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Use of Magnetic Nanoparticles in Cancer Detection with MRI

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***Abstract*—**Magnetic Nanoparticles (MNPs) have great potential to overcome many of the shortcomings of the present diagnostic and therapeutic approaches used in cancer diagnosis and treatment. This Literature review discusses the use of Magnetic Nanoparticles focusing mainly on Iron oxide based MNPs in cancer imaging using MRI.

***Keywords*—**Cancer, Imaging, Magnetic Nanoparticles, MRI.

I. Introduction

CANCER is a leading cause of death globally and is responsible for 7.6 million deaths in 2008 [1]. One of the reasons is that present diagnostic and therapeutic approaches are based mainly on invasive and crude unfocused techniques [2]. Evidence-based strategies such as cancer prevention, early detection and management of patients with cancer can be applied to decrease and control cancer [1]. Nano-medicine, application of nanotechnology to medicine, has great potential to influence various aspects of cancer diagnosis and treatment [3]. Early detection of cancer can reduce mortality and increase the chance of full recovery in numerous types of cancers [1], [3]. However, detection of cancer at an early stage remains a challenge as clinical symptoms seldom become apparent before cancer proceeds to a fatal stage [3]. Nanotechnology can lead to improved cancer management by better diagnostic imaging for various cancers and targeted chemotherapeutic drug delivery. More sensitive imaging will lead to early cancer detection and improved prognosis [4].

Magnetic Nanoparticles (MNPs) are most widely researched nanoprobes in cancer imaging. This has prompted the researcher to choose this area of study as the topic for this mini-review. The present literature review clarifies the role of Iron oxide based MNPs in cancer detection using MRI. First some physiochemical and magnetic properties of iron oxide based MNPs will be reviewed. This section will describe the physics Concepts associated with MNPs. Then an overview of clinical applications of Superparamagnetic Iron Oxide NP (SPIOs) and Ultrasmall Paramagnetic Iron Oxide Nanoparticles (USPIOs) will be provided. The next section will describe briefly the multimodality feature and Theragnostics of MNPs. Finally the role of USPIOs in detecting metastatic Lymph Nodes (LNs) will be reviewed. Fig. 1 lists the objectives of this literature review.

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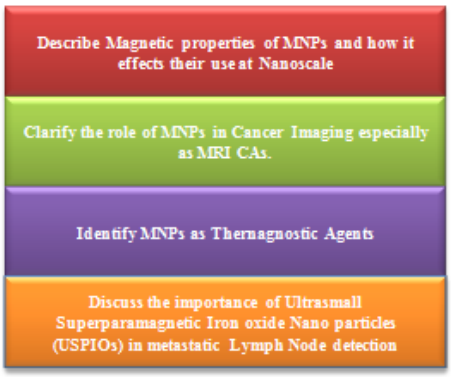

Fig. 1 Objectives of Literature Review

The mini-literature review is written for informed reader and prior medical knowledge is assumed. Definitions have been provided for more ambiguous terms in the glossary and in the text.

II. Materials

Articles were searched using online databases and text books.

III. Literature Review

A. What is Nanotechnology?

Nanotechnology is defined as science of small structures and phenomena that are in the range of 1-100 nm [5], [6]. Nanoparticles (NPs) have unique physical and chemical properties and are smaller than biological organelles [7].

B. Drawbacks of Modern Cancer Imaging

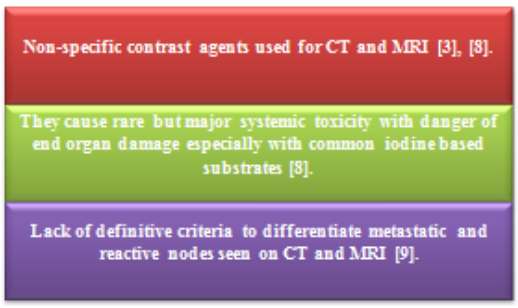

Fig. 2 Drawbacks of Modern Cancer Imaging

C. Iron Oxide Based Magnetic Nanoparticles (MNPs) as MRI Contrast Agents

Different types of nanoparticles have been used to develop MRI contrast agents such as polymers, micelles, dendrimers, liposomes, carbon nanotubes and magneto-nanoparticles. The most commonly investigated material in biomedical applications is Iron oxide (USPIOs, SPIOs) owing to its superb biocompatibility compared to other magnetic materials including both oxides and pure metals [10]-[12]. Another reason for their wider applications is ease of manufacture [10], [13]. Several forms of iron oxide are found in nature and can be made in laboratory. Presently Magnetite (Fe_3O_4) and Maghemite (γ-Fe_2O_3) are the most widely used forms as they satisfy the essential requirements for biomedical applications [10], [14]. Table I gives a summary of these requirements for biomedical application. Based on their size, which in turn influence their plasma half-life and biodistribution properties, iron oxide NPs can be divided into two classes namely super-paramagnetic Iron oxides (SPIOs) and Ultrasmall paramagnetic iron oxides (USPIOs) [10], [12], [14].

USPIOs are smaller than 50 nm in size and are eventually removed by lymphatic system [15]. They exhibit longer blood circulation times and wider tissue distribution. This is mainly because of their small size which enables them to avoid RES (reticuloendothelial system) sequestration [9]. The RES consists of spleen, liver, lymphoid tissue/lymph nodes and bone marrow [9]. Hence they are ideal for detecting metastases in Lymph nodes [9]. Larger particles (SPIOs) exhibit shorter half-life and are quickly taken up by Kupffer cells – macrophages of RES system [15]. These ferrites have spinel crystal architecture with oxygen ions forming a closed packed cubic structure and iron ions situated at interstices. In case of magnetite, magnetization results from electrons jumping between the Fe^{2+} and Fe^{3+} that are situated at the octahedral sites [13]. Table I lists characteristics of magnetic iron oxide nanoparticles.

TABLE I
CHARACTERISTICS OF IRON OXIDE BASED MAGNETIC NANOPARTICLES, ([14] Modified)

Magnetic NPs	Size (nm)	Advantages	Disadvantages	Applications
Superparamagnetic Iron Oxide (SPIO)	> 50	Magnetism in external magnetic field allows control over distribution [14].	Aggregates leads to thrombosis and embolization [14]	Targeted delivery of drug and genes [14]
		Loss of magnetism without magnetic field	Lower magnetic moment than pure iron NPs [10], [11]	Magnetic cell separation for cancer diagnosis and monitoring
		minimizes risk of aggregation in vivo [14]		Negative enhancer MRI CAs [14]
		Biocompatible [13], [14]	Opsonization & quick clearance by phagocytes [6]	Thermotherapy [14]
	//////	Nontoxic [14]		
		Amenable to surface functionalization		Different agents have been used to functionalize MNPs e.g. antibodies, short peptides, glycoproteins etc [6]
		Chemically stable [12], [14]		
		Easy to manufacture [13], [14].		
Ultrasmall Paramagnetic Iron oxide (USPIO)	< 50	Same as above except the 2nd advantage.		MRI CAs especially for Detection of LN Metastasis [9], [14].

D. Mechanism of Action

Depending on their size and surface, iron oxide NPs can target the tumour either passively and/or actively in order to deliver contrast agents and drugs [12]. Part of cancer process emits vascular endothelial growth factors (VEGF) which causes neo-angiogenesis. Neo-angiogenesis is a process by which multiple disordered, leaky blood vessels arise to supply the tumour with blood and nutrients [12]. Passive targeting makes use of this leaky and porous tumour vasculature [12]. The leaky tumour vasculature enables the macromolecules and nanoparticles to extravasate and accumulate in the interstitial spaces [16]. In addition to it the disturbed tumour vessel bed leads to reduced lymphatic drainage from tissue. As a result extravasated particles are not drained from the interstitial spaces thereby enhancing local concentration of nanoparticles. This process is called Enhanced Permeability and Retention Effect (EPR) and can be used for delivery of different drug carrier systems including iron oxide based NPs [16].

Active targeting is achieved by conjugating iron oxide NPs with targeting molecules such as Ligands having high affinity towards unique cell surface receptors or antigens on tumour cells [12], [17]. This functionalization of NPs has shown their increased uptake by tumour cells compared to non-functionalized Nanoparticles [12].

IV. MAGNETIC PROPERTIES OF MNPS

In this section some of the magnetic properties of the MNPs and the physics concepts behind magnetism and biomedical applications of MNPs will be briefly reviewed to have an improved understanding of the advantages of MNPs as MRI contrast agents and drug deliver carriers.

A. Ferrimagnetism

Spinning of some of the electrons in magnetic materials produces magnetic dipoles. The individual dipoles in a crystal can align either parallel or antiparallel to other adjacent dipoles giving rise to macroscopic magnetic effect [12]. Magnetic materials can be categorized as paramagnets, ferromagnets, ferrimagnets or antiferromagnets depending on the magnetic response detected[15]. Magnetite and Maghemite fall into the category of Ferrimagnetism [12]. In ferromagnetic

materials spins are aligned antiparallel but the values or magnitude of the moment in each direction are not equal causing a net magnetic moment different than zero that will still magnetize the material even without the presence of an external magnetic field [12], [18]. Readers are directed to article [18] for illustrations of spin alignment in ferro, ferri and anti-ferromagnetism. The magnetic susceptibilities and behaviour of magnetic materials rely on their atomic structures, temperature, external field, H and size [13], [12]. Table II gives a summary of properties of Iron oxide MNPs.

TABLE II
PROPERTIES OF IRON OXIDE MNPS [19]

Property	Type of Magnetic Iron Oxide	
	Magnetite	**Maghemite**
Molecular Formula	Fe3O4	γFe2O3
Density (g/cm3)	5.18	4.87222
Crystallographic system	Cubic	Cubic or tetrahedral
Type of Magnetism	Ferrimagnetic	Ferrimagnetic
Structure type	Inverse Spinel	Defect Spinel
Ms at 300 K (A-m2/kg)	92-100	60-80

B. Hydrodnamic Size, Coercivity and Superparamagnetism

Reduction in the size of magnetic materials/particles can make them more appropriate for therapeutic and diagnostic applications compared to their bulk counterparts. This is because as size of a magnetic NP is reduced, its Coercivity decreases [13]. Coercivity is a measure of resistance to demagnetization (See Glossary for explanation). Reduction in size below the Superparamagnetic radius (r_{SP}) causes magnetic transformation in particles thereby making both ferro and ferrimagnetic (FM) NPs, Superparamagnetic (SPM). Superparamagnetism is desirable feature of MNPs such as SPIOs because under superparamagnetism high magnetic moments are detected in the presence of an external magnetic field but no remnant magnetic moment is present when the external magnetic field is removed [12], [13]. In other words initial net zero magnetic moment is achieved and no extra energy is required (absence of Coercivity) to demagnetize the particle [12]. This provides the particles with colloidal stability and minimizes aggregation of MNPs thereby reducing the risk of embolism. This makes them viable for use in biomedical applications [13]. The magnetic susceptibility of Superparamagnetic materials lies between ferromagnetic and paramagnetic material [20].

C. MNP Size and Surface Effects

Reduction in NP size has some associated disadvantages as well. As the particle size decreases the surface to volume ratio increase which means higher surface curvature. This causes noticeable surface effects such as spin canting, spin glass behaviour and non collinear spins. These surface effects influence magnetic properties of NPs. The high surface curvature results in disorder crystal structure thereby reducing saturation magnetization of NPs compared to their corresponding bulk phases. A study showed that as the diameter of the NP decreases the size dependent saturation magnetization decreases [37].

D. Magnetic Anisotropy (Ku) and Magnetization

In all materials the magnetization decreases with Nanoparticle size reduction, the association between magnetization and size relies on the *magnetic anisotropy constant* (K_u) which is different for each material [12]. Hence the lowest the constant, the fastest the decrease of magnetization with size will be. ***Magnetic anisotropy constant*** **(K_u)** measures the energy to be overcome in order to reverse the direction of the magnetic dipoles of the material and is influenced by crystal lattice symmetry, the surface co-ordination with the core of the NP and shape of the NP[15]. For instance $MnFe_2O_4$ has a low value of K_u, compared to of $CoFe_2O_4$ and hence its magnetization is more size dependent. In addition to it NPs with high magnetic anisotropies have a significant magnetization even at very small sizes and this feature can be utilized in many biomedical applications [12]. It has been noted that metal alloys have higher magnetization values than their oxide counterparts. Hence they can act as potentially more efficient contrast agents. A study showed that FePt NPs can significantly reduce T2 times in target tissue with values of r_2 300-400 s^{-1} for a 1mM solution of NPs [22]. Another study demonstrated high magnetization values for graphite coated 7nm FeCo Nanoparticles with r_2 values of 600-700 s^{-1} for a 1Mm solution of Nanoparticle [23]. This resulted in a high image contrast by using low doses of NPs compared to commercially available iron oxide contrast agents. Moreover FeCo NPs also acted as T1 Contrast Agents even at very small sizes (4nm) and this dual contrast functionality is difficult to achieve with iron oxide NPs.

E. Effects of Zeta Potential on MNP Stability

Negative zeta potential NP formulations help repel each particle in the suspension thereby providing long term stability and avoiding aggregation whereas positive zeta potential shows some aggregation [24]. It has been observed that engineering of NPs to carry drugs changes their surface charge and their pharmacokinetic properties. For instance when NPs are loaded with doxorubicin it gives a positive charge to the NP surface. As a result NP circulation time is reduced and their passive delivery to brain lesions after intravenous injection is reduced [47]. This could be handled by using carotid artery route for NP administration which could be more useful than intravenous route. This is because intra carotid administration along with magnetic targeting (300mT) results in 1.8 fold higher concentration of MNPs in tumour and is not complemented with a respective increase of NP concentration in contralateral brain thereby improving tumour selectivity of NP delivery [25].

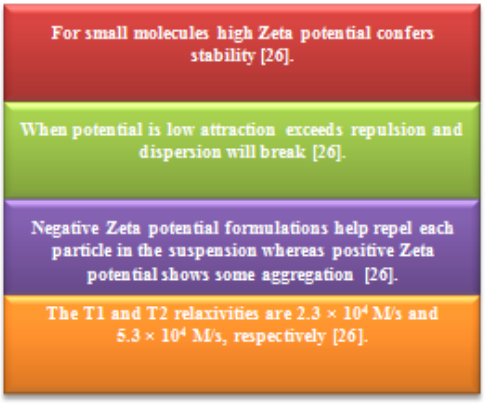

Fig. 3 Effects of Zeta Potential on MNP Stability

F. MRI Contrast Enhancement

MRI provides information on local biology and pathophysiology via nuclear magnetic resonance signals and these signals originate from hydrogen nuclei within the organism. MRI is used to create 3D images for better visualization of many types of tissues. To improve the image contrast for diagnosis and the assessment of treatment response, MRI contrast agents are used to highlight tumours [17]. In case of MRI the main focus has been on the development of MNPs especially SPIOs for contrast enhancement [12], [15].

Under normal conditions Protons or hydrogen nuclei in the water molecules in the body are spinning about their axes and are randomly oriented [12]. When an external magnetic field, B_0 is applied the nuclear spins align with the field and also precess around the axis of the external magnetic field producing a net magnetic moment, m [12]. Upon application of a 90^0 radiofrequency pulse to the object to be imaged causes the nuclear spins to be tipped away from the z axis and these excited spins start precessing in the transverse plane (i.e. xy plane in the laboratory frame) [12], [13]. When radiofrequency pulse is switched off the tipped net magnetic moment, m continues to wobble around the external magnetic field, B_0 giving off RF waves and hence producing NMR signal. This is because rotating magnetic fields produce electromagnetic radiation [12]. The readers are directed to article [12] to see illustration for the Magnetic Resonance principle. The protons recover their original state of equilibrium by two relaxation processes namely T_1 and T_2 to generate an MR image. T_1 is recovery of magnetic moment in the direction of the B_0 and T_2 is the loss of signal in transverse plane [12].

SPM particles have large magnetic moments [15] and generate an additional magnetic field, B_1 which induces local field inhomogeneties. Consequently when SPM particles accumulate within tissues of interest they disturb homogeneous magnetic field i.e. they reduce transverse relaxation times significantly in target tissue/organ [12], [13]. This decrease in T2 produces a negative contrast on T2 weighted images. This is one of the main reasons that MNP such as Iron oxides are considered primarily as T2 contrast Agents [12].

Global magnetization of a nanocrystal is smaller than their bulk counterparts or smaller than larger particles. Hence increase in magnetization of NPs is directly proportional to their size. Larger the particle size, larger is its magnetic moment and greater is T2 weighted image contrast [12].

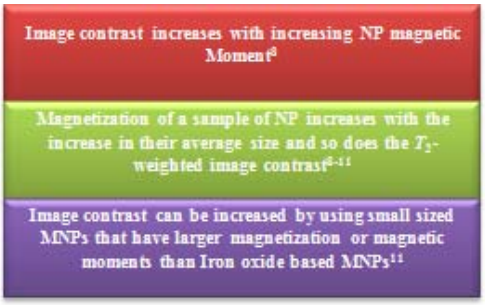

Fig. 4 Effect of Particle size and Concentration on contrast enhancement

V. Pharmacokinetics of MNPs

A. Surface Coating and Functionality

Within each class, particles differ on the basis of their different surface coatings which stops their aggregation and overseas their biodistribution [27]. Surface coating also prevents degradation of naked MNPs during or after synthesis thereby providing chemical stability [10]. Surface coating assist in dispersal of MNPs to form homogenous suspensions [9]. Another study showed that coating iron oxide NP with Cyclodextran (CD- a polymer) and F127 (a polymer) reduces particle size and attenuates their cluster behaviour consistently from > 300nm to 90nm [26]. A study showed that PEG coating can decrease NP recognition and uptake by macrophages [28]. Similarly dextran coating provides NPs with stability in blood and aqueous medium, prevents aggregation of NPs, minimizes their entrapment by liver and spleen and increase their circulation times [9]. Examples of surface coatings include Dextran and its derivatives, Albumin, starch, Silicon, PEG-Poly (ethylene) Glycol, PEI-Polyethyleneimine, Chitosen, Co-polymer and Liposomes and micelles [27], [29].

B. Biocompatibility

Iron oxide based MNPs are highly biocompatible. This means that the iron is metabolized in hepto-renal system and then is added to body's iron reserves via haematopoiesis. It finally becomes part of red blood cells as hemoglobin [24]. Excess iron is effectively cleared from the body without adverse effects to human health [10].

C. Nanoparticle Clearance, Permeability and Plasma Concentration

The hydrodynamic size of the NP also influences its removal from the body [30], [31]. Particles less than 20 nm are excreted by kidneys, NP in the range of 30-150 nm tend to accumulate in heart, bone marrow, kidney and stomach [32],

[33] and particles ranging from 150-300 are cleared by liver and spleen [28]. NP size also influences their permeability across vessels and also dictates their concentration in blood vessels. Particles < 150 nm in diameter are capable of crossing most of the endothelial barriers [29]. However BBB poses stricter restriction upon NP extravasation from the vessels. It has been suggested that NPs are able to cross BBB via diffusion and convection by exploiting the disrupted BBB in the tumour [34], [35] and in case of PEG coated NPs by the likely interaction of PEG with endothelial cells of the blood vessels [34]. Other possible mechanisms by which NPs can cross the BBB include receptor mediated endocytosis, phygocytosis, passive transport of NPs across defects (due to malignancy) in blood brain barrier [36] and tumour can increase permeability of BBB by increase in pinocytosis [37].

VI. Detection of Lymph Node Metastasis

The Lymph node metastases can greatly influence cancer patient management and prognosis [38]. It can significantly reduce survival rates [9]. Presently used imaging modalities like CT and MRI are not reliable in detecting metastatic LNs accurately [9]. CT and MRI are principally anatomical based imaging modalities and mainly rely on size of the LN for detecting metastases [38]. Hence there are susceptible to false positives and false negatives [38]. Other imaging modalities used to assess lymph node status have their own associated disadvantages. Table III lists some of these imaging techniques and their disadvantages in differentiating benign from malignant LNs.

TABLE III
Different Imaging Techniques Used to Detect Lymph Node Metastases

Imaging Modality	Category	Disadvantage
CT	Anatomical	Staging of LN is size dependent [38].
MRI	Anatomical	Staging and assessment of LNs is difficult & Size dependent [39].
PET	Functional	PET describes metabolic activities of the involved LNs but at the expense of anatomical [9].
US	Functional	US is restricted by anatomical definition[18]. Differentiation between metastatic, normal and hyperplasia is difficult. USFNA has a wide range of sensitivity[71] and is less sensitivity in detecting small nodal metastases < 5mm and micrometastases < 2mm[71]. USFNA sensitivity also varies with introduction of sampling error and is highly operator dependent [9].
CT/PET		Promising technique but is dependent mainly on metabolic activity to detect malignancy and is size dependent. Some tumours are not metabolically active e.g. bladder and prostate cancers. Besides bladder also receives FDG as part of physiologic excretion thereby concealing positive LNs [9].
Direct lymphangiography		High invasiveness, challenging technique for contrast administration and side effects [9].

A. Clinical Studies

USPIO MR Contrast agents for detection of metastatic LNs have been investigated in a number of studies for different anatomical areas. Some of which are discussed below and for a summary of these clinical studies see Table IV. A study carried out MRI on 80 patients with prostate cancer before and after administration of intravenous administration of USPIO [40]. The USPIO (Combidex) enhanced MRI correctly diagnosed all patients with metastases, giving a sensitivity of 100% compared to 45.4% on MRI before contrast administration with a specificity of 96% vs. 35.4% on conventional MRI. The USPIOs increased diagnostic specificity from 90.4% to 97.8% (on a node by node basis). USPIO enhanced MRI scans also identified nodal metastases less than 2mm in diameter within normal sized LNs. Such microscopic metastases are under the threshold of detection of other imaging modalities [40]. Other studies listed in Table IV also showed high sensitivity, specificity and accuracy over conventional MRI scans [41].

TABLE IV
Clinical Trials Evaluating USPIO-Enhanced MRI For Lymph Node Metastases

Site	First Author	Year	No of Patients	Sensitivity	Specificity
HN	Curvo-Semedo [41]	2006	19	96%	79%
Neck and body	Anzai [42]	2003	153	83%	77%
Breast	Harada [43]	2007	33	100%	80%
Prostate	Harisinghani [40]	2003	80	91%	96%
Bladder	Deserno [44]	2004	58	96%	95%
Endometrium	Rockall [45]	2005	44	100%	94%

A review performed in 2006 [9] showed high sensitivity and specificity of USPIO as MRI contrast agents for differentiating normal from malignant LNs regardless of size or morphological features. They reported no serious adverse effects with the use of USPIOs. Some mild-moderate side effects were observed in the range of 3-28% and include lumbar pain, rash, transient decrease in blood pressure and arrhythmia. A meta-analysis performed in 2006 [46] to establish diagnostic accuracy of USPIO-enhanced MRI found a net sensitivity of 0.88 and specificity of 0.96 compared to 0.63 and 0.93 sensitivity and specificity respectively for unenhanced MRI. The meta-analysis established the diagnostic precision of Ferumotran-10 for detection of LN metastases in MR imaging.

Another more recent meta-analysis involving 34 studies was carried out in 2010 [39]. This meta-analysis showed an overall sensitivity of 0.90 vs. 0.39 and specificity of 0.96 vs. 0.90 for USPIO-enhanced MRI and non enhanced MRI respectively. Post contrast MRI alone had sensitivity and specificity of 0.85 and 0.93 respectively. This meta-analysis concluded that USPIO-enhanced MRI has better diagnostic precision than conventional MRI in terms of sensitivity and specificity for diagnosing metastatic LNs. This analysis also confirmed that post contrast MRI images alone are equal to combined (pre and post contrast) study for LN characterization.

VII. Theragnostics

It is the combining of therapeutic and diagnostic approaches for simultaneous cancer diagnosis and therapy and has a huge potential to personalize and advance medicine. NP systems have the capability to incorporate a wide range of chemotherapeutic agents and diagnostic agents for delivery to target cells thereby making theragnostics practicable [47].

In one study multifunctional poly (aspartic acid), PA_{sp} nanoparticles (MPAN) containing Superparamagnetic iron oxide crystals and chemotherapeutic drug, doxorubicin (DOX) were prepared for cancer diagnosis and therapy [67]. PA_{sp} was used as a drug delivery carrier due to its biodegradable and acid containing water soluble properties in order to incorporate poorly water soluble drugs. MPAN containing DOX – MPAN (DOX) showed high T_2 relaxivity coefficient (r_2 values) of 2999 L $mmol^{-1}$ s^{-1} which is higher than Ferridex (a commercially available contrast agent). To evaluate the therapeutic potential of MPAN (DOX) breast cancer cells, SKBR-3 were treated with MPAN (DOX). The cytotoxic potency increased as the amount of DOX in NP increased indicating successful delivery of DOX into the nuclei of cancer cells. This study demonstrated that MPAN (DOX) has a great potential to act both as a T_2 weighted MRI Contrast agent and anti-tumour drug delivery system.

In another study tumour targeting Docetaxel containing SPIO NPs were developed and tested for the diagnosis and treatment of prostate cancer invitro [47]. An antibody specific to Prostate Stem cell Antigen which is over expressed in prostate cancer, was conjugated to the NP to achieve active targeting. Tumour targeting agent facilitated specific delivery of Docetaxel to the target cells and inhibited effectively the growth of prostate cancer cells, PC3. NP with antitumour drug did not show any significant cytotoxic effects on the PC3 cells suggesting that antiproliferative effect was due to the activity of the encapsulated chemotherapeutic drug loaded in NP.PC3 cells were scanned under 1.5T scanner. The T_2 weighted MRI images of the tumour specific targeting Docetaxel conjugated SPIO NPs appeared darker than Endorem (a commercial Iron oxide NP MRI CA).

A study showed that multiple drugs can be loaded in iron oxide based MNPs with great efficiency and without affecting the imaging properties of the magnetic NPs [48]. Combination drug therapy was done by using two chemotherapeutic drugs: doxorubicin (DOX) and Paclitaxel (PTX) which were loaded to oleic acid coated iron oxide and pluronic stabilized MNPs. Drug delivery and magnetic resonance imaging properties of these multifunctional NPs were investigated for MCF7 breast cancer cells and compared with Feridex IV. The T_2 relaxivities of drug loaded MNPs were near to that for Feridex (4.4, 5.3 vs. 4.8- for DOX, PTX, Feridex IV). Feridex has highest T_1 relaxivity followed by MNP without drugs and MNP with PTX and finally MNP with DOX. The combination treatment showed highly synergist effect in the concentration range 0.5-100ng/mL. The authors of this study suggested that beside cancer diagnosis and drug therapy these MNPs can be used for hyperthermia under an alternating magnetic field.

VIII. Clinical Applications of Iron Oxide NPs in MRI Imaging

A. Liver Imaging

The first clinical use of SPIO was for imaging liver tumours as these NPs are quickly taken up by Kupffer Cells of hepatic parenchyma [49]. The normal hepatic parenchyma consists of these macrophages whereas liver tumours are mostly devoid of kupffer. This difference in macrophage specific uptake of SPIO between normal and malignant liver tissue gives rise to contrast between healthy and diseased tissue on MRI imaging.

A study by Zheng et al. [50] evaluated the value of SPIO (Feridex) in characterizing focal hepatic lesions. This study involved 43 patients and 12 kinds of benign and malignant lesions including Hepatic Cellular Carcinoma (HCC). All HCC lesions appeared bright after SPIO administration on T2 weighted images whereas signal intensity of normal hepatic parenchyma decreased both on T1 and T2 weighted images after SPIO enhancement especially on T2 weighted images. On T1 weighted images 50% of HCC appeared slightly hyperintense, 45.5% appeared isointense and 4.5% appeared hypointense. It was not easy to differentiate HCC from other malignant lesions due to its non characteristic appearance on pre and post SPIO enhanced T2 images. Detection of HCC after SPIO enhanced greatly improved. SPIO-enhanced imaging seems to be advantageous in diagnosis cirrhotic nodules and Focal nodular hyperplasia (FNH) as both lesions contain Kupffer cells that take up SPIO. This study concluded that lesion where diagnosis is indefinite on Gd-DTPA enhanced images, SPIO enhanced MRI could provide additional information and increase confidence for diagnosis of focal hepatic lesions.

Another study evaluated the value of SPIO (Ferucarbotran) in detection and characterization of hepatic lesions [51]. This study showed that combined non-enhanced and SPIO enhanced images provided the best output for lesion detection and significantly better lesion characterization than SPIO enhanced images alone. The combined approach resulted in higher accuracy as compared with SPIO enhanced T2 MR imaging or Contrast enhanced spiral CT alone (85.3% vs. 73.1%).

B. Brian Imaging and Active Targeting

Different iron oxide based NPs have been investigated for imaging brain tumours using different NP matrix such as Dextran coated, PEG coated Iron oxide NPs, polyacryamide, stearic acid and other matrix formulations [46], [52]-[56]. Feasibility studies to broaden the application of dextran-coated USPIOs to human brain tumour were performed with Sinerem [35], [52], [53]. USPIO enhanced MRI imaging showed better and prolonged tumour delineation especially tumour boundaries were sharply demarcated on MRI scans [35], [52]. All the brain tumours showed T_1 signal enhancement. This contrast enhancement took place gradually with a peak at 24-48 h whereas contrast enhancement with Gd chelate occurred instantly and decreased quickly. Unlike Gd chelates the tumour margins stayed sharp. This study concluded that iron

oxide based NPs will not replace Gd enhanced MRI imaging but will add to the information seen on MRI image with respect to detecting inflammatory component and disrupted BBB involved in invading brain tumours [53]. Polyacrylamide (PAA) NPs with incorporated SPIO crystals were used for in-vivo MRI imaging of brain tumour in a rat model containing 9L gliosarcoma cells [57]. The results of this study showed that MRI signal decrease for normal brain, tumour core and tumour periphery were about 20, 30 and 40-50% respectively. Increase in NP plasma half-life was observed with increase in the size of the PEG units. PEGylation thus can be used to delay NP clearance from plasma. Solid Lipid NPs (SLN) coated with stearic acid (Endorem) was compared with Endorem alone [54]. The results showed that SLN is capable of crossing BBB as it had a longer lasting brain uptake and slower clearance than Endorem and therefore SLNs can act as potent MRI CAs.

1. Active Targeting MNPs for brain:

Active targeting MNPs offer better tumour diagnosis and localization by utilizing biomarker of these diseases as shown in a study [58]. In this study involving a PEG coated iron oxide NP capable of specifically targeting glioma tumours via surface bound targeting moiety, chlorotoxin, CTX demonstrated preferential accumulation of CTX targeted iron oxide NPs in 9L glioma cells (*in vitro*) and xenograft mouse model (*in vivo*). This resulted in improved MRI contrast enhancement of tumours than non-targeted control NPs. No acute side effects were observed by histological analysis [58]. Another application of active targeting for MRI imaging is the use of lactoferrin conjugated SPIO Nanoparticles (Lf-SPIONs) for detecting brain gliomas [59]. This invivo study was conducted using 75nm diameter NPs in a rat model. Magnetic saturation and T2 relaxivity were 51 emu/g Fe and 75.6Mm^{-1} S^{-1} respectively. Substantial decrease in MR signal intensity was observed invivo suggestive of that Lf-SPIONs selectively targeted brain glioma. Lf also seemed to enhance SPION uptake by glioma cells as indicated by decrease of signal intensity with Lf-SPIONs in C6 glioma cells compared to SPIONs. This is because Lf was able to cross the BBB via unidirectional receptor mediated transcytosis. No noticeable cytotoxicity was observed. This study concluded that glioma targeting Lf-SPIONs could be useful CAs for MRI imaging for both pre and post-operative tumour delineation.

C. Head and Neck Cancer Imaging

Successful in-vivo MRI imaging of folate expressing tumours actively targeted by foliate conjugated Iron oxide based NPs was demonstrated [60]. Only Folate conjugated NPs showed internalization of these NPs into target cells when foliate receptors were available. In-vitro analysis showed 97.5% KB cells (a human nasopharyngeal epidermal carcinoma cell line) cultured with Folate conjugated NPs internalized the NPs by endocytosis. In-vivo MRI imaging showed 38% decrease in signal intensity from precontrast to post contrast images of the tumour. This was approximately 3 times the intensity reduction noticed at a non-tumour bearing muscle (cells devoid of folate receptors).

1. Toxicity

Table V covers main points of the toxicity reactions observed in all the above mentioned studies.

TABLE V
TOXICITY OVERVIEW

In all studies and reviews no serious adverse effect was observed
In general MNPs are haemocompatible [9], [12]. SPIOs due to their high magnetic moment cause higher signal change. Hence small quantities of SPIOs are needed limiting cellular toxicity [12]. SPIOS are biocompatible and biodegradable [10].
USPIO: Two Meta-analysis showed all of the adverse events were mild-moderate and accounted for 3-28% [9]
Back pain was most common [9].

2. Cost Effectiveness and Safety

A study evaluating metastatic LNs in ca Prostate patients showed that costs and outcomes of MRI with USPIOs could provide cost benefit compared to pelvic dissection to diagnose pelvic LNs [40]. Pelvic LN dissection is expensive and requires hospitalization. Cost per metastases detected in patients with low risk of post-surgical complications is about $43,600 [40]. Preparation of MNPs is not expensive [9]. Post-contrast USPIO study equates to combined USPIO Post and pre study for metastatic LN detection making it labour saving and cost effective [22]. Imaging with MNPs is non invasive compared to axillary or pelvic dissection and has low toxicity. [16].

IX. CONCLUSION

Nanotechnology has great potential for more accurate and earlier cancer diagnosis as well as for targeted cancer therapy. MNPs especially iron oxide based have shown more accurate and cost effective detection of metastatic LNs in various cancers, something which is not well performed by other imaging modalities. Active Targeting can further increase contrast enhancement by Iron oxide based MNPs. Further studies into miniaturization, functionalization and engineering of Nanoparticles will make them more selective, improve their delivery, increase their stability and will reduce their doses. It will reduce the toxicity of magnetic nanoparticles and will improve image enhancement of tumours.

APPENDIX

Active targeting

Drug targeting involves *passive*, *active* or *physical* targeting. *Active targeting* is achieved with mechanisms that allow direct targeting of drugs and/or carriers to specific cells, tissues or organ systems through specific recognition mechanisms.

Coercivity resistance to demagnetization

Erythropoiesis:

This is the process by which RBCs are produced. The initiating cell is the haemopoitic stem cell from which identifiable proerythroblast develop. This process goes through several stages before a normoblast loses its nucleus to become an erythrocyte.

Her2/neu: human epidermal growth factor receptor 2 and is a protein giving higher aggressiveness in breast cancer.

Herceptin: one of the monoclonal antibody drug used in treating early stage breast cancer

Ligand: molecule that binds to another molecule, as in antigen-antibody and hormone-receptor bondings.

Macrophage: a large scavenger cell present in connective tissue and many major organs and tissues including the bone marrow, spleen, lymph nodes and liver

Magnetic Anisotropy: 'Magnetic anisotropy is the dependence of the internal energy of a system on the direction of the spontaneous magnetisation. An energy term of this kind is called magnetic anisotropy energy. In general most kinds of magnetic anisotropy are related to the crystal symmetry of a material and this is known as magneto-crystalline anisotropy. Anisotropy can also be related to mechanical stress in the system and this is known as magnetostrictive anisotropy'

Magnetic susceptibility: ratio of induced magnetism (M) to the applied magnetic field (H_0); (sun et al, 2008.)

Monoclonal antibody (MAb): an antibody produced artificially from a cell clone and therefore consisting of a single type of immunoglobulin.

Passive Targeting

In passive targeting the distribution of the drugs within the body occurs through drug and carrier properties that are unchanged

Phagocytosis: the engulfment and digestion of bacteria and other foreign particles by a cell

Physical Targeting allows distribution of drugs and carrier systems through external influences, such as magnets in the case of SPION or heat (Neuberger et al 2005)

Pinocytosis: A cellular process that permits the active transport of fluid from outside the cell through the membrane surrounding the cell into the inside of the cell. In pinocytosis, tiny incuppings called caveolae (little caves) in the surface of the cell close and then pinch off to form pinosomes, little fluid-filled bubbles that are free within the cytoplasm of the cell.

Reticuloendothelial system:

A community of cells – phagocytes- spread out throughout the body. It includes macrophages and monocytes. The RES is concerned with defence against microbial infection and with removal of worn out blood cells from the blood stream.

Sensitivity: Ability of a test to identify correctly those who have the disease

Specificity: To identify correctly those who don't have the disease

Transcytosis: 'A mechanism for transcellular transport in which a cell encloses extracellular material in an invagination of the cell membrane to form a vesicle, then moves the vesicle across the cell to eject the material through the opposite cell membrane by the reverse process. Also called *vesicular transport*'

Xenograft: A transplant from one animal to another of a different species.

Zeta potential: Electrical potential at the slipping plane. The plane that separates mobile fluid from the fluid that remains attached to the surface. Value of the potential can be related to the stability of the colloidal dispersion

Fig. 5 Glossary

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DISCLOSURE

This report was internally published (anonymously) by The Open University as a list of theses submitted by The Open University Students. This article is not published in any journal or book.

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