Pre-final

An arsenal of magnetic nanoparticles; perspectives in the treatment of cancer

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Abstract

Common options for managing cancer include chemotherapy, radiotherapy, surgery, and immunotherapy. Nanomedicine is an emerging field encompassing the application of nanoparticles for the treatment of cancer. Magnetic nanoparticles (MNPs) are usually sized between 2-100nm and can circumvent vascular tissue to concentrate at the site of the tumour. Under the influence of an external, alternating magnetic field, MNPs generate high temperatures within the tumour and ablate malignant cells whilst inflicting minimal damage to healthy host tissue. Due to their theranostic properties, they constitute a promising candidate for the treatment of cancer. a critical review of the type, size and therapeutic effect of different MNPs is presented, following an appraisal of the literature in the last five years.

Keywords: magnetic nanoparticles, hyperthermia, cancer, nanotechnology, nanomaterial, superparamagnetic nanoparticles, iron nanoparticles, breast cancer, lung cancer.

Introduction

Cancer is an increasingly common group of disorders characterized by abnormal cell growth. Depending on their type, cancerous cells may have the ability to metastasize, in which case the tumour is referred to as “malignant”, and is associated with a poorer prognosis for the patient. Even though the pathophysiology and progression of all cancers are not the same, there are six changes that occur which are well described: sustained proliferative signalling, evasion of growth suppression, resistance to apoptosis, replicative immortality, induction of angiogenesis and capability of invasion or metastasis[1]. Several risk factors have been identified, such as but not limited to: a genetic predisposition after a positive family history, exposure to UV light or other environmental carcinogens (eg asbestos) and lifestyle (eg smoking). The aforementioned habits comprise only a few from the list of factors which render an individual susceptible to DNA damage. Accumulation of this damage over time eventually becomes irreparable and, in combination with the failure to execute apoptosis, cancer develops [2].

The absence of a panacea for the treatment of cancer highlights the complexity underlying the development of the disease. However, a variety of different therapeutic modalities are available. Treatment options range from medication chemotherapy, to immunotherapy, radiotherapy, and surgery.

Systemic chemotherapy involves the administration of agents, which interfere with DNA replication or transcription in rapidly dividing cells, and induce apoptosis. Examples include alkylating agents, antimetabolites, anthracyclines, topoisomerase inhibitors, taxanes and vinca alkaloids. In an attempt to reduce the systemic side effects, newer and more targeted therapies have been developed. Monoclonal antibodies have shown impressive results, especially in monogenic cancers [3]. Unfortunately, this constitutes only a small percentage of cancers.

In specific cases, radiotherapy may be required. Both photon and particle radiation are available, with the radioactivity targeted towards the specific site of the tumour. However, it may not constitute the best option for tumours with several metastases, or women in close proximity to children (eg pregnant or breast-feeding). Surgery can be performed to remove tumours after diagnosis is confirmed via imaging. The help of a pathologist interpreting a biopsy will guide the surgeon to decide between aggressive or conservative excision.

Advances in the 21st century have given rise to a novel, rapidly growing field, with promising results in cancer therapy, namelynanomedicine, and the use of magnetic nanoparticles. In this review, the different types of Magnetic nanoparticles (MNPs) that have been used for the treatment of cancer both *in vitro* and in vivo in recent years will be discussed while the effectiveness and suitability of the MNPs available will be critically appraised. A concise summary of the type, size and therapeutic effect of each MNP will be presented, following an appraisal of the available literature in the last five years.

Magnetic nanoparticles

MNPs are very small agents, which can be manipulated by a magnetic field. Their size varies; depending on the method of synthesis. They usually have a diameter of 2 – 100nm, but in some cases they can reach a few tenths of a micrometre (eg 300nm = 0.3µm). These particles are highly applicable. They can be used as magnetic resonance contrast agents, substrates for drug delivery or for the treatment of cancer.

The structure of MNPs is linked directly to their properties. For biomedical applications, such as the treatment of cancer, an illustrated example is shown below (Figure 1)[4]. A core of magnetic nanoparticle is coated with a biocompatible substance (eg dextran) and carries spacer arms, which possess binding sites for active molecules [5]. Magnetic nanoparticles can be metallic (eg containing cobalt), and iron MNPs along with iron oxide MNPs are amongst the most common in a biomedical setting.

However, gold and silver nanoparticles are not uncommon. Metallic MNPs may exhibit toxic or even immunogenic properties and thus, their use is mostly, yet not only, confined to industrial applications[6]. High levels of iron can also pose threats of toxicity and hence, appropriate coating is required for patient safety. This complex can carry "arms" containing binding sites for substances. In this manner, labelling molecules for imaging or drugs for cancer therapy can be co-administered.

Magnetic nanoparticles in the treatment of cancer

The uptake of MNPs by tumour tissue can either be passive or active. The former takes advantage of the enhanced permeability and retention effect (EPR). In a site of a developing malignant tumour, the increased demand of proliferating cells for oxygen and nutrients exceeds the normal supply of the tissue. To cope, cytokines such as transforming growth factor beta 1 (TGF-β1) and tumour necrosis factor alpha (TNF-α) together with vascular endothelial growth factor (VEGF), promote angiogenesis [7]. However, these rapidly growing vessels contain fenestrae wider than usual. As a result, small molecules (eg nanoparticles) can circumvent the aberrant endothelial barrier and leave the circulation to concentrate at the site of neoplasia. Once a high concentration is achieved locally, an alternating magnetic current (AC) can be used to target the tumour and the surrounding magnetic nanoparticles. Alternating magnetic field in the range of radiofrequencies raises the temperature of the medium due to heat transfer originating from two well characterised loss mechanisms: neel relaxation as a result of coherent rotation of the magnetisation and lack of mechanical movement, and the Brownian losses due to the viscous friction between the nanoparticle and the medium(Figure 2)[8,9].. This is the widely used principle of hyperthermia (Figure 3), which may soon be available in hospitals [8]. Alternatively, a steady magnetic field gradient can be utilized to confine drug loaded MNPs at the desirable site and induce drug release (figure 4)[10]. A magnet may also be used for directing the particles to the site of the tumour after an IV infusion. This allows for better targeting but requires appropriate nanoparticles; the method has been well described using superparamagnetic iron oxide nanoparticles (SPIONs)[5].

Advances in technology have given rise to more targeted approaches in the domain of nanoparticle uptake in tissues. Unlike the aforementioned category, these are "active", and are orchestrated by surface labelling molecules coating the nanoparticles. As a result, the complex can be targeted to a specific tissue. Examples include but are not limited to folate- and transferring-coated nanoparticles, gold antibody conjugated nanoparticles, and inhaled EGFR-targeting nanoparticles[22,40,61]. These agents are further explored in the tables that follow.

Types of magnetic nanoparticles in the treatment of cancer

Numerous research groups around the globe have been investigating the role of different MNPs in cancer treatment. This area of research is a novel one, with increasing interest. In the past five years, magnetic hyperthermia has been tested using different nanocores and appropriate coatings, SPIONs, MNPs loaded with chemotherapeutic agents (eg 5-fluorouracil) and more. Experiments involve *in vitro*, *ex vivo,* in vivo*,* or all 3 techniques. However, there is still no data available for trials on humans. The closest to the aforementioned case is *in vitro* experiments using human cell lines.

Research groups have employed an *in vitro* approach to test essential parameters of the nano-drugs, such as safety, minimum dosing, tissue concentration and ability to induce hyperthermia in the presence of an external, alternating magnetic current. Safety and minimum dosing are directly linked and it is essential for a drug to be safe in order for it to be considered for clinical use in humans. "Safe" is, indeed, a vague concept; most often an agent will be associated with adverse reactions, but the real challenge is weighting the benefits against these unwanted effects. It should be noted that if a drug is only "safe" in negligible doses, it may be inappropriate for certain individuals. In some cases, patients may take other agents which interact and hide the effects of the drug. In other circumstances, fast metabolisers of a drug may show no improvement upon administration with the minimal dose, as the agent will fail to

reach an appropriate tissue concentration and exert its effects. Even though data on humans is not available yet, the experiments have yielded promising outcomes so far. Combined with the selectivity of MNPs and progress on minimizing toxicity, it should not take long for data on humans to come to light. The results of the most recent studies *in vitro* are shown in table 1.

An *ex vivo* approach has also been useful in assessing nanoparticle uptake in isolated tissue and in evaluating strategies for improved biocompatibility[92,93] . Exposure of whole isolated vessels that are maintained under near-physiological conditions to iron oxide nanoparticles, demonstrates their rapid uptake by endothelial cells (Figure 5)[92,93]. This technique will assist investigators in assessing strategies for enhanced nanoparticle biocompatibility, including surface modifications to decrease uptake, improve monodispersion and reduce aggregation in biological fluid, therefore increase retention time in blood.

An *in vivo* approach has constituted one of the most popular in this field. As the dynamics and kinetics of a drug *in vitro* may not always correspond to an *in vivo* environment, many researchers have investigated the direct influence of magnetic nanoparticles on tumour regression…etc, other clinical parameters…., via 2/ 3? Modes of exposure (intravenous, inhalation, or subcutaneous routes ….)in a number of species (mice, rats or rabbits). Table 2 summarises the recent *in vivo* work in the domain. Other research groups have undertaken a more thorough experimental approach, by investigating the direct influence of the nanoparticles on isolated cells in cultures, followed by their translation in the whole animal, to confirm their hypotheses. These are shown in table 3.

Discussion

MNPs have a great therapeutic potential. After a comprehensive review of the literature, it becomes evident that the majority of research groups using magnetic nanoparticles? have used an iron oxide nanoparticle, either Fe_3O_4 or γ-Fe_2O_3 [11].

Favourable properties that make these nanoparticles two of the most ideal candidates for magnetic hyperthermia are their high biocompatibility, non-toxicity and potential to exhibit superparamagnetic properties if sized below 20nm [12]. However, toxicity to healthy skin and lung tissue with Fe_3O_4 has been reported *in vitro* [13]. Appropriate coating (eg with chitosan) could help reduce systemic toxicity whilst improving specificity to cancerous tissue, at the same time [65]. Even in this case, complete safety is not ensured and hence more time needs to be invested into investigating potential side effects, before these agents can be used for therapy in humans. It should be noted that clinical trials on humans are significantly more challenging due to differences in intra- and inter-cellular environments[91]. Apart from magnetite and maghemite, iron is present in a variety of other nanoparticles. Examples include FeSi[36], FePt[34], Greigite (Fe-S)[41], $Mn_{0.4}Zn_{0.6}Fe_2O_4$[43], $Gd_{0.01}Fe_{2.99}O_4$[64], Mn-Zn ferrites[72], $ZnFe_2O_4$[73] and $Zn_{0.4}Fe_{2.6}O_4$[77]. Iron is essential in the human body and comprises the core of several indispensable molecules essential for survival, such as haemoglobin. These MNPs combine the useful stability and magnetic properties of iron with the unique properties other compounds have to offer. $Gd_{0.01}Fe_{2.99}O_4$ can be used for long term eradication of a neoplasm[64] whereas Mn-Zn ferrites have various effects on the tumour site, such as inhibition of angiogenesis[72]. Others, such as FeS and FePt are preferred for multifunctionality and versatility, respectively. Even though all particles seem biocompatible, some did not show an important therapeutic effect (eg FeSi[36]). Repetitions of the experiments as well as multicentre experimentation using the same MNPs will enlighten researchers on the potential of these particles for theranostics. A few years ago, MNPs came under the spotlight as a hitherto unknown option for treating tumours with hyperthermia. Human trials to come, along with more, supportive findings in the scientific literature, may soon offer MNPs an established status and a firm position in the list of theranostic options for cancer. Results in preclinical trials have been promising so far, and the possibility of using MNPs in hospitals over the next five years is increasing.

Only a number of research groups have investigated non-Fe containing MNPs. These include silver NPs (AgNPs)[32], gold nanorods[44], manganese perovskite[63] and As_2O_3[88]. AgNPs reduce tumour size in the presence of a magnetic field, and the effect becomes

more prominent with a reduction in size of the nanoparticle. Gold nanorods, which can be used along with Fe for theranostics, are effective in reducing tumour size[44], and the same holds true for manganese perovskite[63]. The use of As_2O_3 proved very effective in a quick and significant reduction of tumour size. Even though research has been more extensive with iron-MNPs compared to other compounds, the shift to new, alternative biocompatible coatings or potentially different compounds, may encourage the use of these non-Fe MNPs in pre/clinical trials.

Another important factor to take into consideration is the microenvironment of the tumour. Researchers have shown that the pH around the area of cancer sites is lower than normal; it is estimated to vary between 5.7-7.8[94]. Therefore, several groups have exploited the acidity of the tumour compared to the general circulation, by designing drug-loaded nanoparticles which unload their contents at the acidic pH of the tumour. This technique has been particularly effective with doxorubicin-loaded iron oxide nanoparticles[19]. Furthermore, the slow, insignificant release of the agent in a neutral or slightly basic pH (eg in blood) contributes to reducing systemic toxicity[17]. In another study, quantum dot- and adenovirus-based nanoparticles were modified to undergo charge reversal in acidic conditions. The results showed that not only nanoparticles, but also imaging agents as well as viruses can be surface-engineered to improve specificity and uptake in a tumour microenvironment[95].

Add a paragraph here about toxicity, including immunological responses to nanoparticles and uptake by blood cells/ phagocytic cells, as well as non-phagocytic cells (as clearly illustrated by figure 5, which demonstrated the extension of cytoplasmic projections and engulfment of clusters of iron oxide nanoparticles by endothelial cells surrounding blood vessels….also can include something about excretion and removal of these nanos after injection (see some refs on iron oxide in vivo biocompatibility studies). This will satisfy the requirement for one of the reviewers and makes the article more balanced.

Findings from the present review highlight the variation in the techniques utilised for the chemical characterisation of the nanoparticles va. This includes estimation of

nanoparticle size, charge and polydispersity. Even though many researchers performed electron microscopy (eg SEM or TEM) to determine particle size, others have used dynamic light scattering, also known as photon correlation spectroscopy[96], either separately, or in conjunction with SEM/TEM measurements Dark-field microscopy and acoustic spectrometry comprise other, less commonly used methods. Each method has discrete advantages and disadvantages which are beyond the scope of this review. It is, however, important to appreciate that this can contribute to discrepancy in nanoparticle size estimation.Polydispersity refers to the degree to which a distribution lacks uniformity and is an important measure of size distribution. Size, charge, magnetic field distribution, pH and coating are all factors which affect this property[96]. As a result, different MNPs have high or low degrees of polydispersity. Identification of individual polydispersity can help in better selecting an imaging modality to estimate the size of the particle, or even choose an appropriate treatment plan, in the near future. In terms of charge, a key concept, is corona formation, whereby, based on surface charge, nanoparticles can attract proteins from the surrounding material (need a ref here- eg. Oberdorster) which consequently alters the overall charge of the nanoparticle. This has implications on biocompatibility of the nanoparticle, in vitro and in vivo. ..can expand further….

Variation in the experimental approach adopted by researchers may further lead to discrepancy in findings by research groups. The studies critiqued herein, are based on two main approaches …. Can expand a little here about this…

In conclusion, the use of MNPs to induce targeted hyperthermia under the influence of an external alternating magnetic field has yielded very promising results. Despite it being a novel field, research interest is immense and a variety of particles have been tested. Most results have been positive so far, but human trials are required to provide more useful and generalizable data. Could it be possible that MNPs are a double-edged sword? Arguably, the consensus at this moment is in favor of their use in *in vitro* studies and *in vivo* experiments in animals but not in humans, yet. Even though MNPs are generally safe, it should not be forgotten that every substance is a poison, if given at an inappropriate dose (*Paracelsus,*1965).

Future perspective

The next few years may behold surprises in the treatment of cancer. If MNPs continue to show promising results, which are transferrable to humans, they may soon be the treatment of choice for many tumours. In the years to come, a great amount of focus will be given in developing targeted nanoparticles, rather than relying on EPR. The ability to coat nanoparticles with biocompatible, non-toxic agents, which recognize and are attracted to specific host targets, will bring nanotherapies one step closer to everyday clinical practice. Currently, several centres around the world including us are working on toxicology and surface modification, designing a clinical trial pathway within the next five years.

Certainly, research in the field is expanding and as a result, progress is accelerating. The multifunctionality of MNPs is probably the most promising aspect of the domain; with advances in imaging techniques or other diagnostic tests, researchers may be capable of detecting cancer at asymptomatic stages, and markedly improve the prognosis. One can only hope that MNPs will withstand the wear of time and suffice as a treatment for human malignant tumours. A task anything but simple. A task, which most of the agents available nowadays can only delay, causing a notable burden in resources of health systems worldwide.

Disclosure

The authors report no conflicts of interests in writing this review.

Executive Summary

Magnetic nanoparticle-induced hyperthermia for the treatment of cancer

- MNPs can be manipulated by an external magnetic field with the use of an alternating current.
- The spinning of MNPs generates heat, which ablates the tumour.

Drug-loaded magnetic nanoparticles for the treatment of cancer

- Several MNPs have the capacity to carry chemotherapeutic agents and release them selectively at the site of the tumour.
- The release is mediated by parameters in the tumour microenvironment, such as temperature and pH.

Types of magnetic nanoparticles used

- Metallic, Au-, Ag-, As- and Co-based MNPs.
- Fe-based MNPs are amongst the most common and can be enhanced with addition of specific elements (eg Pt, Gd).
- Selection of appropriate MNP depends on the type of tumour, capacity for drug loading and type of experiment (*in vitro* or *in vivo*).

Pathway to clinical study

- Preclinical *in vivo* test has been successful in killing cancer cells.
- Toxicology of nanoparticles in progress.
- Basic science of delivery has been established.
- Next stages are GMP/GLP manufacturing of the MNPs.
- GLP testing of preclinical trial.
- Feasibility clinical trial within 5 years.

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References of (particular) interest***
8**: Seminal paper on the mechanisms of MNP-induced hyperthermia.
50*: Topical administration of MNPs has a more specific effect.
63*: Successful use of manganese perovskite for malignant tumours.
96*: Importance of the technique used in determining nanoparticle size.

Captions for figures

Figure 1. Schematic structure of a magnetic nanoparticle (left) and a transmission electron micrograph of a metallic cobalt nanoparticle coated with carbon (right).

Figure 2. Demonstration of the “spinning” of MNPs, a prerequisite for the generation of high temperatures to ablate malignant cells.

Figure 3. Application of magnetic hyperthermia for targeted ablation of tumours.

Figure 4. Using magnetic nanoparticles for the treatment of tumours; a representative schematic.

Figure 5. Transmission electron microscopy image of an aortic vessel section after a 10 minute incubation in iron oxide nanoparticles. The nanoparticles are seen being engulfed into endosomal structures by an endothelial cell lining the vessel wall. Magnification x6300.

Table 1. The use of magnetic nanoparticles for cancer hyperthermia *in vitro,* in the last five years. Keys: Magnetic mesoporous silica, MMS; Polymer stabilized iron oxide-graphene, PIG; Au-coated iron oxide superparamagnetic nanoparticles, SPIONs@Au.

Year/Ref	Nanoparticle	Size (nm)	Experiment	Outcome
2015[14]	Fe_3O_4	11	PIG along with paclitaxel for cancer in HeLa cell lines.	A combination of hyperthermia with paclitaxel yields the best results. PIG alone shows effective.
2014[15]	PEG-Fe_3O_4	30	Photothermal therapy with near-infrared light for cancer, using HeLa cells.	Promising results with low cytotoxicity and high biocompatibility.
2014[16]	Cinnamaldehyde tagged Fe_3O_4	20	Administration of cinnamaldehyde using nanoparticles for breast cancer.	Nanocoating increases bioavailability of cinnamaldehyde and results in reduced viability of breast cancer cell lines.
2014[17]	MMS encapsulating Fe_3O_4	150	Efficacy of MMS nanoparticles in HeLa cells; combination of chemotherapy and hyperthermia.	Even though drug release is slow at blood pH, generation of heat can be effectively generated.
2014[18]	SPIONs@Au	6.8	Evaluation of combined DOX chemotherapy and SPION hyperthermia for cancer.	Promising results in cancers of two different cell lines.
2014[19]	Fe_3O_4	12	Investigation on temperature and pH responsivity of iron oxide carrying DOX in HeLa cells.	DOX was released effectively with both pH and temperature changes.
2014[20]	Fe_3O_4 doped with Co(II)	7	Doping with Co(II) and efficacy of heat mediation.	Increased apoptosis after doping; potential improvement as a theranostic platform.
2014[21]	Ferucarbotran (Resovist)	-	MNP administration with cisplatin in terms of efficacy and dosing.	Potential reduction in dosing of cisplatin with hyperthermia, to induce the same ablation effects.
2014[22]	SPIONs	10±3	Efficiency of SPIONs loaded with curcumin and 5-fluorouracil and coated with folate/transferrin.	Remarkable multimodal efficacy; initiation of both early and late apoptosis
2014[23]	Pluronic F127-chitosan	300	Thermally responsive MNPs loaded with curcumin for adenocarcinoma in human prostate cells.	Only mild hyperthermia is required to induce a 7-fold reduction in tumour viability.
2014[24]	Fe_3O_4	11	Evaluation of the efficacy of bortezomib (BZ) with MNP hyperthermia for cancer cell lines.	Combination therapy provided the best results, both in sensitive and resistant lines.
2014[25]	Fe_2O_3	13	Dextran coated MNPs for targeted delivery.	Aptamer tagged SPIONs can be applied at substantially lower doses than non-targeted MNPs.
2013[26]	Fe_3O_4	97	Design of a platform for concurrent chemotherapy delivery and mild hyperthermia in ovarian cancer cells.	Most effective treatment when combining hyperthermia with chemotherapy.
2013[27]	Au – coated Fe_3O_4	30	Photothermal ablation of a human pancreatic cell line.	Phagocytosis of Au shells and more efficient and targeted

				ablation, with minimal tissue damage.
2013[28]	SPIONs	-	Investigation of magnetic thermochemotherapy combining MNP hyperthermia with methotrexate	Great potential for clinical application; therapeutic results more significant in the combined approach.
2013[29]	Citric acid covered SPIONs	8-10	SPIONs as a theranostic tool in cancers of HeLa cell lines.	Low cytotoxicity and high specificity, with great theranostic value.
2013[12]	Fe_3O_4	-	Investigation of genotoxicity in human skin and lung epithelial cell lines.	A degree of genotoxicity observed.
2013[30]	Fe_3O_4	72	Investigation of the mechanism of potentiation of chemotherapy with MNP hyperthermia.	MNP hyperthermia greatly increases cell membrane permeability of the tumour to external therapeutic agents
2013[31]	Fe_3O_4	11±2	5-fluorouracil-loaded magnetoliposomes for combined hyperthermia and chemotherapy in a human colon cancer cell model.	High loading capability, and hyperthermia-triggered burst release suggest potential benefits in combined anti-tumour therapy.
2012[31]	Silver nanoparticles (AgNP)	10	Combined AgNP and radiation therapy for glioma cells on human cell lines.	AgNPs enhance the effect of radiation with hyperthermia and result in increased ablation of the tumour.
2013[33]	Fe_3O_4	10-20	MNPs in the treatment of microscopic tumours in human prostate cells with hyperthermia.	Minimum tumour threshold of 1 mm^3 below which MNP hyperthermia is ineffective.
2013[34]	FePt (alloy composition: $Fe_{34}Pt_{66}$)	12±1	Photothermal cancer therapy via femtosecond-laser-excited MNPs.	FePt NPs are very versatile and a viable option for cancer therapy.
2012[35]	Fe_2O_3	10±3	Co-nanoencapsulation of MNPs for breast tumour treatment.	Potential therapeutic effects.
2012[36]	FeSi	5-30	Efficacy of MNP microspheres in hyperthermia for cancer on rat cultured cell lines.	Biocompatible but **no** significant inhibition to tumours was observed.
2012[37]	Fe_3O_4	10	Herceptin conjugated and docetaxel loaded MNPs for treatment of cancer.	Combination of targeting, chemotherapy and MNPs hyperthermia is significantly better in ablating tumours
2012[38]	Fe or Pt-Fe nanoparticle polymers	14	Bladder cancer therapy using conjugated cisplatin inside polymeric nanoparticles.	Better delivery system, combined with SPION-induced hyperthermia.
2012[39]	SPIONs and ferromagnetic NP	13 & 44	Effect of MNPs on the living rate of cultured human breast cancer cells.	Ferromagnetic NPs showed a higher heating efficiency than SPIONs.
2012[40]	Gold nanoparticles (Au NPs)	10	Investigation on stability of antibody-conjugated AuNPs for cancer therapy.	AuNP solubility is pH dependent and exposure to radiofrequency based field leads to dissipation of energy as heat.
2011[41]	Greigite (Fe-S)	50-100	Application of greigite MNPs for human cell line adenocarcinomas.	Greigite MNPs were able to induce more damage with hypothermia to cancerous
2011[42]	$FeFe_2O_4$	9.4	Docetaxel-embedded magnetoliposomes (DML) for human cancer cell lines implanted in mice.	The tumour cell death rate increased in the group injected with DML

Table 2. The use of magnetic nanoparticles for cancer hyperthermia *in vivo,* in the last five years. Keys: Au-coated iron oxide superparamagnetic nanoparticles, SPIONs@Au, Ultra-small superparamagnetic iron oxide, USPIO.

Year/Ref	Nanoparticle	Size (nm)	Experiment	Outcome
2015[43]	$Mn_{0.4}Zn_{0.6}Fe_2O_4$	4-26	Test of the biocompatibility and anti-tumour effect of the nanoparticle in mice.	Reduce the weight and volume of in vivo and xenograft live tumours.
2014[44]	Au nanorods	10	Evaluation of therapeutic response of photothermal therapy mice.	Anti-tumour effects and appropriate for image-guided assessment of therapy.
2015 [45]	USPIO	<20	Injection of USPIOs to lymph nodes in the neck of rabbits containing metastases and application of hyperthermia.	100% tumour regression (USPIO group) vs. 20% (control group).
2015 [46]	MF66	12	Comparison of MF66, MF66-N6L, MF66-DOX (doxorubicin) and MF66-N6LDOX in anti-cancer hyperthermia.	Enhancement of MF66 with N6L and, especially DOX, strongly increases cytotoxicity to tumour cells.
2015 [47]	Fe_3O_4 core, Au - coated	12-15	Development of a new tumour targeting PEGylated gold nanoshell delivery system of DOX.	Promising theranostic results and no toxicity recorded.
2015 [48]	FeS	32-36	PEG-FeS for cancer theranostics.	Highly effective photothermal tumour ablation and no appreciable toxicity.
2014 [49]	Fe-powder-dispersed PLGA (Fe/PLGA)	-	Injectable smart phase-transformation nanoliquid for hyperthermia.	The properties of the liquid prove highly effective in tumour regression.
2014 [50]	Ferucarbotran (Resovist)	70.3±31.5	Comparison of therapeutic effect of MNPs or DOX alone vs. in combination.	Combining Resovist with DOX results in maximum ablation.
2014 [51]	Fe_3O_4	10.5	MNPs for hyperthermia and controlled DOX release for multiple myeloma.	Destruction of the entire tumour and complete cure, without recurrence.
2014 [52]	Fe_3O_4	19	Dynamic interactions of PEGylated Fe_3O_4 with the tumour milieu.	Improved drug penetration and ability to modify tumour stroma after hyperthermia.
2014 [53]	Fe_3O_4	-	Use of MNP induced hyperthermia for oxygenation of hypoxic tumour tissue.	Increased oxygen delivery and thus, potential for better drug delivery in combination with MNP hyperthermia.
2014 [54]	Starch coated Fe_3O_4	100 (total particle size)	Evaluation of MNP hyperthermia for metastatic spine disease in rats.	Effective clearance of the tumour without damage to spinal cord or lymphatics of the area
2014 [55]	Fe_3O_4	110 (total particle size)	Comparison of MNP hyperthermia and microwave hyperthermia.	Both result in equal ablation, but MNP hyperthermia is more targeted, destroying fewer healthy cells.
2014 [56]	Nano-iron	-	Action of MNP hyperthermia on rat brain gliomas.	Significant shrinkage in brain gliomas.
2013 [57]	Fe_3O_4	50	Ability of MNP hyperthermia to enhance cisplatin chemotherapy.	Combination of cisplatin with MNP hyperthermia is

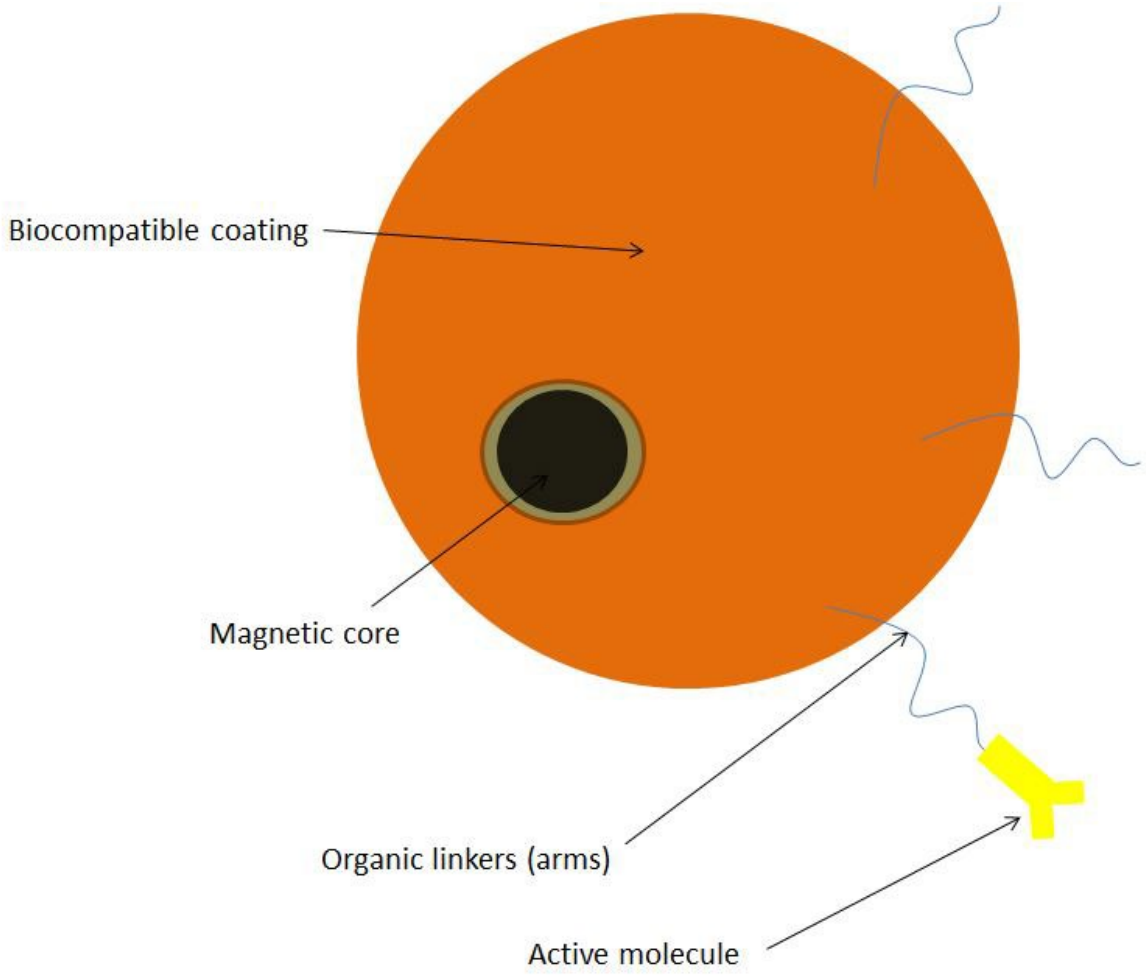
				significantly safer and more therapeutic.
2013 [58]	Fe_3O_4	50-150	Targeted hyperthermia in a VX_2 rabbit liver tumour model.	Feasible treatment, without significant effect on healthy liver parenchyma.
2013 [59]	Fe_3O_4	20	Magnetic fluid hyperthermia for bladder cancer in rats.	Well-localised heated in bladder lumen, ablation of neoplasm and minimal heating to surrounding tissue.
2013 [60]	Magnetite SPION	7-9	Theranostic applications of SPIONs in mice with tumours.	Reduction of tumour volume to a tenth of the original size 35 days after treatment.
2013 [61]	Fe_3O_4	-	Inhalable EGFR-targeted MNPs for hyperthermia in non-small cell lung cancer in mice.	Promising results, good specificity for tumour site and inhibition of growth.
2013 [62]	Au NP-TNF (CYT-6091)	-	Delivery of vascular disrupting agents inside gold nanoparticles for mice cancer.	Significant improvement in cancer therapy by rendering tumour vasculature susceptible to subsequent insults.
2012 [63]	$La_{1-x}Sr_xMnO_3$	-	Investigation of the effectiveness of manganese perovskite for cancer hyperthermia.	Manganese perovskite is effective as an inducer of tumour hyperthermia.
2012 [64]	$Gd_{0.01}Fe_{2.99}O_4$ – $Gd_{0.04}Fe_{2.96}O_4$	12-33	Use of tailored nanoparticles for tumour hyperthermia in mice.	A first cycle of treatment ablated most of the tumour. A second cycle resulted in complete regression for at least 5 years.
2012 [65]	Fe_3O_4	30	Chitosan encapsulated MNPs for cancer hyperthermia in mice.	Eradication of malignant tissue through caspase-mediated apoptosis, without any severe toxicity to healthy tissue.
2012 [66]	γ-Fe_2O_3	18	Mediated drug release from MNPs by alternating magnetic current for treatment of liver tumours in rabbits.	Effective dual therapy employing hyperthermia and chemotherapy.
2012 [67]	Fe_3O_4	-	Use of tumour homing cells loaded with MNPs for hyperthermia treatment in mice.	Increased survival after tumour transplantation up to 31%.
2012 [68]	$La_{1-x}Sr_xMnO_3$	-	Testing the use of manganese perovskite nanoparticles for cancer therapy in mice.	Manganese perovskite nanoparticles are an effective inducer of hyperthermia.

Table 3. The use of magnetic nanoparticles for cancer hyperthermia in vivo and *in vitro*, in the last five years.

Year / Ref	Nanoparticle	Size (nm)	Experiment	Outcome
2015 [69]	Fe_3O_4@Au nanostars	5.3	Use of theranostic nanoplatforms for diagnosis and treatment of cancer.	Photothermal ablation of tumour cells both in vivo (xenografts) and *in vitro.*
2014 [70]	*Magnetosomes (MNs) from Magnetospirillum gryphiswaldense*	35-50	MNs in thermotherapy in an *in* vitro model of colon cancer.	MNs increase the efficacy of thermotherapy.
2014 [71]	γ-Fe_2O_3	10-20	Investigation of efficacy of Fe_2O_3 magnetic induced hypothermia for hepatocarci-noma.	Promotes apoptosis of tumour cells by decreasing mutant p53. Also enhances Bax expression and Hsp70.
2014 [72]	Mn-Zn ferrites	15	Evaluation of PEGylated Mn-Zn ferrite nanocrystals for cancer theranostics.	Prolonged hyperthermia ablates tumour, inhibits angiogenesis and suppresses further neoplasia.
2014 [73]	$ZnFe_2O_4$	23	Delivery of lethal miRNA (let-7) within MNPs for enhanced apoptosis in brain cancer cells.	miRNA delivery in MNPs followed by magnetic hyperthermia is significantly more effective than either of the therapies alone.
2014 [74]	Fe@Fe_3O_4	8.9	PEGylated Fe@Fe_3O_4 for tumour targeting, imaging and photothermal therapy.	The MNP has intrinsically high thermal conversion and ablates tumour cells.
2014 [75]	Starch coated Fe_3O_4	100	Interaction of MNP induced hyperthermia with responses from the immune system.	After hyperthermia, a marked response of $CD8^+$ T cells acts as an anti-tumour response, reducing risk of metastasis and recurrence.
2014 [76]	Fe_3O_4 crystals	5-15	Mutlifunctionality of polypyrrole@Fe_3O_4 nanoparticles in HeLa cells and nude mice.	Potential role for thermal imaging, MRI and photothermal ablation of cancer cells.
2014 [77]	$Zn_{0.4}Fe_{2.6}O_4$	15	MNPs used for overcoming resistance apoptotic resistance.	High efficacy resistance-free hyperthermia both in vivo and *in vitro*
2014 [78]	Fe_3O_4	-	^{188}Re labelled folate targeting albumin MNPs with cisplatin for the treatment of ovarian cancer using $SKOV_3$ cells and mice.	Hyperthermia, chemotherapy and targeted radionuclide radiation inhibit growth of ovarian cancer.
2013 [79]	$MnFe_2O_4$	1	Thermography as a theranostic tool.	Promising results both in terms of early cancer detection and hyperthermia treatment for subcutaneous tumours.
2014 [80]	Au-coated silica	120	Photothermal cancer therapy in mice and *in vitro* melanoma cells.	Potential for ablation, alongside imaging.
2014 [81]	Au shells coated in iron oxide doped silica	180	Theranostic approach for pancreatic cancer.	Specific targeting and successful photothermal therapy.
2013 [8]	Fe_3O_4	11	Intravenous MNP injections for tumour ablation with hyperthermia.	Appropriate choice of MNP allows good and targeted concentration to the site of tumour, making hyperthermia effective.

2013 [82]	Fe_3O_4	-	Carboxymethyl chitosan (CMCTS) for stabilization of MNPs.	More targeted drug delivery, increased tumour cell drug uptake reduced toxicity after CMCTS addition.
2013 [83]	$Mn_{0.5}Zn_{0.5}Fe_2O_4$	15-20	MNP hyperthermia combined with radiation for cancer therapy.	Combined therapy provides the best results.
2013 [84]	$Mn_{0.5}Zn_{0.5}Fe_2O_4$	15-20	Therapeutic effect of MNPs with radiation on hepatomas.	Viable approach for the treatment of cancer.
2013 [85]	Fe_3O_4	100	Novel administration for peritoneal tumours and involvement of tumour-associated macrophages (MΦ).	Intra-peritoneal injections of MNPs are more accessible to tumour-associated MΦ and result in greater MNP concentration at the site of tumour.
2012 [86]	Fe_3O_4	5	Encapsulated chemotherapy agents and on-demand drug release.	Induction of burst-like release of the contents of the nanoparticle for precise control of the drug.
2012 [87]	Fe_3O_4	14-24	Fighting the problem of drug resistance in tumours using multifunctional MNPs.	MNPs with hyperthermia may be associated with reversal of multidrug resistance in leukaemia.
2011 [88]	As_2O_3	100	Use of thermosensitive magnetolipo-somes for hepatoma treatment.	Strong anti-hepatoma role, with tumour shrinkage to around a tenth of its original size.
2011 [89]	Silver nanoparticles (Ag NPs)	21, 53,137	Effect of Ag NPs of different sizes on gliomas.	All Ag NPs exhibited cytotoxicity and genotoxicity against tumour cells, but the effects were most prominent for small- sized Ag NPs.
2011 [90]	Fe_3O_4	~20	Investigation of hyperthermia treatment of pancreatic cancer in mice.	The technique was both feasible and effective, significantly prolonging the life of the mice in which it was applied

Figure 1


Biocompatible coating
Magnetic core
Organic linkers (arms)
Active molecule

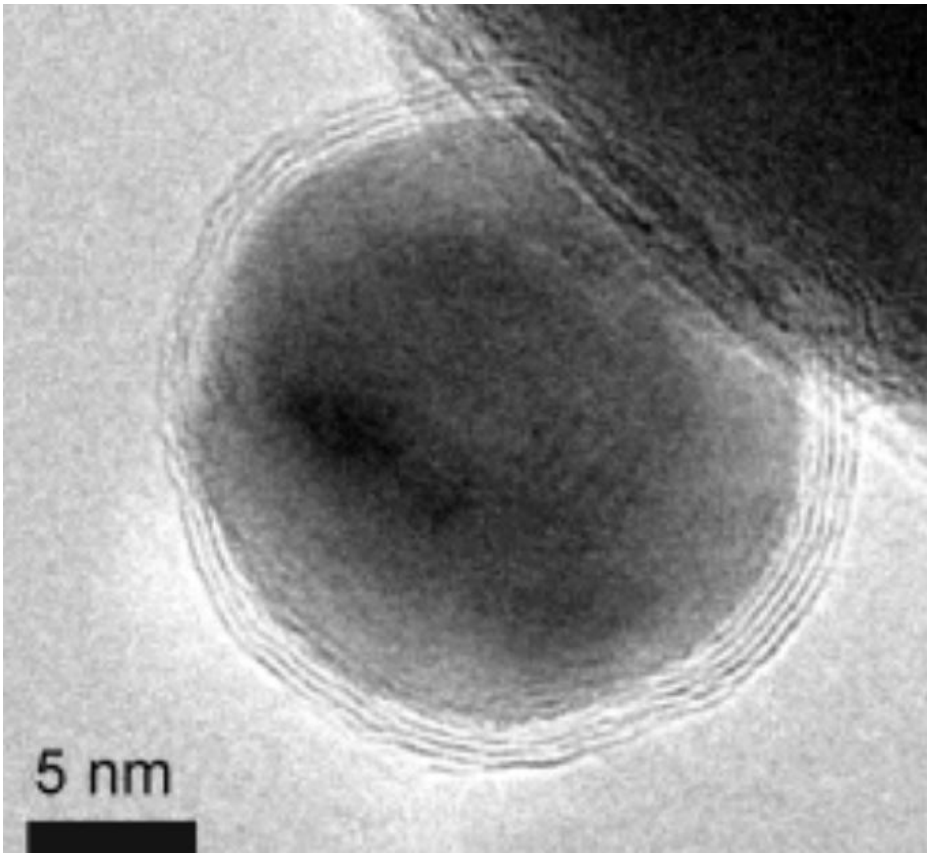

5 nm

Figure 2

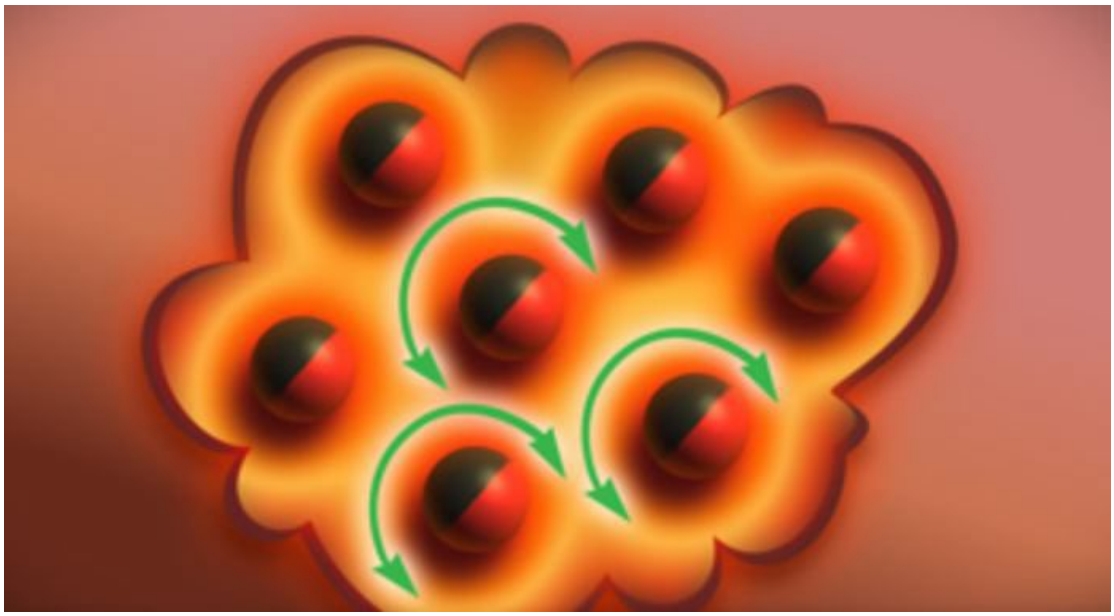
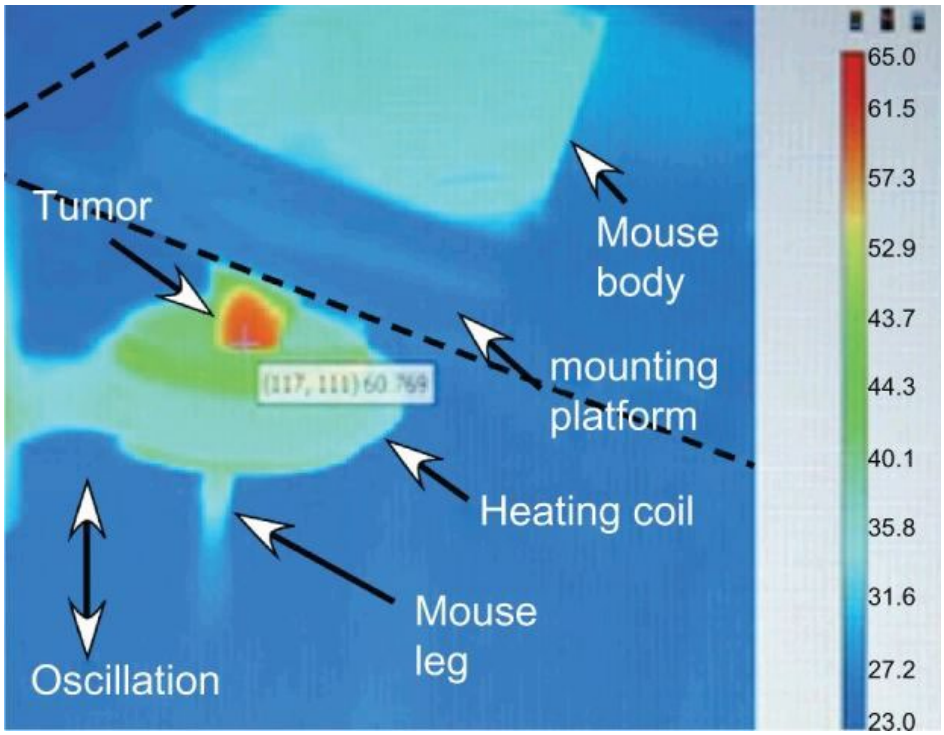


Figure 3


Tumor
Mouse body
mounting platform
Heating coil
Mouse leg
Oscillation
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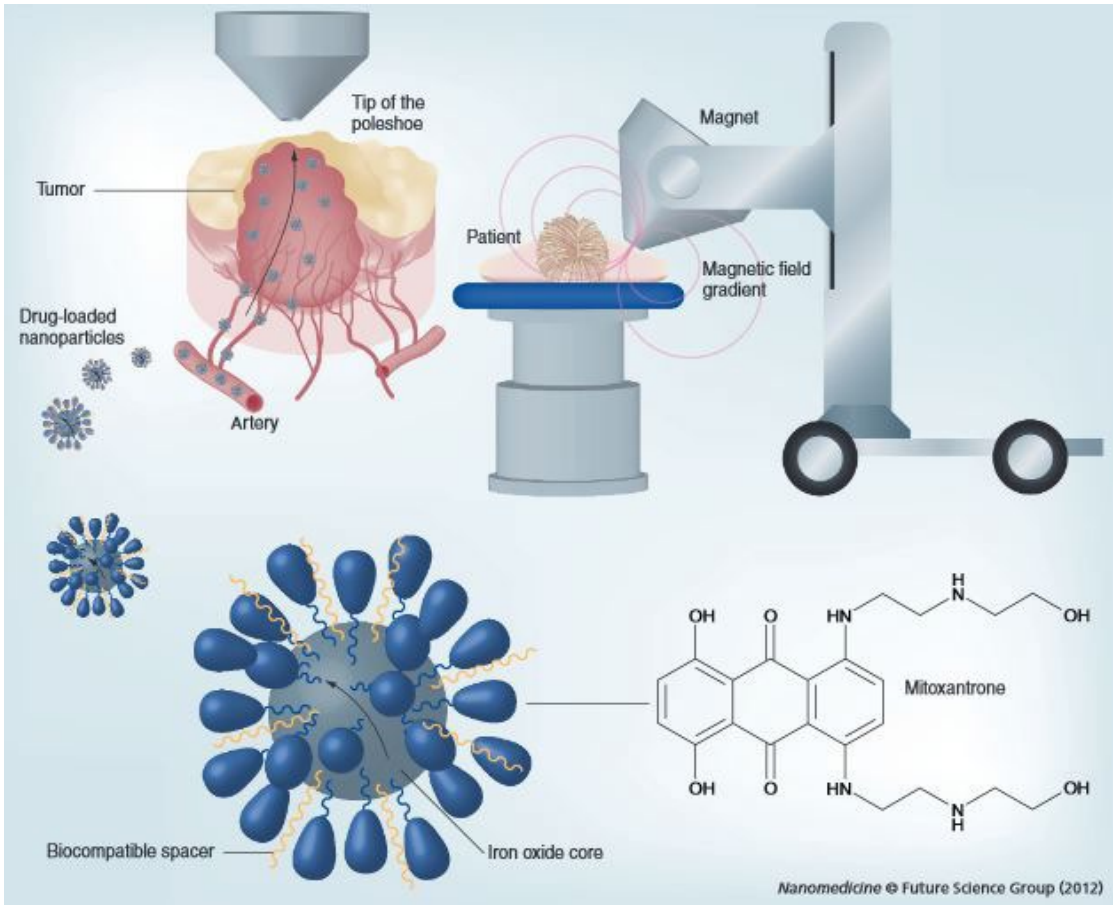
23.0

Figure 4


Tip of the poleshoe
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Drug-loaded nanoparticles
Artery
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Patient
Magnetic field gradient
Biocompatible spacer
Iron oxide core
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Nanomedicine © Future Science Group (2012)

Figure 5