (e) site-targeted PDT. Parts b, d, and f correspond to the optical images of a, c, and e (Reproduced from reference Wan et al. 2015 with permission from American Chemical Society)

nanomaterial was shown toward MCF-7 cells, upon irradiation with 980 nm laser source (20 mW/cm^2) for 3 min. The authors reported a decrease of 70% of cancer cell viability upon this treatment. Moreover, the nanoparticles were found to accumulate on the MCF-7 surfaces. The toxicity to cancer cells was attributed to the ability of AIPcS4 to generate singlet oxygen (Zeng et al. 2013).

The nanocomposite $\text{Fe}_3\text{O}_4\text{@NaYF}_4\text{:Yb-E}$ conjugated with folic acid (FA) was synthesized and applied as PDT agent (under NIR irradiation) toward in vitro and in vivo (MCF-7 tumor-bearing nude mice) evaluations (Zeng et al. 2015). In vitro studies demonstrated that the viabilities of HeLa and MCF-7 cells decreased to 18% and 31%, upon incubation with the nanocomposite and 980 nm laser irradiation. Moreover, in vivo studies revealed 95% of MCF-7 tumors in bearing nude mice, indicating the promising application of this nanomaterial to combat cancer (Zeng et al. 2015). A DNA damage caused by Fe_3O_4 nanoparticles in PDT application was reported (Zhang and Chai 2012). Magnetite-silica core-shell nanomaterial was synthesized by sol-gel technique to promote DNA damage by a photoactive platinum-diimine complex under red light (Zhang and Chai 2012).

Zhao et al. (2014) reported the incorporation of the photosensitizer methylene blue (MB) into folate-conjugated $\text{Fe}_3\text{O}_4\text{/mesoporous silica}$ core-shell nanoparticles. The toxicity of the nanoparticles was demonstrated toward different cell lines: HeLa cells, mouse fibroblast cells (NIH3T3 cells), mouse sarcoma cells (S-180 cells), and

human ovarian cancer cells (SK-OV-3 cells). Folic acid (FA) was used to target the nanoparticles to the cancer cells, since cancer cells are known to overexpress folate receptors. The cell irradiation was performed by using a 650 nm laser beam (70 mW/cm² for 4 min). The results demonstrated a significant decrease in cell viability (reduction of 80% of viable cells compared to control group) by incubating SK-OV-3 cells with 200 µg/mL¹ of the nanoparticle. Moreover, the nanoparticles were found to internalize to cancer cells. In vivo experiments were performed with S-180 tumor-bearing mice treated with the nanoparticles and laser irradiation at 650 nm. The results demonstrated the potential anticancer activity of the nanoparticles by suppressing tumor growth (Zhao et al. 2014).

Finally, the photosensitizer indocyanine green (IR820) was conjugated with chitosan quaternary ammonium (CSQ) magnetic iron oxide nanoparticles, leading to the formation of IR820-CSQ-Fe nanoparticles (Zhou et al. 2016). The phototoxicity of the nanoparticles (808 laser source, 8 Wcm⁻², 7 min) was demonstrated with breast cancer cell line (MDA-MB-231 cells) (Zhou et al. 2016). Taking together, several important and recent publications described successfully the synthesis of magnetic iron oxide-based nanoparticles as targeting nanocarriers in PDT to combat cancer.

1.4 Conclusions

The recent advantages in the design of iron oxide magnetic nanoparticles as nanocarriers of photosensitizers are able to generate therapeutic amounts of singlet oxygen directly in the target site of application. The targeting therapy can be achieved upon the application of an external magnetic field due to the superparamagnetic grapheme of iron oxide nanoparticles. It is clear from the reviewed literature that there is a great interest on the preparation of hybrid nanomaterials with multifunctions (e.g., cancer treatment and cancer diagnosis).

It should be noted that although tremendous progress has been made in this promising field, still more studies are required to translate the research to the clinical settings. In this direction, the ideal nanocarrier needs to meet the following requirements: (i) biocompatibility, (ii) biodegradability, (iii) small size and high loading capacity of the photosensitizer, (iv) minimum side effects, (v) prolonged body circulation with minimum tendency to aggregation, (vi) enhanced ERP effect, and (vii) ability to generate therapeutic amounts of singlet oxygen directed to the desired site and no dark toxicity.

Although PDT has been used in oncology for several years, some challenges need to be overcome, for instance, (i) minimization of systemic toxicity, (ii) adequate generation and permeation of singlet oxygen, and (iii) no dark toxicity.

Finally, it can be concluded that the combination of magnetic iron oxide nanoparticles as nanocarriers of photosensitizers in PDT is a promising field in cancer treatment. However, more studies are necessary in this area of nanomedicine.

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