Review

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Imaging modalities using magnetic nanoparticles – overview of the developments in recent years

Abstract: The use of nanoparticles in tumor imaging, molecular imaging, and drug delivery has significantly expanded in the last few years. The relatively new field of "theranostics" combines their capacity for drug delivery with their potential as contrast agents. Depending on the imaging modality used, several types of nanoparticles are available, such as gold for optical imaging or superparamagnetic iron oxide for magnetic resonance imaging. This review will give a short overview of the different types of nanoparticles as well as their development and potential application in recent years. Furthermore, it describes the research on classic imaging modalities as well as on new techniques to image nanoparticles *in vivo* and focuses on magnetic-based imaging modalities.

Keywords: magnetic particle imaging; magnetorelaxometry; multifunctional nanoparticles; nanoparticles.

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1 Introduction

The term "nanoparticles" usually refers to solid particles between 1 and 100 nm in diameter (Figure 1) [1].

Because the production method and size of magnetic nanoparticles influence their relaxometric properties, Roca et al. concluded that, for new advanced contrast agents, highly crystalline magnetic nanoparticles would be desirable [2]. They also observed that r_2 values increased with saturation magnetization as a consequence of particle size at 1.5 T. Samples with larger particle sizes showed different r_2 values but similar saturation magnetization, which affected initial susceptibility values at low field strengths [2].

The orbital motions and spins of a material determine its magnetic characteristics [3]. The key factors in magnetic moment are the electron's spin and its angular momentum, which determine how the magnetic forces of each atom influence one another in solid materials. Due to their extremely small particle volume, magnetic nanoparticles possess superparamagnetic properties. One consequence is that even fluctuations in thermal energy may be sufficient to change the magnetic moment spontaneously. An electromagnetic field will align the magnetic moments as in normal paramagnetic materials (Figure 2). However, the magnetic moment of superparamagnetic nanoparticles is up to 10^4 times larger than that of paramagnetic materials [4].

Magnetic nanoparticles based on iron oxide are currently being investigated in clinical trials for their potential as contrast agents in magnetic resonance imaging (MRI) [4].

2 Materials

Various applications such as molecular imaging and drug delivery have taken advantage of the special characteristics of polymer-coated metal nanoparticles. Such materials include synthetic polymer-coated cores for molecular imaging [5, 6], gold nanoshells for therapy, carbohydrate-coated iron oxide nanoparticles for magnetic applications [7–9], and biocompatible quantum dots [10, 11].

2.1 Gold

Nanoparticles based on colloidal gold are useful for various applications in cell biology. Kim et al. showed

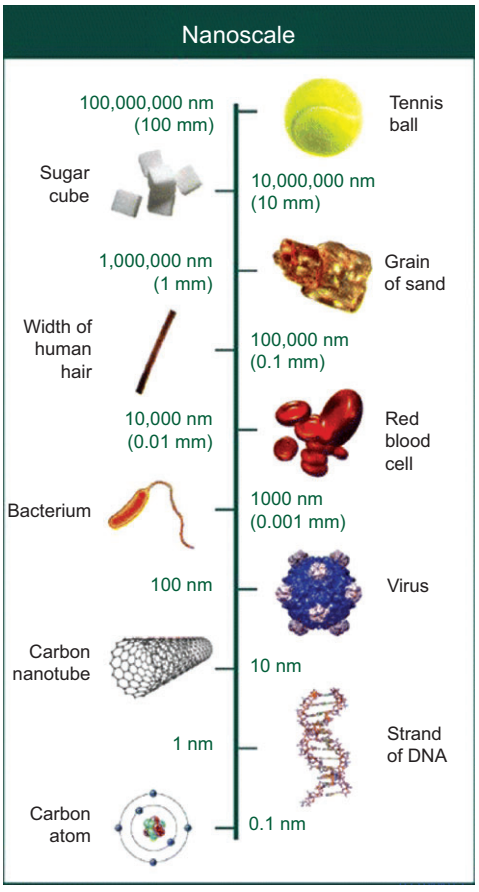

Figure 1 Nanochart showing relative scales of nanosized items to everyday objects [1].
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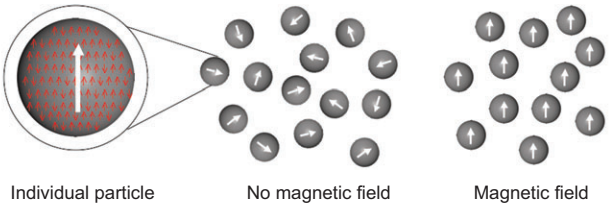

Figure 2 Schematic representation of a superparamagnetic particle.
Note that although the moments within each particle are ordered (red arrows), the net magnetic moment of a system containing MNPs will be zero in the zero field and at high enough temperatures. In the presence of a field, there will be a net statistical alignment of magnetic moments [4]. Permission/license was obtained from RightsLink; license number: 3091760402470. Reproduced with kind permission from the Royal Society of Chemistry.

in animal models that polyethylene glycol (PEG)-coated gold nanoparticles used as a computed tomography (CT) contrast agent overcame some limitations of iodine-based contrast agents, such as short imaging times, renal toxicity, and vascular permeation [12]. With gas chromatography-mass spectrometry, it was shown that functionalized gold nanoparticle sensor arrays could be used to differentiate between volatile organic compounds in breath samples from healthy volunteers and patients suffering from different forms of cancer, such as lung, breast, colorectal, and prostate cancers [13].

Aptamers have several advantages over traditional antibody-based applications for molecular targeting [14, 15], and they also have been used for such applications. One promising approach is the development of aptamer-based gold nanoparticles as contrast agents, where the aptamer serves as a molecular targeting agent and the gold particles provide the image contrast. This contrast agent has been successfully used for the detection of prostate-specific membrane antigen, and its design strategy can easily be modified to incorporate multifocal agents as part of a multimodal platform [16].

Some applications in cancer imaging and therapy take advantage of the ability of metal nanoparticles to absorb and scatter electromagnetic radiation, resulting in the possibility of a dual use for molecular imaging and cancer treatment. Gold nanorods have already been used for cancer cell imaging and photothermal therapy [17].

In another study, an all-optical method based on photothermal interference contrast was used to visualize membrane proteins labeled with gold nanoparticles. The high sensitivity of this method and the stability of the signal allowed the 3D imaging of individual nanoparticles without the drawbacks of photobleaching and blinking inherent to fluorescent markers [18].

2.2 Liposomal nanoparticles

Liposomes are phospholipid vesicles that are widely used as pharmaceutical agents. They make it possible to image a broad range of pathologic processes with CT or positron emission tomography (PET) once they have been labeled with radioisotopes. Liposomes have a half-life of approximately 6–12 h. This allows them to accumulate in target cells [19], after which they are cleared and excreted by the kidneys. The formulation of liposomes for cancer detection depends on the location and type of the cancer. The highest uptake of diethylenetriaminepentaacetic acid (DTPA)-labeled PEGylated liposomes was found in head-and-neck cancer, with lesser uptake for lung cancer and considerably lower uptake for breast cancer [20, 21]. Several studies have shown that ^{111}In-labeled liposomes can be used to detect skin cancer, AIDS-related Kaposi's

sarcoma, soft-tissue sarcoma, metastatic brain tumors, and even glioblastomas [19–21].

2.3 Magnetic nanoparticles

Ultrasmall iron oxide particles were initially developed for magnetic resonance lymphography. After intravenous administration, they are taken up by macrophages in the lymph nodes, where they accumulate. They can be detected by a reduction of the signal intensity in T_2- and T_2^*-weighted sequences due to the susceptibility effects of the iron oxide [22].

Magnetic nanoparticles have been shown to be biocompatible [23] and have low clinical toxicity [24]. The use of lymphotrophic superparamagnetic nanoparticles to identify lymph node metastases in prostate cancer increased sensitivity to 100% compared with 71.4% if conventional MRI was used [24]. These were among the first nanomaterials to be clinically tested [25].

Lee et al. produced antifouling polymer-coated superparamagnetic iron oxide nanoparticles (SPIONs) that enabled them to detect tumors *in vivo* using T_2-weighted fast spin-echo and T_1-weighted spoiled gradient echo sequences for MRI at 1.5 T [26]. Synthesis of magnetic iron oxide nanoparticles [27] as well as their characteristics [28] has previously been described.

2.4 Hybrid nanoparticles

The dual use of magnetic nanoparticles as a combined drug delivery and contrast agent for MRI is a relatively new application. Serrano-Ruiz et al. developed hybrid nanoparticles based on magnetic nanoparticles embedded in poly(e-caprolactone) that could be used as a smart coumarin delivery system, because drug release from the particles could be triggered by increasing their temperature with magnetic induction. Because these magnetic nanoparticles also introduced magnetic field inhomogeneities that could be detected by MRI, it was possible to track the particles within the body. This system could therefore be used to release doses of the drug on-demand in response to an external magnetic field after ensuring the presence of the nanoparticles in the target tissue [29].

2.5 Quantum dots

Because the size of quantum dots is in the nanometer range, they can be classified as nanoparticles. Their great advantage is that highly luminescent semiconductor quantum dots that have been covalently coupled to biomolecules for use in ultrasensitive biological detection have 20 times the brightness, 100 times the stability against photobleaching, and a 30% greater spectral line width than organic dyes such as rhodamine [30]. These characteristics make quantum dots a promising tool for microscopic protein detection in cell cultures.

Using quantum dots linked to immunoglobulin G and streptavidin, the breast cancer marker Her2 was labeled on the surface of fixed and live cancer cells [31]. It was even possible to detect two cellular targets with one excitation wavelength by using quantum dots with different emission spectra conjugated to immunoglobulin G and streptavidin [32], demonstrating that probes based on quantum dots could be very effective in cellular imaging, in addition to having advantages over organic dyes in multiplex target detection.

Multifunctional nanoparticle probes based on semiconductor quantum dots have now been developed for cancer targeting and imaging in living animals. For example, Gao et al. used subcutaneous injection of quantum dot-tagged cancer cells and systemic injection of multifunctional quantum dot probes to obtain sensitive multicolour fluorescence images of cancer cells *in vivo* [33].

3 Nanoparticle imaging

3.1 MRI

Several modalities are available for anatomical, vascular, and tumor imaging, including PET, ultrasonography, CT, and MRI. MRI is best option due to its accuracy, the possibility to generate 3D images, excellent spatial resolution, optimal contrast with soft tissue, very good signal-to-noise-ratios, and the fact that patients are not exposed to radiation. However, MRI is usually time-consuming, and depending on the goal of the investigations, many sequences require the use of contrast agents. Nevertheless, the US National Cancer Institute has identified *in vivo* molecular imaging as an extraordinary opportunity for the noninvasive study of diseases at the molecular level [33].

3.2 Contrast agents

Most contrast agents for MRI are based on chelates of Gd^{3+} compounds and require the use of T_1-weighted sequences.

Gd^{3+} compounds have seven unpaired electrons, resulting in high magnetic moments and the possibility of detection at micromolar concentrations, which are very much lower than those required for the iodine-based contrast agents used in CT.

However, SPIONs can also be used as contrast agents for MRI, although they require the use of T_2-weighted sequences. SPION-based contrast agents are composed of 3–5 nm particles of iron oxide, which are coated with dextran, starch, or citrate [34]. Superparamagnetic nanoparticles were first used in 1985 as a contrast agent for MR investigations of the reticuloendothelial system [35]. Further development led to the use of more suitable coatings and reduction of the hydrodynamic diameter and to the term ultrasmall superparamagnetic iron oxide (USPIO) for iron oxide-based nanoparticles of diameters less than 50 nm. USPIO particles have a long plasma half-life of approximately 24 h and are eliminated from the circulation by the reticuloendothelial system [24]. Due to their half-life and small size, USPIO particles are capable of penetrating tissues such as myocardium, atherosclerotic plaques, and tumors [34]. Sparsely expressed targets can be detected *in vivo* with sufficient sensitivity [34] because of the high relaxivity (R_2=50 mm l^{-1} at 0.5 T) [36]. Whereas Gd^{3+}-based contrast agents increase the T_1 signal where they accumulate and can therefore be referred to as "positive contrast agents," superparamagnetic nanoparticles cause a decrease in the T2 signal, so that they can be regarded as "negative contrast agents."

A great advantage of SPIONs is their safety at the concentrations that are normally used. SPIO has an effective dose/LD_{50} ratio of 1:2400, whereas the safety margin of Gd-DTPA, a gadolinium-based contrast agent for MRI investigation, is much less with an effective dose/LD_{50} ratio of 1:50 (Gd-DTPA) [37].

3.3 Micro-CT

CT is an X-ray-based technique using an X-ray source that rotates around the object. Micro-CT can be used for nanoparticle imaging in medical research. The technique is identical to clinical CT, with the difference that small experimental animals such as rats can be scanned.

Superparamagnetic nanoparticles have been used as contrast agents for MRI for at least 20 years [38], but they can also be used as a CT contrast agent for micro-CT [39]. Using 3D analysis with this technique, Tietze et al. demonstrated how iron nanoparticles are spatially arranged inside intact blood vessels [40].

3.4 PET

PET is based on short-lived radioactive tracer isotopes that are chemically bound to a carrier substance. Several radionuclides are available, with half-lives ranging from approximately 1 min to 2 h. Because ^{18}F has the longest half-life and is widely available, it is often used as a radioactive tracer. However, labeling functionally complex materials with ^{18}F is still very challenging. Devaraj et al. were among the first researchers to synthesize and characterize an ^{18}F-labeled aminated cross-linked dextran iron oxide (^{18}F-CLIO; Figure 3) [41]. These particles consisted of cross-linked dextran held together in core-shell formation by a superparamagnetic iron oxide core and could be detected with PET, fluorescence molecular tomography, and MRI [41].

4 Development

4.1 Imaging of magnetic nanoparticles

SPIONs were first synthesized for magnetic imaging in the 1980s. This ensured high relaxivity (2.4×10^5 s^{-1} $м^{-1}$ l), good chemical stability, singular biodistribution, and a considerable safety margin as shown by its efficacy dose to lethal dose ratio of 1:2400 compared with 1:50 for Gd-DTPA [37].

In 1994, Halavaara et al. investigated the value of superparamagnetic iron oxide particles for the detection of focal liver lesions using MRI at 1.0 T. They found that the iron oxide contrast medium had significant effects on liver signal-to-noise ratios and tumor-to-liver contrast-to-noise ratios and reported that lesion-to-liver contrast improved markedly after infusion of superparamagnetic nanoparticles using T_1, T_2, proton density-weighted spin-echo and short T_1 inversion recovery sequences. This showed that superparamagnetic iron oxide represented a promising contrast medium for MR examinations of the liver [42]. Ferumoxides-enhanced MRI of the liver showed a trend toward higher than dual-phase helical CT [43]. However, the specificity of helical CT was superior to that of enhanced MRI [43].

The accuracy of contrast-enhanced MRI techniques was compared with that of spiral CT, as used in preoperative assessment of colorectal liver metastases with arterial portography. It was shown that this noninvasive MRI technique is safe and as accurate as spiral CT under these conditions. It was also found that this MRI technique resulted in comparable clinical decisions and outcomes after hepatectomy [44].

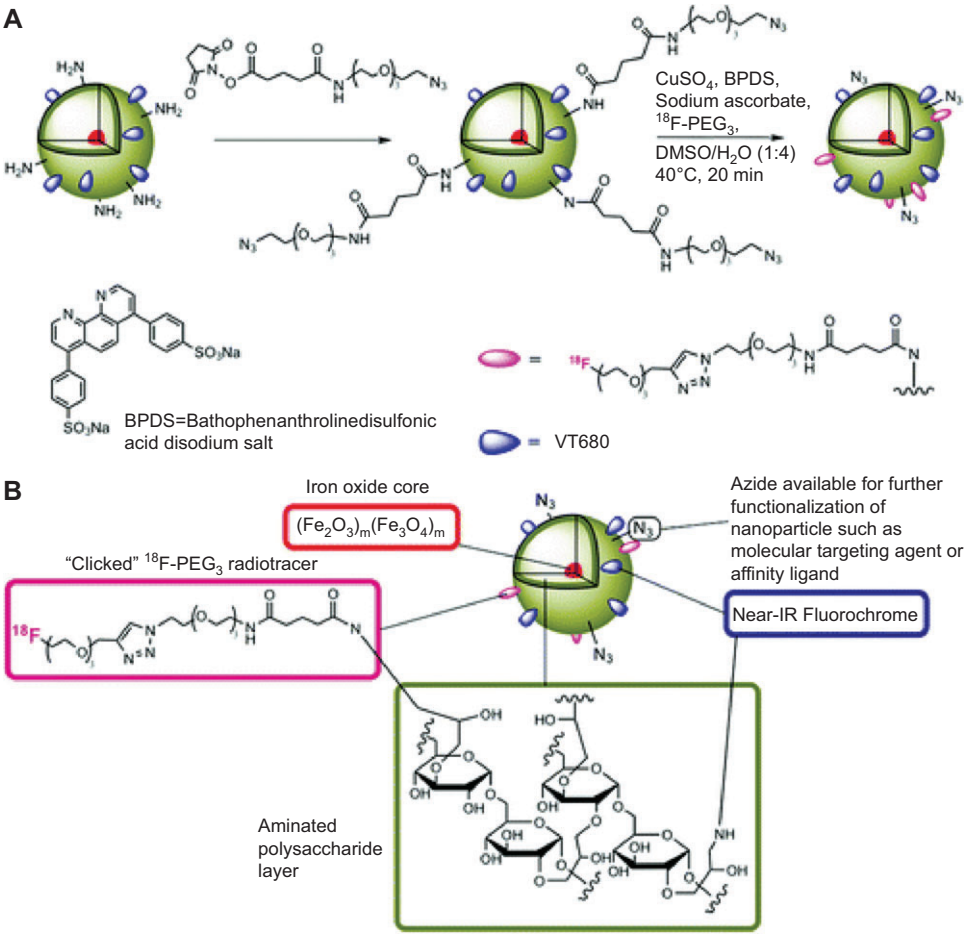

Figure 3 Preparation of ^{18}F-CLIO.
(A) Derivatization of primary amines on CLIO-VT680 with the NHS ester of 1-azido-13-oxo-3,6,9-trioxa-12-azaheptadecan-17-oic acid followed by chemoselective "click" of ^{18}F-PEG3 radiotracer. (B) Schematic of ^{18}F-CLIO [41]. Permission/license was obtained from RightsLink; permission/license is granted. Reproduced with kind permission from the American Chemical Society.

Onishi et al. compared the accuracy of contrast material-enhanced multidetector row CT, superparamagnetic iron oxide-enhanced MRI, and both modalities together in detection of hepatic metastases and concluded that addition of superparamagnetic iron oxide MRI to contrast-enhanced multidetector row CT improved the sensitivity of detection of these metastases [45]. It has also been reported that CT and SPIO-enhanced MRI are more sensitive but less specific than standard PET in the detection of liver metastases [46].

In another study, the pharmacokinetics and CT imaging efficacy of colloidal gold nanoparticles were investigated as a blood-pool agent for X-ray CT in mice, and these PEG-coated nanoparticles were found to have good biocompatibility. It was also possible to visualize the vascular system immediately after injection as well as 24 h after the application of these nanoparticles [47].

Popovtzer et al. were the first to describe a targeted molecular imaging platform that enables cancer detection at the cellular and molecular levels with standard clinical CT. Their approach was based on gold nanoparticles that selectively target tumor antigens with high sensitivity and good contrast in CT imaging, and in cultured human head-and-neck cancer cell lines, they reported an attenuation coefficient for the molecularly targeted cells over five times higher than that for identical but untargeted cancer cells or for normal cells [48].

A comparison of ^{18}F-deoxyglucose (FDG)-PET, CT, and MRI, including unenhanced single-shot spin-echo planar imaging and small paramagnetic iron oxide enhancement in the context of detecting liver metastases from colorectal carcinoma, found that MRI had good sensitivity and positive predictive values for detecting such metastases and even outperformed FDG-PET for small liver metastases [49].

Tanabe et al. performed a study to determine an appropriate MR sequence for the detection of hepatocellular lesions with increased iron uptake as shown by superparamagnetic iron oxide-enhanced MRI and compared T_1-weighted in-phase gradient recalled echo images, T_2-weighted fast spin-echo images, and T_2^*-weighted gradient recalled echo images [50]. Using echo times of 7 or 12 ms at 1.5 T, they found that a T_2^*-weighted gradient recalled echo sequence with an echo time of 7 ms showed high lesion-to-liver contrast-to-noise ratios for hepatocellular lesions with increased iron uptake in superparamagnetic iron oxide enhanced MRI [50]. The 3D spatial distribution of rat cardiac progenitor cells that had been labeled with iron oxide nanoparticles was demonstrated inside the infarcted rat heart early after injection using X-ray micro-CT. Giuliani et al. also concluded that such 3D images are an improvement over experimental 2D histologic analysis [51].

Chou et al. stated that water-soluble FePt nanoparticles with sizes of 3, 6, and 12 nm could be conjugated with monoclonal antibodies for use as a dual-modality contrast agent for combined CT and molecular MRI to identify transplanted MBT2 tumors in mice [52]. These nanoparticles showed excellent biocompatibility and hemocompatibility. The highest serum concentration and circulation half-life was observed for 12 nm FePt nanoparticles, and the highest brain concentration was found with 3 nm FePt nanoparticles. The authors concluded that these alloy nanoparticles could serve as a dual-modality contrast agent for CT/molecular MRI and could be cleared from the body after about 1 week. They also showed that 12 nm FePt particles provide the highest serum concentration and circulation half-life and the highest image contrast in both CT and MRI, whereas the 3 nm FePt particles provided the highest plateau concentration in the brain [52].

Glaus et al. developed a new nanoparticle-based dual-modality PET/MRI contrast agent consisting of a superparamagnetic iron oxide core coated with PEGylated phospholipids that were radiolabeled with ^{64}Cu. They characterized the physical and pharmacokinetic properties of this tomography/MRI nanoparticle probe and demonstrated its potential as a dual-modality imaging agent with quantitative analysis of biodistribution and *in vivo* micro-PET/CT imaging [53]. Another approach used a novel bifunctional chelator containing a dithiocarbamate group for binding the PET isotope ^{64}Cu and a BP group for strong binding to Fe_3O_4 and other inorganic materials to create a dual-modality PET-MRI agent [54].

Despite the advantages of MRI, achieving high accuracy in the imaging of biological targets still remains a challenge. As described above, positive contrast agents are available that enhance signals and there are also negative contrast agents that decrease signals. A new approach combining both contrast agents was developed using dual-mode nanoparticle contrast agents (Figure 4). According to the authors, this provides the unique capability of displaying "AND" logic signals in both the T_1 and T_2 modes, enabling self-confirmation of images and leading to greater diagnostic accuracy [55]. Another recent development involves the use of gadolinium-based nanoparticles as a potential contrast agent for MRI for monitoring the migration of mesenchymal stem cells and neural stem cells *in vivo*. The nanoparticles synthesized by Shen et al. possessed hexagonal mesoporous structures with appropriate assembly of nanoscale Gd_2O_3 clusters. These had little cytotoxicity against proliferation and only a minor effect on the inherent differentiation potential of the labeled cells [56]. In recent work by Li et al., a composite agent was developed by introducing SPIONs into perfluorooctylbromide nanoparticles. *In vivo* studies based on this contrast agent found that superparamagnetic perfluorooctylbromide nanoparticles produced higher echogenicity than unmodified perfluorooctylbromide nanoparticles and possessed strong magnetic susceptibility and radiopacity for multimodal imaging. Furthermore, macrophages incubated with different concentrations of magnetic perfluorooctylbromide nanoparticles demonstrated dose-dependent cellular uptake. The authors concluded that the composite agent could be used as a multimodal contrast agent for enhanced ultrasound, MRI, and CT imaging [57].

Furthermore, it was shown that magnetoferritin nanoparticles could be used to target and visualize tumor tissues without the use of any targeting ligands or contrast agents. Using iron oxide nanoparticles that were encapsuled inside a recombinant human heavy-chain ferritin protein shell enabled. Fan et al. to distinguish cancerous

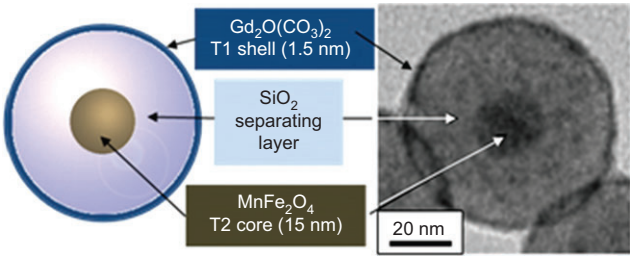

Figure 4 Schematic and transmission electron microscope image of core-shell type DMCA [$MnFe_2O_4$@SiO_2@$Gd_2O(CO_3)_2$] [55]. Permission/license was obtained from RightsLink; permission/license is granted. Reproduced with kind permission from the American Chemical Society.

cells from normal cells with a sensitivity of 98% and a specificity of 95% [58].

4.2 Multifunctional nanoparticles

Multifunctional nanoparticles combine the ability to serve as contrast agent for at least two different imaging modalities or may serve as a contrast agent plus carrier for drug targeting, detection of hyperthermia, or molecular imaging. Magnetic nanoparticles can be used as multimodal nanoprobes if their characteristics as contrast agents for MRI are supplemented by adding modality-specific tags to the magnetic core. Potential enhancements include radioactive nucleotides for PET, antibodies for molecular targeting, or optical tags. Dual-mode PET/MRI active probes that target vascular inflammation were developed in 2008 [59]. Other nanoparticles that target fibrin and activated factor XIII could be used to image thrombi using fluorescence and MRI *in vitro* and *in vivo* [60]. A similar hybrid system could also be used for the early detection of plaques in *in vivo* studies of Alzheimer's disease [61].

Thermoresponsive polymer-coated magnetic nanoparticles loaded with anticancer drugs such as doxorubicin can be used for magnetic drug targeting followed by simultaneous hyperthermia and drug release [62]. A very interesting approach for the use of iron oxide nanoparticles is the remote regulation of protein production *in vivo*. A modified TRPV1 was successfully decorated with antibody-coated iron oxide nanoparticles. Using this setup, local temperature increment caused TRPV1 to gate calcium, which resulted in the stimulation of synthesis and release of bioengineered insulin gene [63].

Nowostawska et al. investigated the use of multimodal porphyrin-magnetite nanoparticle composites for marking macrophage cells and suggested that this technique might be useful in the future for *in vitro* biological imaging [64]. Huang et al. used anti-α-fetoprotein-mediated Fe_3O_4 magnetic nanoparticles to examine the characteristics of magnetic labeling of liver tumors in mice using MRI and scanning superconducting-quantum-interference-device biosusceptometry. They concluded that magnetic labeling is also feasible during surgical operations using this technique [65]. Multifunctional Fe_3O_4-PEI-RITC was first described in 2011. Fe_3O_4-PEI-RITC magnetic nanoparticles that were rapidly taken up by astrocytes have the potential for use in bimodal MRI-fluorescence imaging [66]. Another recent study showed that iron oxide nanoparticles acting as drug carriers also played a role in decreasing tumor volume induced by hyperthermia [67]. Xu et al. synthesized few-layer, carbon-coated, iron magnetic nanoparticles and loaded them with two anticancer drugs. They were able to trigger release of the drugs with radiofrequency heating, thereby combining hyperthermia with targeted chemotherapy [68]. Miyaki et al. analyzed the suitability of multimodal magnetic nanoparticles labeled with rhodamine B for detection of cells in culture and demonstrated internalization of these nanoparticles as well as their stability and imaging properties [69]. Another recent publication deals with the attachment of DNA-binding fluorochromes to nanoparticles. This showed that TO-PRO1 attached to Feraheme nanoparticles bound to DNA *in vitro* and formed microaggregates that were characterized by fluorescence, light scattering, and T_2 changes [70].

Magnetic nanoparticle clusters have the capability to be used as both contrast agent for T_2 sequences and heating substance to target the tumor environment and the tumor itself if using hyperthermia [71]. In the last case, magnetic nanoparticles show a high efficiency of magnetic thermal induction and therefore offering a high potential for noninvasive therapies [72], which makes them an excellent choice for the combination of therapy and diagnostic [73–75].

4.3 Magnetic particle imaging

Magnetic particle imaging (MPI) is a new and promising imaging modality for applications in human or small animal angiography, cancer imaging, *in vivo* cell tracking, and inflammation imaging [76]. Narrowband MPI was first introduced in 2009 [77]. This technique reduces bandwidth requirements and increases the signal-to-noise ratio for a fixed specific absorption rate [77]. Mathematical modeling can be used to analyze MPI sensitivity and spatial resolution and suggest ways in which imaging performance could be optimized [78]. Knopp et al. performed a simulation study on different trajectories moving the field-free point through the field of view [79]. They also calculated the system function using a model of the signal chain, which enabled fast generation of system functions on arbitrarily dense grids [80]. The quality of a least-squares approach, which represents a mathematical solution to linear equations, was improved by incorporating a weighting matrix using the reciprocal of the matrix-row energy as weights [81]. In 2010, the spatial resolution of MPI was investigated by analyzing the modulation transfer function of the imaging process [82].

For image reconstruction in 3D MPI, a system function is used that describes the relation between the acquired MPI signal and the spatial origin of the signal. Rahmer

et al. carried out a detailed analysis of a measured system function to give experimental evidence that 3D MPI encodes information using a set of 3D spatial patterns or basis functions that is stored in the system function. This resulted in a simple formula that qualitatively describes the basis functions to be expected at a certain frequency [83].

MPI might be able to improve breast cancer treatment if the particles can be used as tracers for sentinel lymph nodes [84], because it has been demonstrated that SPIONs accumulate in the cortex region of lymph nodes [84].

Goodwill et al. presented an analytical description and tests of a projection magnetic particle imager that enabled reconstruction without precharacterization of the imaging tracer, harmonics, or matrix inversion techniques [85].

A stochastic dynamical model of rotating Brownian nanoparticles has also been developed using a Langevin equation approach [86]. Using this model, it was demonstrated that harmonics of the magnetization carried enough information to infer environmental parameters such as viscosity and temperature [86]. MPI also represents a technique that could be very helpful in interventional radiology. Non-signal-generating instruments such as catheters were coated with superparamagnetic iron oxide, making it possible to detect a signal [87]. Furthermore, Ferguson et al. showed that the performance of MPI could be optimized by selecting phase-pure magnetite tracers of a particular size and narrow size distribution. They found that a median diameter of 20 nm, a log-normal distribution shape parameter of 0.26, and a hydrodynamic diameter of 30 nm gave the best performance [88]. These parameters provided four times the signal intensity and 20% better spatial resolution than commercial nanoparticles [88].

4.4 Magnetorelaxometry

In 2003, a new method was developed that made it possible to characterize magnetic nanoparticles based on an analysis of the Néel relaxation signal. This method directly delivers the energy barrier distribution of the magnetic system for ferrofluid particles or their aggregates [89]. One of the first applications was the quantification of ferrofluids delivered after magnetic drug targeting. Samples of VX2 tumors were successfully measured by magnetic relaxation and the amount of iron was determined using the original ferrofluid suspension as a reference [90].

Heim et al. used magnetic nanoparticles as tracer replacements to investigate the quantity of physically entrapped nanoparticles in a hydrogel network, because superparamagnetic nanoparticles show a different behavior if they are entrapped in a hydrogel or mobile [91].

Since 2008, a method has been available for quantification of biomolecules in the form of a bead-based magnetic relaxation assay. It has been shown to be suitable for quantitative monitoring of the functionalization of magnetic nanoparticles [92].

Magnetic nanoparticles have also been used to combine cell transduction and positioning in the vascular system, where it was possible to measure the superparamagnetic behavior of transduced cells with magnetorelaxometry [93].

The accuracy of magnetorelaxometric investigations was found to be at least one order higher than necessary for the reconstruction of the magnetic nanoparticle accumulation along an artery after magnetic drug targeting [94]. This degree of accuracy is necessary for both noninvasive quantification and localization of magnetic nanoparticle accumulation to ensure that these particles are located in the tumor area before performing hyperthermal therapy [95]. It was shown that most of the magnetic nanoparticles applied within the tumor remained in the tumor region over a period of 7 days [96].

Hydrogels are currently being investigated as long-term delivery systems for pharmaceutically active biomacromolecules and it is therefore important to tailor the release behavior during the fabrication process. Fluxgate magnetorelaxometry is a tool that enables characterization of the release properties of such long-term depots [97]. Based on the use of incorporated superparamagnetic core-shell nanoparticles, magnetorelaxometry makes it possible to evaluate the influence of different cross-linking conditions during hydrogel production and to follow the increase in nanoparticle mobility during hydrogel degradation [98]. Furthermore, it was demonstrated that magnetorelaxometry could also be used to get information on the size of magnetic multicore nanoparticles [99] and that the particle size significantly affects the detection sensitivity [100].

Table 1 gives an overview of the development of multifunctional nanoparticles, MPI, and magnetorelaxometry.

5 Future directions

Past research on nanoparticles has revealed great potential in many different areas. Experience and probable outcomes make future trends for the role of magnetic nanoparticles in medical imaging look very exciting, because the technique will make it possible to design new drugs

Table 1 Summary of articles showing the development of the past years regarding multimodal nanoparticles, MPI, and magnetorelaxometry.

Multifunctional nanoparticles
Jarrett et al. [59]: Development of a PET/MRI probe using superparamagnetic iron oxide
McCarthy et al. [60]: Development of thrombosis-specific molecular imaging agents
Skaat and Margel [61]: Selective marking of $A\beta_{40}$ fibrils using superparamagnetic iron oxide
Purushotham and Ramanujan [62]: Synthesis of composite nanomaterials for hyperthermia and drug release
Nowostawska et al. [64]: Analysis of porhyrin magnetic nanoparticle composite
Huang et al. [65]: Characterization of anti-α-fetoprotein mediated Fe_3O_4
Yiu et al. [66]: Development of Fe_3O_4-PEI-RITC with MR-fluorescence imaging and transfections capabilities
Ren et al. [67]: Investigation of the efficiency of Fe_3O_4 combined with chemotherapy and hyperthermia for overcoming multidrug resistance
Xu et al. [68]: Synthesis of few-layer, carbon-coated, iron magnetic nanoparticles for controlled drug release and hypothermia
Miyaki et al. [69]: Analysis of multimodal magnetic nanoparticles-rhodamine B
Cho et al. [70]: Development of nanoparticles that bind to DNA through fluorochome-mediated interactions

MPI
Weaver et al. [98]: Exploration of the signal from magnetic nanoparticles
Goodwill et al. [77]: Introduction of narrowband-MPI
Ferguson et al. [78]: Presentation of mathematical modeling results that show the dependence of core design on physical properties
Knopp et al. [79]: Simulation study on different trajectories moving the field-free point through the field-of-view
Knopp et al. [80]: Calculation of the system function using a model of the signal chain
Knopp et al. [81]: Demonstration of how the quality of the least-squares solution can be improved
Knopp et al. [82]: Investigation of the spatial resolution of magnetic particle imaging
Rahmer et al. [83]: Presentation of a detailed analysis of a measured system function
Finas et al. [84]: Investigation of superparamagnetic iron oxide and magnetic particle imaging as sentinel lymph node biopsy tracer
Goodwill et al. [85]: "Derive" of 2-D x-space signal equation and 2-D image equation
Reeves and Weaver [86]: Development and validation of a stochastic dynamical model of rotating Brownian nanoparticles from a Langevin equation approach
Haegele et al. [87]: Test of various commercially available catheters, guide wires, and a catheter experimentally coated with SPIONs regarding signal characteristics using magnetic particle spectroscopy
Ferguson et al. [88]: Presentation of experimental magnetic particle imaging measurements acquired using a homemade magnetic particle imaging magnetometer

Magnetorelaxometry
Romanus et al. [89]: Presentation of a method for the characterization of magnetic nanoparticles based on the analysis of the dependence of the Néel relaxation signal on the sample temperature
Wiekhorst et al. [90]: Quantification of ferrofluids using magnetorelaxometry delivered after magnetic drug targeting
Heim et al. [91]: Analysis of the magnetic relaxation behavior of superparamagnetic nanoparticles used as replacement for biomolecules in hydrogels
Eberbeck et al. [92]: Quantification of the binding reaction of streptavidin and antibiotin-antibody, both labeled with magnetic nanoparticles, to biotin coated agarose beads
Hofmann et al. [93]: Use of magnetic nanoparticles for cell transduction, positioning in the vascular system and investigation of the superparamagnetic behavior of transduced cells
Richter et al. [94]: Demonstration of the potential of magnetorelaxometry for quantifying the distribution of magnetic nanoparticles in an artery
Richter et al. [95]: Demonstration of the capability of magnetorelaxometry for noninvasive quantification and localization of magnetic nanoparticle accumulation in small animal models
Kettering et al. [96]: Investigation of the biodistribution of intratumorally injected magnetic nanoparticles in mice using magnetorelaxometry
Wöhl-Bruhn et al. [97]: Investigation of the applicability of fluxgate relaxometry as a tool to characterize the release properties of hydrogels

that can be tracked *in vivo*. Furthermore, there is great potential in the field of the detection of tumor detection and follow-up monitoring.

Acknowledgments: The authors thank the Emerging Fields Initiative of the University of Erlangen-Nuremberg, the DFG (AL 552/3-3), the BMBF-Spitzencluster (01EX1012B), and the Else Kröner-Fresenius Foundation, Bad Homburg v.d.H., for their support.

Received March 12, 2013; accepted May 25, 2013; previously published online July 2, 2013

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Department of Otorhinolaryngology, Head and Neck Surgery, Section for Experimental Oncology and Nanomedicine (SEON): The SEON emerged from Prof. Christoph Alexiou's working group after he received the first chair for Nanomedicine in Germany, which was endowed by the Else Kröner-Fresenius Stiftung in 2009. The group can look back on more than 15 years of experience in the application of iron oxide nanoparticles in cancer treatment. The favored therapy approach is "magnetic drug targeting". The main goal of SEON is to enhance cancer treatment and simultaneously reducing the side effects of chemotherapy, by accumulating the nanoparticle-bound drug with strong external magnetic forces. In the nearer past, SEON has broadened its activities to the use of iron oxide particles in the treatment of arteriosclerosis and also in regenerative medicine.

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Christina Janko studied Biology at the Friedrich-Alexander-University Erlangen-Nuremberg from 2002 to 2007. After her diploma thesis in 2007, she was a PhD student in the group of Prof. Dr. Martin Herrmann at the Institute of Clinical Immunology and Rheumatology at the University Hospital Erlangen from 2007 to 2012. In her dissertation in 2012, she focused on the CRP-mediated effects in the clearance of dying and dead cells. Since 2013, she has worked as a postdoctoral research fellow in the group of Prof Dr. Christoph Alexiou in the SEON at the Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Erlangen, where she analyzes the toxicology of nanoparticles.

Philipp Tripal studied Biology at the Friedrich-Alexander-University Erlangen-Nuremberg. In September 2003, he graduated in the field of Microbiology. From 2003 to 2006, he studied for his PhD within the field of tumor research, which he received in May 2007. From 2007 to 2011, he worked as a postdoctoral research fellow for the Department of Psychiatry and Psychotherapy, studying the pharmacologic consequences of antidepressants on neuronal cells. In 2011, he moved back to tumor research. In the Department of Nuclear Medicine, he investigated the use of radioactive, tumor-specific compounds for tumor therapy and cell labeling. In January 2013, he joined the Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Erlangen. Within the SEON, he is investigating the use of magnetic nanoparticles for their application in tissue engineering and 3D cell cultures.

Iwona Cicha studied Biology at the Jagiellonian University, Cracow, Poland. After obtaining her PhD in Medical Sciences at the Ehime Medical School, Ehime University, Japan, she moved to the University of Erlangen. She was a postdoctoral fellow in the Department of Nephrology in 2003, before joining the Department of Cardiology, where she obtained her Habilitation in Experimental Medicine in 2012. Currently, she is a group leader in the Laboratory of Molecular Cardiology. She has extensive research experience in the field of atherosclerosis, with focus on the role of inflammation and blood flow dynamics in plaque development and destabilization.

Stephan Dürr earned a Medical Degree from the Friedrich-Alexander-University Erlangen-Nuremberg and received his MD at the Institute of Pathology at the University Hospital Erlangen. Since 2004, he has been working at the Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital Erlangen, where he specialized as an otorhinolaryngologist/head and neck surgeon in 2009. Within the ENT Department, he joined the SEON in 2010. There he has been working as a research fellow on magnetic drug targeting.

Christoph Alexiou received his MD from the Technical University of Munich, Medical school in 1995. After finishing his internship at the Department of Gastroenterology, University Hospital of the Technical University of Munich, he started as a physician and researcher at the Department of Otorhinolaryngology, Head and Neck Surgery and founded a research group working in the field of local chemotherapy with magnetic nanoparticles (magnetic drug targeting). In 2000, he received his degree as an ENT-Physician and in 2002, he moved to the ENT-Department in Erlangen, Germany, where he performed his postdoctoral lecture qualification (Habilitation). He worked as an assistant medical director in the clinic and lead the SEON. Since 2009 he holds the Else Kröner-Fresenius-Foundation-Professorship for Nanomedicine at the University Hospital Erlangen. His research focuses on the translation of magnetic drug targeting and the application of magnetic nanoparticles into clinical application. He has received several national and international awards for his work.

Stefan Lyer studied Biology at the Friedrich-Alexander-University Erlangen-Nuremberg. After finishing his PhD thesis at the German Cancer Research Center (DKFZ)/Ruprecht-Karls-University Heidelberg he continued as a postdoctoral research fellow at the Department of Genome Analysis at the DKFZ. In 2008, he moved back to Erlangen starting a postdoc position at the group of Prof. Dr. Christoph Alexiou at the ENT-Department of the University Hospital Erlangen, which was renamed SEON in 2009. Here, he focussed on the application of nanoparticles in cancer therapy. Since 2011, he has been assistant group leader of SEON.