# Iron oxide nanoparticles for therapeutic applications

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*Teaser*: Owing to their reassuring toxicity profiles and multiple mechanisms of action, iron oxide nanoparticles have been considered for a wide range of medical applications, including treatment of iron-deficiency anemia, cancer, Parkinson's, Alzheimer's, antibacterial and antifungal diseases.

In nanomedicine, iron oxide nanoparticles are at an advanced stage, being commercialized for cancer treatment and iron-deficiency anemia treatment. Their therapeutic efficacy comes from their ability to target a tissue, activate a drug, locally produce reactive oxygen species or a temperature increase following (or not) the application of an external source of energy, modify genes or activate various biological materials, or replace diseased cells by stem cells. Owing to these various mechanisms of action, they can potentially be used for treating a whole range of different diseases, making them more appealing than conventional drugs that target a more limited number of indications.

## Introduction

Nanomaterials have been developed for medical applications owing to their appealing properties. First, their size is of the same order of magnitude as that of various biological materials such as enzymes, proteins and lipids, hence promoting interactions with them, and they are larger than most individual molecules used as standard drugs, resulting in better tissue interaction and larger accumulation in targeted organs for nanomaterials than for standard drugs. Second, nanomaterials have a large exposed surface area, which enables them to adjust their properties by changing their surface charge, composition or chemistry, or by binding to their surface molecules of interest such as specific ligands or chemotherapeutic molecules.

These properties, which are stimulating from the point of view of research, make it hard to set up a well-established regulatory framework, hence delaying the use of nanomedicine for human treatments. In addition, because each group working on a different nanomaterial tries to highlight the advantages of its system compared with others, it is difficult to identify a nanosystem that is better than others. However, iron oxide nanoparticles (IONPs) have paved the way among the most widely studied nanomaterials, owing to their biocompatibility and multiple ways in which they can trigger therapeutic activity. In this review, after a brief presentation of IONP manufacturing methods and physicochemical properties, the different mechanisms of action as well as treatments for specific diseases in which IONPs are involved are described.

## Fabrication and physicochemical properties of IONPs

Methods to synthetize IONPs include: (i) co-precipitation between $Fe^{2+}$ and $Fe^{3+}$ in basic conditions [1]; (ii) thermal decomposition of organo-iron in high boiling point organic solvents in the presence of surfactants [2]; (iii) nanoparticle formation inside micelles [3]; (iv) sol-gel methods [4]; (v) hydrothermal synthesis [5]; and (vi) cathodic electrochemical deposition in which anode oxidation leads to the formation of metal ions that are further reduced to metals by the cathode in the presence of a stabilizing material [6]. These synthesis methods enable obtaining IONPs with various sizes ~1–120

nm, size distributions, compositions [essentially consisting of $Fe_3O_4$ (magnetite) and $\gamma$-$Fe_2O_3$ (maghemite)], crystallinity (amorphous or crystalline structure), magnetic properties (essentially superparamagnetic for IONPs <10–20 nm and ferrimagnetic for IONPs >10–20 nm) and geometries (spherical, round, square, cubic, cubo-octahedric, needle, cylindrical, cigar, bullet type). To prevent IONPs from aggregating, which can lead to embolism, these nanoparticles can be coated by various materials including polymers and fatty or amino acids. Furthermore, the coating material can influence nanomaterial biodistribution by controlling opsonization mechanisms; the capacity of specific cells such as macrophages to capture IONPs, for example polyethylene glycol (PEG), leads to a longer blood circulation time than citric acid [7–10].

**IONP targeting methods**

For IONPs to be efficient, they need to reach the organ of interest. As illustrated in Figure 1, this can be achieved through three different types of targeting mechanisms:

- Passive targeting also called enhanced permeability and retention (EPR) effect in which IONPs target tumors by passively diffusing through the holes of abnormal vessels resulting from angiogenesis that irrigate the tumor.
- Active targeting in the absence of magnetic field application, which relies on the use of IONPs conjugated with a targeting ligand (TL), which specifically targets a specific receptor that is overexpressed at the surface of cancer cells (T). Examples of pairs (TL:T) are: (i) antibody:antigene, such as antibody-Her-Neu:receptor-Her-2-neu [11]; (ii) aptamer:tumor-cell-receptor, such as anti-Muc-1:Muc-1 [12]; (iii) protein:tumor-cell-receptor, such as RGD:$\alpha\nu\beta_3$ [13]; (iv) peptide:various-tumor-parts, such as F3::tumor-vasculature [14]; (v) enzyme:cell-receptor, such as uPA:uPAR [15]; (vi) vitamin:tumor-cell-receptor, such as folic-acid:folate-receptor [16]; (vii) chemical-compound:tumor-cell-receptor, such as methotrexate:folate-

receptor [17]; and (viii) hormone:tumor-cell-receptor, such as luteinizing hormone releasing hormone (LHRH):LHRH-receptor [18].

- Magnetic drug targeting, in which a strong external magnetic field gradient is applied to drive IONPs into the area of interest.

Taking the literature as a whole, it appears that the targeting efficacy is relatively similar for different targeting, yielding a percentage of injected IONPs in the tumor of $5.10^{-4}$% to 3%, 0.1% to 7% and $5.10^{-3}$% to 2.6% for passive, active and magnetic targeting, respectively [19]. Next, the different mechanisms through which IONPs can generate a therapeutic activity, as summarized in Figures 1 and 2, are reviewed.

**IONP drug delivery without a mechanism of drug release**

Although, in several cases, drug delivery using IONPs was described without mentioning a specific mechanism of drug release, this does not mean that such a mechanism did not occur. Disregarding such a mechanism, the conjugation of various chemotherapeutics to IONPs can be used to deliver these drugs to tumors, yielding the following advantages compared with the injection of fee drugs: (i) the possibility to localize IONPs and therefore also possibly the drug conjugated to IONP, using MRI for example; (ii) the increase in drug accumulation in the tumor, for example IONPs containing doxorubicin (DOX) led to a higher concentration of DOX in tumor cells compared with free DOX treatment in an ovarian cancer model [19]; (iii) the association of drugs to IONPs can improve antitumor activity, for example IONPs conjugated with vinblastine led to enhanced MCF-7 breast cancer cell inhibition compared with vinblastine alone [20]; (iv) the combination of IONPs with a chemotherapeutic drug such as gemcitabine or mitoxantrone and a targeting agent such as anti-CD44 or prostate-specific membrane antigen (PSMA) antibodies can enhance drug targeting and antitumor efficacy on tumor cells compared with free drugs [21–24]; and (v) IONPs can be combined with a specific entity to prevent DNA pro-tumor activity, for example DNA was captured by cytarabine attached to IONPs [25].

**Drug delivery and activation with a mechanism of drug release**

Delivery and activation of drugs associated to IONPs can occur via various mechanisms:

- pH variation, which has been described for drugs such as 5-FU, curcumin, DOX and tamoxifen that either involve magnetic-polymer, PEG or IONPs inserted within the large pores of mesoporous hydroxyapatite-coated IONPs, or bind to monoclonal antibody (mAb)-conjugated magnetic nanocarriers, where release is more pronounced at an acidic cellular pH of 5.8 than at the blood pH of 7.4, leading to an increase of the percentage of drug release from 10–55% at pH 7.4 to 70–85% at pH 5.8 [26,27].
- Temperature variation, which has been highlighted in systems containing: (i) curcumin incorporated in a nanogel containing a thermosensitive polymer (PNIPAM) and IONPs, releasing 35% and 70% of curcumin at 37°C and 41°C [28]; (ii) DOX bound to IONPs through an Azo linker, which is destroyed above ~43°C, when exposed to near infrared (NIR) irradiation, leading to DOX release and inhibition of tumor growth inhibition [29]; (iii) gemcitabine encapsulated together with IONPs inside a phospholipid bilayer to form a magneto-liposome, leading to an increase of drug release from 17% at 37°C without alternating magnetic field (AMF) application to 70% following AMF application [30].
- Magnetic field through either an indirect mechanism (i.e., the magnetic field causes nanoparticle cellular internalization of a drug bound to IONPs, such as BCNU-loaded nanocomplex in HG cells, resulting in drug release in the acidic cellular environment) [31] or a direct mechanism {i.e., the magnetic field itself causes drug release, as observed for IONP nanocapsules containing drugs surrounded by thermo-labile polymer (PEO-PPO-PEO) [32], IONP surrounded by a hydrophobic poly(vinyl alcohol) coating containing hydrophilic DOX, fluid MAG-CMX conjugated to DOX or magnetic polymerosomes loaded with DOX [33]}.
- Water or buffer surrounding IONPs that can trigger drug release as observed for 5-FU or curcumin progressively detaching from IONPs in water or different buffer solution [34],

possibly leading to enhanced cytotoxicity against tumor cells as highlighted for F@BSA@CUR in MCF-7 cells [34].

- IONP shape can have an impact on drug release mechanism, as observed for 5-FU bound to IONPs of various shapes [35].
- Drug circulation time in the organism can influence drug release, as observed for polyplex-coated magnetite (Nano-co-Plex) loaded with BCNU, which shows a time-dependent release efficacy with maximum release of 75% of the loaded BCNU [36].
- Breaking of the bond between the drug and the IONPs, using either acidic conditions or specific biological materials (proteinase), as described for: (i) L-lysine surface-coated IONPs conjugated with methotrexate (MTX), where the peptide bond is cleaved in the presence of proteinase K at low pH [37]; (ii) for IONPs bound to polyethylene glycolated methotrexate (MTX-PEG) and NIR fluorescent cyanin dye (Cy5.5) that could be hydrolyzed at intracellular pH, preventing drug release in systemic circulation while allowing it inside endosomes or lysosomes [38]; (iii) for IONPs linked to drugs through bounds that are cleaved by tumor enzyme-cleavable peptides such as lysosomal proteases [39].

These mechanisms of selective drug release can prevent side effects as highlighted when the highly cytotoxic drug azademethylcolchicine (ICT) was conjugated to the dextran layer of the IONP ferumoxtol using a peptide linker, which was cleavable by matrix metalloproteinase-14 (MMP-14), leading to significant tumor cell apoptosis while sparing healthy cells [40].

**Intracellular drug delivery with IONPs**

If a drug is not delivered intracellularly it can lose its activity, for example by diffusing in the bloodstream or by being eliminated. Drugs associated with IONPs can more easily internalize in cells than free drugs because the size of the drug–IONP complex is significantly larger than that of free drugs, a ligand can be bound to the IONP surface that specifically binds a cell receptor. As an example,

direct intracellular drug delivery of DOX by IONPs was shown to be much more efficient than that of free DOX for targeting and concentrating DOX within a Hep-G2/MDR tumor site [41].

**IONP therapy working through the production of ROS**

IONPs could trigger some activity by releasing radical oxygen species (ROS) as demonstrated either when IONPs are dissolved into $Fe^{2+}$ and/or $Fe^{3+}$, for example following IONP cellular internalization and degradation by lysosomes [42], or when a substance bound to IONPs produces ROS, as shown for cisplatin attached to IONPs, which activated nicotinamide adenine dinucleotide phosphate oxidase (NOX), transforming di-oxygen ($O_2$) to superoxide radical ($O_2$ •−) and $H_2O_2$, resulting in the apparition of toxic hydroxyl radicals (•OH) through the Fenton's reaction between $H_2O_2$ and $Fe^{2+}$ [43]. Furthermore, ROS, such as •OH, have been shown to induce oxidative damage to lipids, proteins and DNA in the tumor site while minimizing systemic toxicity [43].

ROS can also be produced by exposing a photosensitizing agent associated with IONPs to a laser through a technique called photodynamic therapy (PDT). In this case, IONPs are used as a platform or support for binding a large number of photosensitizing agents such as hypericin. The efficacy of such an approach was demonstrated on a complex of red blood cells, DOX, IONPs and chlorine e6 that significantly suppressed tumor growth using PDT [44].

**IONP therapy based on local temperature increase (magnetic hyperthermia and photothermal therapy)**

Localized heat can be produced by exposing IONPs to the following:

- An AMF using a technique called magnetic hyperthermia (MHT), where the AMF should have frequency and strength >20–100 kHz and 1-10 mT, respectively, and IONP should be sufficiently concentrated (i.e., typically above 1 mg of IONP per $cm^3$ or ml of surrounding medium) [41], resulting in specific absorption rate (SAR) of typically 1 to 1000 W/gFe, depending on IONP properties (size, anisotropy stability of magnetic moment) and AMF

parameters, leading to a series of different therapeutic activities (i.e., specific tumor cell destruction without destruction of healthy cells) [41,42]; (ii) significant or full tumor destruction on mice bearing different tumor types (e.g., subcutaneous or intracranial) [45–50]; (iii) activation of a drug such as DOX [51]; (iv) an improvement of GBM patient survival by ~7 months [44,52]; (v) significant lesion reduction by implanting a composition of IONPs and calcium phosphate cement in bone tumors [53]. A typical protocol used for MHT treatment is illustrated in Figure 3.

- A laser using photothermal therapy (PTT), which produces a localized surface plasmon wave using laser wavelengths in the NIR within 650–900 nm to avoid harmful light absorption by healthy tissues, and laser power is maintained below the safe limit of tissue irradiation of 0.3–1.0 W/cm$^2$, resulting in the destruction of tumor cells (e.g., MCF-7 and MDA-MB-231) or full or partial tumor disappearance in mouse without apparent side effects, where therapeutic activity was observed in the absence and in the presence of an absorbing material such as PPy [28].

These nanoparticle-based heating techniques present the advantage of moderately heating the tumor during treatments at typically 41–55°C, which limits side effects compared with 80–90°C reached in cancer thermotherapies that do not use nanoparticles such as HIFU and radiofrequency.

Mechanisms to explain antitumor efficacy with MHT and PTT rely on: (i) structural and functional cancer cell damage, specifically at the level of protein, membrane and cytoskeleton, which can lead to apoptosis [54]; (ii) ‘fragilization’ of cancer cells which increases the sensitivity of these cells to other treatments such as chemotherapy or radiotherapy [55]; (iii) expression of heat shock proteins at temperatures of typically 41–46°C that can attack the tumor [56], although the opposite behavior (protection of the tumor) has also been reported for these proteins [57]; (iv) protein denaturation or unfolding that can occur at high temperature; (v) pH changes, perfusion and oxygenation of the tumor microenvironment that can lead to tumor necrosis [58]; and (vi) apoptosis or necrosis depending on treatment parameters (temperature, duration of heating, etc.) [45].

## Triggering gene therapy with IONPs

IONPs could potentially improve gene therapy efficacy by carrying specific nucleic acids that regulate altered gene expression resulting from carcinogenesis, essentially in the form of siRNA or plasmid DNA. An IONP–gene complex could have a larger circulation time than that of a free gene, avoid potential damages by nucleases and hence enable nucleic acids delivery to the organ of interest such as a tumor [59]. Such therapy could act against a tumor in the following ways:

- Directly, for example when IONPs conjugated with siRNA (siPLK1) act against Pololike kinase-1 (cell-cycle-specific serine/threonine kinase) and two peptides (MPAP and MUC1) were injected in tumor-bearing mice, the result is the accumulation of IONPs in the tumor, efficient PLK1 silencing and tumor suppression through increased apoptosis [60].
- Indirectly, for example when IONPs carrying the phosphatase and tensin homolog (PTEN) gene increased the sensitivity of A549/CDDP lung cells to cisplatin treatment indicating that PTEN can effectively be used against cisplatin-resistant lung cancer cells [61].

Furthermore, to improve the efficacy of gene therapy, the IONP–gene complex can be attracted near the tumor by a magnet, hence increasing gene transfer into cells [62], via a technique called magnetofection. To date, clinical trials testing gene therapy have been unsuccessful owing to the existence of a multitude of different genes responsible for cancer, depending on cancer type, cancer progression and type of cancer patient, which can hardly all be targeted with only one gene therapeutic agent [63]. To further improve the efficacy of gene therapy, it might be suggested to use IONPs to: (i) carry out a pre-screening of pro-tumor genes for each patient taken individually and adapt gene therapy depending on the detected genes; (ii) use a combination of different genes instead of only one gene to increase the probability of targeting pro-tumor genes; or (iii) combine gene therapy with another modality of treatment.

## Stem cell therapy using IONPs

IONPs can be used in stem cell therapy by being incorporated inside these cells, enabling them to localize, for example using MRI, and activating them with the help of magnetic actuation [64]. By using IONPs during the transplantation of oligo-dendrocyte precursor cells (OPCs), it might be possible to help in regenerating the central nervous system by increasing myelin production and hence restore the protective sheath around nerve fibers [65].

**Immunotherapy**

IONPs can act on the immune system in the following ways:

- They modulate macrophage polarization (i.e., inducing a shift from M2-type macrophages with anti-inflammatory and pro-tumor activity to M1-type macrophages with anti-tumor and proinflammatory activity), as was shown for example for THP-1 monocytes that shifted from a M2-like phenotype before IONP exposure toward a M1-like phenotype after IONP exposure [66].
- They increase the efficacy of immune-suppressive drugs used during organ transplantation, as was shown when mycophenolic acid (MPA) combined with IONPs and effectively inhibited the secretion of the cytokines interleukin (IL)-2 and tumor necrosis factor (TNF), with a concentration of MPA that is lower than that necessary to reach a similar effect in the absence of IONPs [67], hence reducing side effects observed at high MPA concentrations.

**Protein and enzyme activation or removal with IONPs**

It has been shown that IONPs can act like enzymes by mimicking the activity of peroxidase [60] and efficiently immobilize proteins at their surface owing to their large surface-area:volume ratio, and then separate or isolate proteins by applying an external magnetic field [68].

**Different types of diseases treated with IONPs**

A summary of the different types of diseases that can be treated with IONPs and the mechanisms of action that are involved in the treatments is provided in Figure 4 and Table 1. Cancer can be treated through the generation of ROS or localized heat by IONPs or via a chemotherapeutic drug associated with IONPs, where the IONP–drug complex can lead to the following:

- An enhanced efficacy of chemotherapeutic drug, for example: (i) IONPs associated with paclitaxel (IONP–PAX) are more cytotoxic than free PAX in destroying prostate tumor cells, as demonstrated *in vitro* on PC3 and CWR22R human prostate cancer cells [69] and *in vivo* on C4-2-cell-derived xenograft tumors in athymic nude mice [69]; and (ii) IONPs associated with DOX are more cytotoxic toward 4T1 cells than free DOX [70].
- A reduction of toxicity of certain anticancer drugs such as docetaxel, mitoxantrone, cisplatin and carboplatin by increasing the efficacy of tumor drug delivery and by simultaneously reducing the dose necessary to reach therapeutic efficacy, as shown for IONPs conjugated to docetaxel, which led to enhanced cellular uptake ability and antiproliferative activity in PC3 prostate cancer cells, and for toxic mitoxantrone where tumor uptake was increased when it was conjugated with IONPs [69], overcoming the cytotoxicity induced by certain chemotherapeutics (cisplatin, carboplatin).
- An increase in drug-targeting efficacy through the use of: (i) stimuli-responsive drug release from IONPs, for example with a higher drug release at pH 5.8 (mimicking the tumor microenvironment) than at pH 7.4 (normal physiological condition); (ii) targeting agents that are able to target specific receptors of cancer cells as summarized above.
- Chemosensitization of tumor cells to chemotherapeutic drug, as was shown through the cytotoxicity enhancement of cisplatin toward cisplatin-resistant A549 tumor cells using IONPs loaded with cisplatin [71].

In addition to the mechanisms described above, it has been reported that IONPs can modify the tumor microenvironment by blocking an artery that irrigates the tumor with blood [72]. Alzheimer's disease (AD) could be treated by IONPs functionalized by a targeting agent that can cross the blood–brain

barrier (BBB) and reach small peptides called amyloid-β (Aβ) and then either detect Aβ using MRI or affect the Aβ fibrillation process, responsible for AD, with an efficacy that seems to depend on IONP size, surface area, charge, concentration and level of opsonization [73]. Parkinson's disease (PD) could be treated by IONPs using the following:

- Gene therapy, by conjugating IONPs with short hairpin RNA (shRNA), promoting neuronal uptake via nerve growth factor (NGF) receptor-mediated endocytosis or neuron apoptosis caused by a lower α-syn expression *in vitro* and *in vivo*, leading to effective repair in a PD model [74].
- Magnetic field therapy, by applying a magnetic field on rats at the site of PD lesion 24 h after IONP administration, 2 h/day for 1 week, leading to an improved behavior, condition, mitochondrial function and attenuation of lesion volume in all treated rats, indicating the neuroprotective effect of this therapeutic approach [75].

Neuro-AIDS, which refers to a reservoir of AIDS viruses in the brain, could potentially be treated by attaching conventional treatments (tenofovir and vorinostat) to IONPs to enable these drugs to cross the BBB and to efficiently eradicate AIDS viruses in the brain, as demonstrated by IONP antiviral activity *in vitro* over a period of 5 days after HIV infection in primary human astrocytes [76]. Iron-deficiency anemia, which cannot be efficiently cured with oral iron treatment owing to drug gastrointestinal digestion, could be treated with intravenous injection of IONPs, improving iron biodisponibility; and this has been commercialized in formulations such as Dexferrum®, Feraheme®, Ferrisat®, Ferrlecit®, Monofer® and Venofer® [19].

Fungi or bacterial contamination can be eradicated by inhibiting the growth of fungi such as *Trichophyton verrecosum, Trichophyton mentagrophytes, Dermatophilus sp., Trichothecium roseum, Cladosporium herbarum, Penicillium chrysogenum, Alternaria alternata and Aspergillus niger* [77] or bacteria such as *Escherichia coli, Bacillus subtilis, Candida albicans, Aspergillus niger and Fusarium solani* [78]. Growth inhibition can be the result of leakage of the cell contents leading to cell death, inhibition of spore germination, membrane destruction, production of ROS with lipid peroxidation, DNA damage, release of metal ions and/or the presence of antibacterial or antifungal substances

associated with IONPs such as vanilla essential oil, antibiotics and chlorhexidine (CHX). Furthermore, IONPs can penetrate inside biofilms and hence yield efficient biofilm destruction, a property that seems to depend upon IONP physicochemical characteristics, such as IONP surface charge, hydrophobicity and high surface-area:volume ratio [79]. Blood contamination can be treated by attaching IONP molecules that specifically bind to contaminant material, such as pathogens or toxic compounds, as shown for IONPs colliding with *Staphylococcus aureus*, internalizing inside these bacteria and then being removed under magnetic field application [80]; or for IONPs surrounded by a specific coating that can bind radioactive material or heavy metal.

## Concluding remarks and future perspectives

Despite their huge potential, IONPs are currently only commercialized for treatment of iron-deficient anemia (e.g., Venofer® commercialized by American Regent) and cancer treatment (e.g., Nanotherm® sold by Magforce). To foster IONP commercialization for other indications, new developments can be carried out such as: (i) IONP synthesis methods that do not contain toxic products and yield sufficient production of highly pure and stable IONPs; (ii) IONPs that can be activated on demand by applying an external source of energy, only when they are located in the organ to be treated, hence avoiding IONP side effects on healthy tissues; and (iii) an energy source that is affordable and can easily be used by doctors, especially within the hospital environment.

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## Conflicts of interest

Edouard Alphandéry has been working at the start-up Nanobacterie.

## Figure legends

**Figure 1.** Schematic representation of iron oxide nanoparticle (IONP) therapeutic mechanisms of action through chemotherapeutic drug release and activation, heat and reactive oxygen species (ROS) production, under application (or not) of an external source of energy. Schematic illustration of the three targeting mechanisms (passive, active, magnetic targeting).

**Figure 2.** Iron oxide nanoparticle (IONP) mechanisms of action can be subdivided between: (i) IONP local temperature increase (PTT, MHT); (ii) gene therapy (intentionally or non-intentionally triggered); (iii) reactive oxygen species (ROS) production (PDT or due to IONP themselves); (iv) drug delivery caused by the release of drugs initially attached to IONPs; (v) IONP targeting of specific cells or biological entities through active, passive or magnetic targeting.

**Figure 3.** Example of iron oxide nanoparticle (IONP) treatment using magnetic hyperthermia by administering IONPs to a tumor and then heating IONPs under alternating magnetic field application. The mechanisms of action involve moderate local heating at typically 42–50°C followed by death of cancerous cells.

**Figure 4.** Iron oxide nanoparticle (IONP) therapeutic applications including treatment of neuro-AIDS, cancer, blood contamination, Alzheimer's disease and Parkinson's disease, where the treatments rely on antifungal, antibacterial, antitumoral, nanozyme or immunotherapy.

## References


1. Lagrow, A.P. *et al.* (2019) Unravelling the growth mechanism of the coprecipitation of iron oxide nanoparticles with the aid of synchrotron X-Ray diffraction in solution, *Nanoscale* 11, 6620−6628

2. Dos Santos Monteiro, D. and Oliveira da Guarda Souza, M. (2016) Thermal decomposition of precursors and iron oxide properties. *J. Therm. Anal. Calorim.* 123, 955–963

3. Groult, H. *et al.* (2018) Micellar iron oxide nanoparticles coated with anti-tumor glycosides. *Nanomaterials* 8, 567

4. Sharafi, Z. *et al.* (2018) Synthesis of silica-coated iron oxide nanoparticles: preventing aggregation without using additives or seed pretreatment. *Iran. J. Pharm. Res.* 17, 386–395

5. Ge, S. *et al.* (2009) Facile hydrothermal synthesis of iron oxide nanoparticles with tunable magnetic properties. *J. Phys. Chem. C* 113, 13593–13599

6. Karimzadeh, I. *et al.* (2017) Superparamagnetic iron oxide (Fe3O4) nanoparticles coated with PEG/PEI for biomedical applications: a facile and scalable preparation route based on the cathodic electrochemical deposition method. *Adv. Phys. Chem.* 2017, 9437487

7. Arias, L.S. *et al.* (2018) Iron oxide nanoparticles for biomedical applications: a perspective on synthesis, drugs, antimicrobial activity, and toxicity. *Antibiotics* 7, 1–32

8. Ghazanfari, M.R. *et al.* (2016) Perspective of Fe3O4 nanoparticles role in biomedical applications. *Biochem. Res. Int.* 2016, 7840161

9. Hu, Y. *et al.* (2018) Construction of iron oxide nanoparticle-based hybrid platforms for tumour imaging and therapy. *Chem. Soc. Rev.* 47, 1874–1900

10. Mosayebi, J. *et al.* (2017) Functionalization, and design of magnetic nanoparticles for theranostic applications. *Adv. Healthcare Mater.* 6, 170030

11. Artemov, D. *et al.* (2003) MR molecular imaging of the Her-2/neu receptor in breast cancer cells using targeted iron oxide nanoparticles. *Magn. Res. Med.* 49, 403–408

12. Aghanejad, A. *et al.* (2018) Mucin-1 aptamer-armed superparamagnetic iron oxide nanoparticles for targeted delivery of doxorubicin to breast cancer cells. *BioImpacts* 8, 117–127

13. Zhang, C. *et al.* (2007) Specific targeting of tumor angiogenesis by RGD-conjugated ultrasmall superparamagnetic iron oxide particles using a clinical 1.5-T magnetic resonance scanner. *Cancer Res.* 67, 1555–1562

14. Reddy, G.R. *et al.* (2006) Vascular targeted nanoparticles for imaging and treatment of brain tumors. *Clin. Cancer Res.* 12, 6677–6686

15. Yang, L. *et al.* (2008) Development of receptor targeted magnetic iron oxide nanoparticles for efficient drug delivery and tumor imaging. *J. Biomed. Nanotechnol.* 4, 439–449

16. Bonvin, D. *et al.* (2017) Folic acid on iron oxide nanoparticles: platform with high potential for simultaneous targeting, MRI detection and hyperthermia treatment of lymph node metastases of prostate cancer. *Dalton Trans.* 46, 12692–12704

17. Kohler, N. *et al.* (2004) A bifunctional poly(ethylene glycol) silane immobilized on metallic oxide-based nanoparticles for conjugation with cell targeting agents. *J. Am. Chem. Soc.* 126, 7206–7211

18. Leuschner, C. *et al.* (2006) LHRH-conjugated magnetic iron oxide nanoparticles for detection of breast cancer metastases. *Breast Cancer Res. Treat.* 99, 163–176

19. Alphandéry, E. (2019) Biodistribution and targeting properties of iron oxide nanoparticles for treatments of cancer and iron anemia disease. *Nanotoxicol.* 2, 1–24

20. Javid, A. *et al.* (2013) Chitosan-coated superparamagnetic iron oxide nanoparticles for doxorubicin delivery: synthesis and anticancer effect against human ovarian cancer cells. *Chem. Biol. Drug Des.* 82, 296–306

21. Albermani, M.S.K. *et al.* (2017) Vinblastine based iron oxide nano drug delivery system. *J. Global Pharma Tech.* 8, 90–96

22. Nagesh, P.K.B. *et al.* (2016) PSMA targeted docetaxel-loaded superparamagnetic iron oxidenanoparticles for prostate cancer. *Colloids Surf. B Biointerface.* 144, 8–20

23. Barar, J. *et al.* (2015) Multifunctional mitoxantrone-conjugated magnetic nanosystem for targeted therapy of folate receptor-overexpressing malignant cells. *J. Nanobiotechnol.* 13, 26

24. Zaloga, J. *et al.* (2016) Pharmaceutical formulation of HSA hybrid coated iron oxide nanoparticles for magnetic drug targeting. *Eur. J. Pharm. Biopharm.* 101, 152–162

25. Shahabadi, N. *et al.* (2016) Improving antiproliferative effect of the anticancer drug cytarabine on human promyelocytic leukemia cells by coating on Fe3O4@SiO2 nanoparticles. *Colloids Surf. B Biointerface.* 141, 213–222

26. Asadi, N. *et al.* (2018) Synthesis, characterization and *in vitro* evaluation of magnetic nanoparticles modified with PCL–PEG–PCL for controlled delivery of 5FU. *Art. Cell. Nanomed. Biotechnol.* doi: 10.1080/21691401.2018.1439839

27. Prabha, G, and Raj, V. (2016) Preparation and characterization of polymer nanocomposites coated magnetic nanoparticles for drug delivery applications. *J. Magnetism Magnetic Mater.* 408, 26–34

28. Estelrich, J. and Busquets, M.A. (2018) Iron oxide nanoparticles in photothermal therapy. *Molecules* 23, 1567

29. Wu, L. *et al.* (2017) Remotely controlled drug release based on iron oxide nanoparticles for specific therapy of cancer. *Colloids Surf. B Biointerface.* 152, 440–448

30. Ferreira, R.V. *et al.* (2016) Thermosensitive gemcitabine magnetoliposomes for combined hyperthermia and chemotherapy. *Nanotechnol.* 27, 085105

31. Akilo, O.D. *et al.* (2016) An *in vitro* evaluation of a carmustine-loaded Nano-co-Plex for potential magnetic-targeted intranasal delivery to the brain. *Int. J. Pharm.* 500, 196–209

32. Liu, J. *et al.* (2015) Design of hybrid nanovehicles for remotely triggered drug release: an overview. *J. Mater. Chem. B* 3, 6117–6147

33. Nadeem, M. *et al.* (2016) Magnetic properties of polyvinyl alcohol and doxorubicine loaded iron oxide nanoparticles for anticancer drug delivery applications. *PLoS One* 11, e0158084

34. Nosrati, H. *et al.* (2018) Bovine serum albumin (BSA) coated iron oxide magnetic nanoparticles as biocompatible carriers for curcumin-anticancer drug. *Bioorg. Chem.* 76, 501–509

35. Tuncelli, G. *et al.* (2015) 5-Fluorouracil intercalated iron oxide@layered double hydroxide core-shell nano-composites with isotropic and anisotropic architectures for shape-selective drug delivery applications. *Mater. Sci. Eng. C* 55, 562–568

36. Akilo, O.D. *et al.* (2016) An *in vitro* evaluation of a carmustine-loaded Nano-co-Plex for potential magnetic-targeted intranasal delivery to the brain. *Int. J. Pharm.* 500, 196–209

37. Nosrati, H. *et al.* (2018) Methotrexate-conjugated L-lysine coated iron oxide magnetic nanoparticles for inhibition of mcf-7 breast cancer cells. *Drug Dev. Ind. Pharm.* 44, 886–894

38. Lin, J. *et al.* (2015) Drug/dye-loaded, multifunctional PEG−chitosan−iron oxide nanocomposites for methotraxate synergistically self-targeted cancer therapy and dual model imaging. *ACS Appl. Mater. Interface.* 7, 11908–11920

39. Anderson, C.F. and Cui, H. (2017) Protease-sensitive nanomaterials for cancer therapeutics and imaging. *Ind. Eng. Chem. Res.* 56, 5761–5777

40. Ansari, C. *et al.* (2014) Development of novel tumour-targeted theranostic nanoparticles activated by membrane-type matrix metalloproteinases for combined cancer magnetic resonance imaging and therapy. *Small* 10, 566–417

41. Wang, Y. *et al.* (2016) *In vivo* dual-targeted chemotherapy of drug resistant cancer by rationally designed nanocarrier. *Biomater.* 75, 71–81

42. Saikia, C. *et al.* (2016) Effect of crosslinker on drug delivery properties of curcumin loaded starch coated iron oxide nanoparticles. *Int. J. Biol. Macromol.* 93, 1121–1132

43. Ma, P. *et al.* (2017) Enhanced cisplatin chemotherapy by iron oxide nanocarrier-mediated generation of highly toxic reactive oxygen species. *Nano Lett.* 17, 928–937

44. Li, K. *et al*. (2017) Next generation superparamagnetic iron oxide nanoparticles for cancer theranostics. *Drug Discov. Today* 22, 1421–1429

45. Alphandéry, E. *et al.* (2017) Development of non-pyrogenic magnetosome minerals coated with poly-L-lysine leading to full disappearance of intracranial U87-Luc glioblastoma in 100% of treated mice using magnetic hyperthermia. *Biomaterials* 141, 210–222

461 46. Alphandéry, E. *et al.* (2017) Chains of magnetosomes with controlled endotoxin release and
462 partial tumour occupation induce full destruction of intracranial U87-Luc glioma in mice under the
463 application of an alternating magnetic field. *J. Control. Release* 261, 259–272
464 47. Le Fèvre, R. *et al*. (2017) Enhanced antitumour efficacy of biocompatible magnetosomes for the
465 magnetic hyperthermia treatment of glioblastoma. *Theranostics* 7, 4618–4631
466 48. Hamdous, Y. *et al.* (2017) Biocompatible coated magnetosome minerals with various
467 organization and cellular interaction properties induce cytotoxicity towards RG-2 and GL-261 glioma
468 cells in the presence of an alternating magnetic field. *J. Nanobiotechnol.* 15, 74
469 49. Mandawala, C. *et al.* (2017) Biocompatible and stable magnetosome minerals coated with poly-
470 L-lysine, citric acid, oleic acid, and carboxy-methyl-dextran for application in the magnetic
471 hyperthermia treatment of tumours. *J. Mater. Chem. B* 5, 7644–7660
472 50. Hilger, I. (2013) *In vivo* applications of magnetic nanoparticle hyperthermia. *Int. J.*
473 *Hyperthermia* 29, 828–834
474 51. El Hajj Diab, D. *et al.* (2018) Combined treatments of magnetic intra-lysosomal hyperthermia
475 with doxorubicin promotes synergistic anti-tumoral activity. *Nanomaterials* 8, 468
476 52. Maier-Hauff, K. *et al.* (2011) Efficacy and safety of intratumoural thermotherapy using magnetic
477 iron-oxide nanoparticles combined with external beam radiotherapy on patients with recurrent
478 glioblastoma multiforme. *J. Neurooncol.* 103, 317–324
479 53. Cortajarena, A.L. *et al.* (2014) Engineering iron oxide nanoparticles for clinical settings.
480 *Nanobiomedicine* doi: 10.5772/58841
481 54. Master, A.M. *et al.* (2016) Remote actuation of magnetic nanoparticles for cancer cell selective
482 treatment through cytoskeletal disruption. *Sci. Rep.* 6, 33560
483 55. Giustini, A.J. *et al*. (2011) Magnetic nanoparticle hyperthermia in cancer treatment. *Nanolife*
484 doi: 10.1142/S1793984410000067
485 56. Kobayashi, T. (2011) Cancer hyperthermia using magnetic nanoparticles. *Biotechnology J.* 6,
486 1342–1347

487 57. Calderwood, S.K. (2016) Heat shock proteins promote cancer: it's a protection racket. *Trends*
488 *Biochem. Sci.* 41, 311–323
489 58. Patel, A. and Sant, S. (2016) Hypoxic tumour microenvironment: opportunities to develop
490 targeted therapies. *Biotechnol. Adv*. 34, 803–812
491 59. Kievit, F.M. and Zhang, M. (2011) Surface engineering of iron oxide nanoparticles for targeted
492 cancer therapy. *Acc. Chem. Res.* 44, 853–862
493 60. Mahajan, U.M. *et al.* (2016) Tumour-specific delivery of siRNA-coupled superparamagnetic
494 iron oxide nanoparticles, targeted against PLK1, stops progression of pancreatic cancer. *Gut* 65, 1838–
495 1849
496 61. Min, L.F. *et al.* (2012) Magnetic iron oxide nanoparticles carrying PTEN gene to reverse
497 cisplatin-resistance of A549/CDDP cell lines. *J. Central South University* 19, 331–339
498 62. Son, S. *et al.* (2015) Magnetofection mediated transient NANOG overexpression
499 enhances proliferation and myogenic differentiation of human hair follicle derived mesenchymal stem
500 cells. *Bioconjug. Chem*. 26, 1314–1327
501 63. Amer, M.H. (2014) Gene therapy for cancer: present status and future perspective. *Mol. Cell*
502 *Ther.* 2, 27
503 64. Zhao, M. *et al.* (2018) A GPC3-specific aptamer-mediated magnetic resonance probe for
504 hepatocellular carcinoma. *Int. J. Nanomed.* 13, 4433–4443
505 65. Connell, J.J. *et al.* (2015) Advanced cell therapies: targeting, tracking and actuation of cells with
506 magnetic particles. *Regen. Med.* 10, 757–772
507 66. Laskar, A. *et al.* (2013) SPION primes THP1 derived M2 macrophages towards M1-like
508 macrophages. *Biochem. Biophys. Res. Commun.* 441, 737–742
509 67. Zanganeh, S. *et al.* (2016) Iron oxide nanoparticles inhibit tumour growth by inducing pro-
510 inflammatory macrophage polarization in tumour tissues. *Nat. Nanotechnol.* 11, 986–994
511 68. Gao, L. *et al.* (2017) Iron oxide nanozyme: a multifunctional enzyme mimetic for biomedical
512 applications. *Theranostics* 7, 3207–3277

69. Chowdhury, P. *et al.* (2017) Magnetic nanoformulations for prostate cancer. *Drug Discov. Today* 22, 1233–1241

70. El-Zahabya, S.A. *et al.* (2019) Reviewing two decades of nanomedicine implementations in targeted treatment and diagnosis of pancreatic cancer: an emphasis on state of art. *J. Control. Release* 293, 21–35

71. Srikanth Vallabani, N.V. and Singh, S. (2018) Recent advances and future prospects of iron oxide nanoparticles in biomedicine and diagnostics. *3 Biotech.* 8, 279

72. Wang, L. *et al.* (2018) Tumour microenvironment-enabled nanotherapy. *Adv. Healthcare Mater.* 7, 1701156

73. Brambilla, D. *et al.* (2011) Nanotechnologies for Alzheimer's disease: diagnosis, therapy, and safety issues. *Nanomed. Nanotechnol. Biol. Med.* 7, 521–540

74. Niu, S. *et al.* (2017) Inhibition by multifunctional magnetic nanoparticles loaded with alpha-synuclein RNAi plasmid in a Parkinson's disease model. *Theranostics* 7, 344–356

75. Umarao, P. *et al.* (2016) Neuroprotective potential of superparamagnetic iron oxide nanoparticles along with exposure to electromagnetic field in 6-OHDA rat model of Parkinson's disease. *J. Nanosci. Nanotechnol.* 16, 261–269

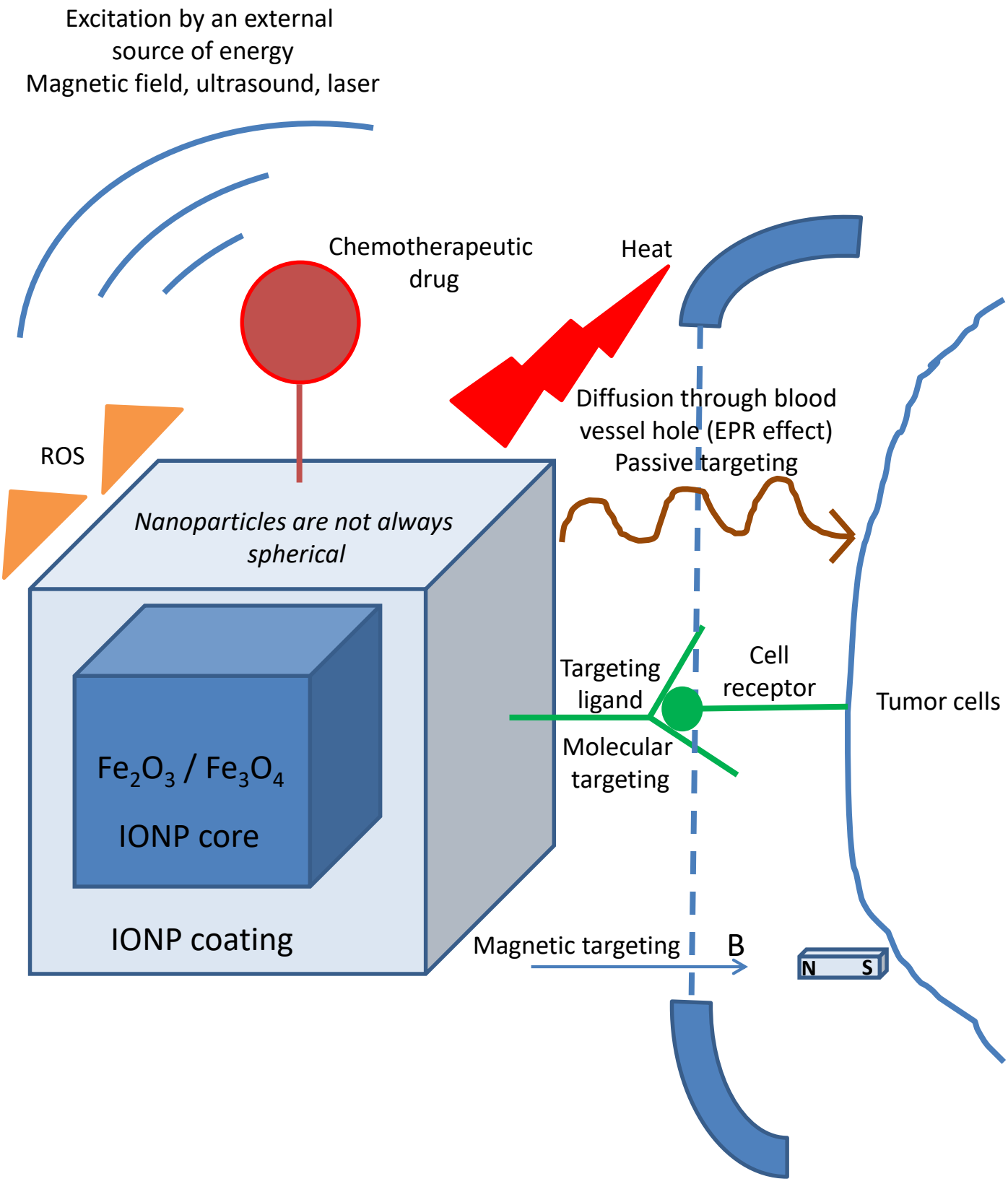
76. Sagar, V. *et al.* (2016) Magnetic nanotherapeutics for dysregulated synaptic plasticity during neuroAIDS and drug abuse. *Molecular Brain* 9, 57

77. Parveen, S. *et al.* (2018) Preparation, characterization and antifungal activity of iron oxide nanoparticles. *Microbial Pathogenesis* 115, 287–292

78. Arakha, M. *et al.* (2015) Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. *Sci. Rep.* 5, 14813

79. Wang, L. *et al.* (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int. J. Nanomed.* 12, 1227–1249

80. Kang, J.H. *et al.* (2015) Optimization of pathogen capture in flowing fluids with magnetic nanoparticles. *Small* 11, 5657–5666


Excitation by an external source of energy
Magnetic field, ultrasound, laser
Chemotherapeutic drug
Heat
ROS
Diffusion through blood vessel hole (EPR effect)
Passive targeting
Nanoparticles are not always spherical
Targeting ligand
Cell receptor
Tumor cells
Molecular targeting
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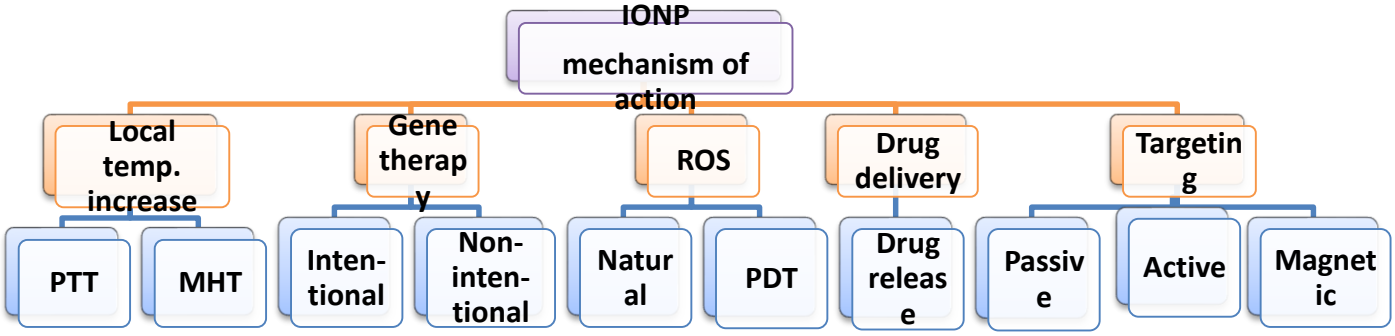
IONP
mechanism of action
Local temp. increase
PTT
MHT
Gene therapy
Inten-tional
Non-inten-tional
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Drug delivery
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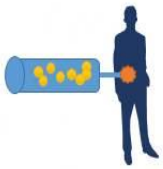
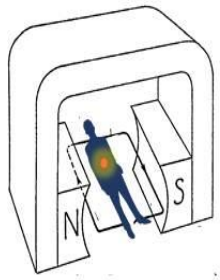
Targetin g
Passiv e
Active
Magnet ic

Administration of IONP to the patient in solid tumors



IONP

Administration of IONP by an alternating magnetic field

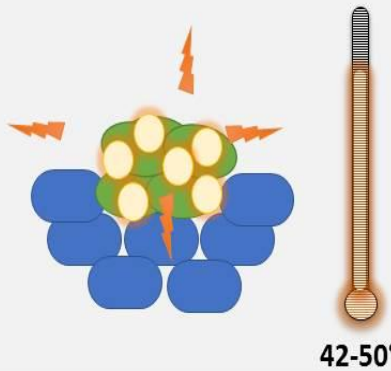




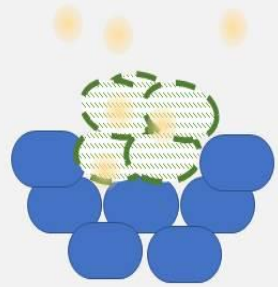
Field-activated IONP

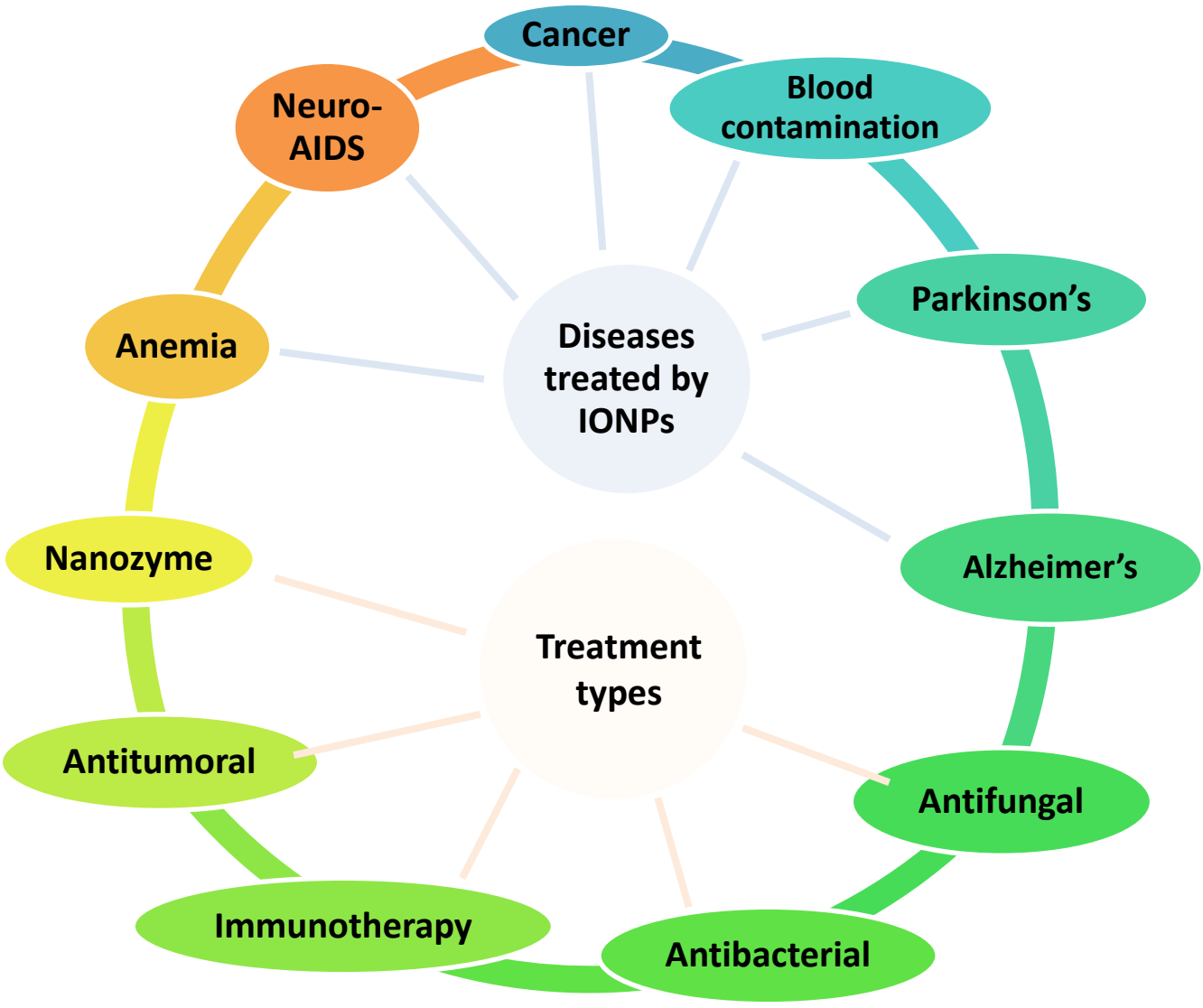
IONP and cancerous cells brought together


Internalisation and heating of IONP when activated




Death of the cancerous cells. Natural metabolism of iron.




Cancer
Blood contamination
Parkinson's
Alzheimer's
Antifungal
Antibacterial
Immunotherapy
Antitumoral
Nanozyme
Anemia
Neuro-AIDS
Diseases treated by IONPs
Treatment types

| Types of treated diseases | Mechanisms involved in treatment | Experimental conditions (Celluar/animal/human) |
|---|---|---|
| Cancer | Enhanced chemotherapy | IONP-PAX more efficient than free PAX:<br>○ *in vitro* on PC3/CWR22R cells [69]<br>○ *in vivo* on C4-2 mouse tumors [69] |
| | Reduction of toxicity of anticancer drugs | Tumor uptake of mitoxantrone increased with IONP by reducing therapeutically efficient dose of mitoxantrone shown *in vitro* and *in vivo* [69] |
| | Increase in drug-targeting efficacy | Stimuli drug release as a function of:<br>○ pH variation (*in vitro* [26])<br>○ Temperature variation (*in vivo* [27])<br>○ Magnetic field (*in vitro* [30])]<br>○ Water/buffer (*in vitro* [34]) |
| | ROS | IONPs produce ROS:<br>○ by themselves (Fenton reaction) (*in vitro* and *in vivo* [43])<br>○ following laser excitation (PDT) (*in vivo* [44]) |
| | Localized heat | ○ MHT (human [52])<br>○ PTT (*in vitro* [28]) |
| | Prevent tumor growth | Blocking the blood artery that irrigates the tumor and promotes tumor growth (*in vivo* [72]) |
| Alzheimer's | Acts on fibillation process | Therapy/diagnosis (*in vitro*, *in vivo* [73]) |
| Parkinson's | Gene therapy | Inhibition by IONP-RNAi (*in vitro*, *in vivo* [74]) |
| | Magnetic field therapy | Neuroprotective ability of IONPs under magnetic field exposure (*in vivo* [75]) |
| Neuro-AIDS | Enables AIDS drugs to cross blood–brain barrier | Proposed model for *in vitro*/*in vivo*/human configurations [76] |
| Iron-deficiency anemia | Enhances iron bioavailability | Commercially available to treat IAD [19] |
| Fungi/bacteria contamination | Destroys fungi/bacteria through ROS production/DNA damages | IONP activity demonstrated directly on fungi/bacteria [77–79] |
| Blood contamination | Removal of blood contaminants using IONP | Capture of blood bacteria by IONP demonstrated *in vitro* [80] |

**Table 1. Types of diseases treated with IONPs; mechanisms involved in the treatment; experimental demonstration; associated references**

Abbreviations: PAX, paclitaxel; PDT, phtodynamic therapy; MHT, magnetic hyperthermia; PTT, photothermal therapy; RNAi, RNA interference; IAD, iron anemia disease.