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Surface-modified Ferrite Nanoparticles as Magnetic Resonance Imaging T_2 Contrast Agents

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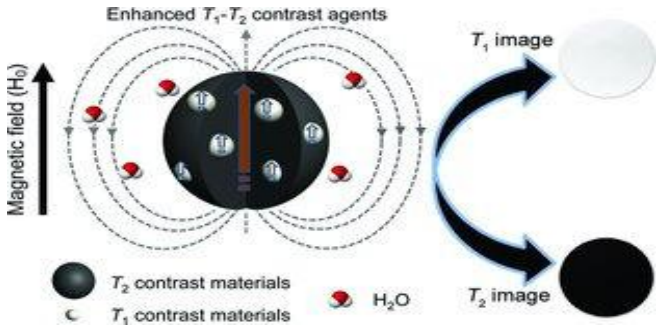
1. INTRODUCTION

Magnetic Nanoparticles (MNPs) are widely used in biological applications as a platform for several immunoassays, drug delivery and targeting, bio-separation techniques like cell sorting, contrast agents for magnetic resonance imaging systems and also as heating mediators for cancer therapy or hyperthermia which is mainly attributed to their unique feature of reacting to a magnetic force [1].

Contrast agents can be defined as a medium to improve the visualization of the internal body structures through an X-ray based imaging system. The property of enhancing the visibility of an organ region makes the contrast agents termed as "contrast enhancing agents" (CE). Iodine, barium are some of the radioactive compounds commonly used as contrast enhancing agents in imaging techniques such as computed tomograghy (CT scan), fluoroscopy and radiography. Other techniques in medical imaging include magnetic resonance imaging (MRI) and positron emission tomography (PET). MRI works on the principle of change in magnetic properties of hydrogen nuclei in the nearest affinity of the contrast agent. Magnetic resonance (MR) imaging is a non-invasive diagnostic procedure with a resolution of about 25-100µm. The relaxivity of the magnetic spins in water protons determines the sensitivity in MR imaging. Inspite of the detailed images produced by MRI itself, a diagnosis purely based on the resulting images may not be accurate, since normal tissues and lesions show negligible or minor variations in relaxation time. MRI contrast agents can aid the imaging technique to clarify images, for a better clinical interpretation.

The contrast arises due to different signal intensities in an MRI image coming from different volume elements. For example, fat, muscle, bone, etc produce different signal intensities due to different concentrations of water protons. But, signal intensity also depends on the rate at which protons relax to the ground state from the excited state due interactions with surroundings and with other protons. A 'longitudinal' relaxation time, T_1, is related to the transfer of energy from the excited protons to their surroundings and a 'transverse' relaxation time, T_2, is related with the exchange of energy between excited state and ground state protons. The relaxation rates, r_1 and $r2$, are reciprocals of the times for the two processes.

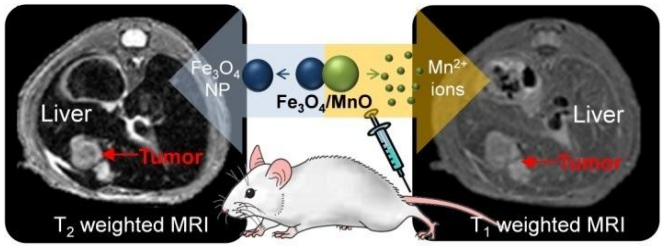
The relaxation rates, $1/T_1$ and $1/T_2$ can be increased by varying degrees by different contrast agents and different applied magnetic fields. For example, Gd(III)-complex based contrast agents tend to increase $1/T_1$ and $1/T_2$ by almost similar amounts, but T_1 weighted images typically give best images because of the dominance of the $1/T_1$ term in calculating the magnitude of the signal intensity. Contrary to this, ferrite particles tend to have a much bigger effect on the $1/T_2$ term and are usually used with T_2 weighted imaging [1(a)].



Contrast agents can be of two categories: (i) Paramagnetic compounds (including lanthanides like gadolinium) which reduce the longitudinal (T_1) relaxation resulting in a brighter

signal; (ii) Super paramagnetic iron oxide nanoparticles which affect the transversal (T_2) relaxation.

Paramagnetic complexes currently in use are gadolinium chelates, among which, Gd-DTPA is widely used. Gadolinium-based contrast agents enhance the signal in T_1-weighted images and are focused on detecting the breakage of the blood brain barrier (BBB), to track the changes in vascularity, flow dynamics and perfusion [2]. The major limitation of the MR imaging is its low sensitivity which results in the unclear diagnosis of the abnormal tissues from the normal tissues which can be considerably overcome by the use of nanoparticle agents as MR imaging contrast agents [3]. Paramagnetic compounds like gadolinium and super-paramagnetic nanoparticles such as iron oxides, ferrites are used as T_1 and T_2 contrast agents respectively, in magnetic resonance imaging (MRI) serving as excellent diagnostic probes resulting in bright and intense signals [4]. There is an increasing need in the engineered nanoparticles with a suitable coating material which maybe a metal oxide or organic polymers or inorganic metals and these surface treatments enhance the probing efficiency of the nanomaterial by overcoming certain drawbacks such as low biocompatibility and solubility or dispersion in water solutions. The contrast efficiency of any magnetic nanoparticle to be applied in MR imaging can be influenced by several factors which includes its size, surface area, magnetic spin, biocompatibility, toxicity, half-life period.



2. FERRITE NANOPARTICLES

Ohgushi et al. (1978) first reported that iron oxides could shorten the T_2 relaxation time (spin-spin relaxation time) of water and thereafter iron oxides have been extensively used as magnetic resonance imaging (MRI) contrast agents. Ferrite nanoparticles are the most preferred amongst metal oxides for therapeutic and diagnostic medical applications due to their relative inertness and properties that can be tailored by alternative synthesis parameters. Magnetic iron oxide nanoparticles are widely used as MRI contrast agents among the inorganic group due to their ability to shorten T_2* relaxation times in liver, spleen and bone marrow, thus providing a strong contrast effect in T_2-weighted images. Furthermore, the nanoparticulate properties exhibited by the nano-sized dimension and shape of Super-paramagnetic Iron Oxide nanoparticles (SPIONs) allow different bio-distribution and opportunities beyond the conventional imaging of chemical agents. SPIONs particles are considered as small, thermally agitated magnets in carrier liquids, known as “ferrofluids". The property of superparamagnetism in SPIONs serves as an activation mechanism, and when the external magnetic field is removed, the magnetization disappears, preventing the embolization of the capillary vessels [5]. Functionalized Iron oxide nanoparticles along with targeting agents have been used for specialized imaging by the site-specific accumulation of nanoparticles at the target of interest.The ferrofluids of Superparamagnetic iron oxide nanoparticles synthesized using sonochemical method showed high magnetization and crystallinity. These nanoparticles coated with oleic acid as a surfactant and dispersed in chitosan were investigated for the properties of contrast agent in MRI. The different concentrations of ferrofluids were checked for agglomeration criteria. The ferrofluids showed good stability in the blood circulation as they did not agglomerate for 30 days. The results of T_1- and T_2-weighted MR images of these ferrofluids were equivalent potential to that of Resovist® (clinically approved super-paramagnetic iron oxide based MRI contrast agent specifically for MRI of the liver) [6].

3. GENERAL SYNTHESIS PROTOCOLS FOR FERRITE NANOCRYSTALS:

The synthesis of MNPs involves iron chemistry with magnetite, iron-based metal oxides, or iron alloys as core material which could be carried out as a single-step procedure or as sequential step process. Precipitation, solution combustion synthesis, reverse micelles, thermal decomposition are some of the methods commonly employed in the synthesis of MNPs.

(i) Precipitation: It is a basic method for the preparation of ferrous ions in an aqueous solution. Precipitation technique can be broadly classified as two categories namely, wet precipitation and co-precipitation method.

Wet precipitation: Nanoparticles are prepared by manipulating the pH of iron salt solution. One of the major drawbacks of this method is that the sizes of the resulting particles are large and it is greatly influenced by the pH parameter. Also the volume of water required for the synthesis is large and so the scale-up process becomes problematic.

Co-precipitation: This method involves the preparation of Iron oxide particles (Fe3O4) by mixing two stoichiometric solutions of Fe2+ and Fe3+ ions and treating them with a suitable base.

(ii) *Solution combustion synthesis (SCS):* This method is frequently used when there is a need of highly pure, homogeneous nanocrystalline powder. The SCS method utilizes fuels in the form of salts such as nitrates, metal sulfates, carbonates as oxidants and reducing agent fuels such as glycine, sucrose, urea. SCS is based on the phenomenon

that if a reaction is initiated using heat, an exothermic reaction occurs which sustains by itself for a time period sufficient to synthesize the final product in the powder form. Also the fuel-oxidant ratio plays an important role in determining the morphology and the pore size of the nanoparticle. As the amount of fuel increases, the greater is the pore size in the resulting product. This method is cost-effective, consumes less time and energy and so it is widely used for the synthesis of oxide nanoparticles [7].

(iii) Reverse micelles: Micelle formation occurs in a high concentration environment of amphiphilic surfactant molecules.

Surface-engineered Super-paramagnetic Iron Oxides (SPIO):

The surfaces of SPIONs can be engineered to enhance various biomedical-related functions such as drug carrier properties, magnetic resonance imaging (MRI) contrast agents and hyperthermia (heat induction effect). The ferrite nanoparticles along with surface coatings in the form of ferrofluids find a major role in nanomedicine for molecular MRI. The core substance of the nanomaterial contributes to the contrasting ability while the biocompatibility and conjugation activity are rendered by the surface properties. A variety of biomolecules such as antibodies, proteins, peptides, polysaccharides and aptamers can be covalently bound to the surface of iron oxide nanoparticles for their site-specific accumulation at the targets of interest.

Most importantly, bio-compatible polysaccharides are widely used in modifying the surface of MNPs. Agarose, alginate, carrageenan, chitosan, dextran, heparin, pullulan, hyaluronic acid, starch are the commonly preferred polysaccharide material for coating MNPs. These polysaccharide agents are neutral or negatively charged (except chitosan which has positive ionic charge) and requires few chemical modifications to enhance the gene interaction with polymer surface coating on the MNPs [8]. Some of the well-studied polysaccharides are discussed below:

(a) Alginates are anionic polysaccharides containing β(1-4) linked D-mannuronic acid and α (1-4) linked L-guluronic acid residues and are found in brown algal cell wall. Alginates are one among the preferred biomaterials, especially in matrix supporting tissue repair and regeneration because of their high biocompatibility, chelating property and non-immunogenic nature.

The alginate-coated iron oxide nanoparticles can be synthesized by the following methods,

Method-1: (i) Gelation of alginate in a solution of ferrous ion, (ii) In situ precipitation of ferrous ion by alkaline treatment, (iii) Oxidation of ferrous hydroxide using oxidizing agents such as O_2 or H_2O_2.

Method-2: (i) Formation of the Fe_3O_4 particles through co-precipitation of ferric and ferrous ions by alkaline treatment, (ii) Surface coating of the Fe_3O_4 particles with alginate.

TABLE: Properties and applications of polysaccharide-coated MNPs used for MR-imaging [8].

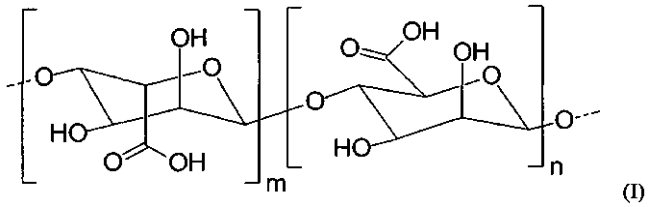
Polysaccharide	Occurrence	Charge	Functional groups	Surface modification for imaging purpose	In vitro/in vivo tests done
Alginate	Brown algal cell wall	Negative	OH COOH	Alginate-poly-L-lysine-alginate (APA)	C2C12 myoblast cell line implanted into the abdominal cavity of mice (ref)
Chitosan	Exoskeletons of shrimp and other crustaceans (treated with NaOH)	Positive	OH NH_2	Chlorotoxin (CTX), Polyethylene glycol (PEG), Near-IR fluorophore (NIRF), Cy.5.5	Brain Autochthonous medulloblastomas in genetically engineered ND2: SmoA1 mice
Dextran	Microbial product in wine	Neutral	OH	FITC-derivatized Tat peptide Chelator DTPA	Hematopoietic and neural progenitor cells
Starch	Green plants	Neutral	OH	Polyethylene glycol(PEG)	Male Fisher 344 rats induced with 9L-glioma brain tumors
Heparin	Animal tissues	Negative	OH OSO_3H	Gold-deposited Glycol chitosan Pluronic F-68	Tumor-bearing mice, induction of Squamous cell carcinoma (SCC-7) cells in male C3H/HeN mice by subcutaneous injection

The MNPs obtained by this method showed a core diameter of 5-10nm and 193.8-483.2nm after the alginate surface coating. The T_2 relaxivity of these SPIONs was higher to that of the clinically employed SPIONs, thereby proving the efficiency of the alginate-coated SPIONs as a negative MRI contrast agent [9].

(b) Chitosan is a hydrophilic polysaccharide, a copolymer of a 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose unit with β (1-4) linkages usually obtained by the deacteylation of chitin. Chitosan is one of the biocompatible and stable particle which is rendered to the presence of its functional amino and hydroxyl groups. The chemical structure of the chitosan allows the surface modification of SPIONs by physical adsorption and electrostatic interactions, thus eliminating the need for a cross-linker.

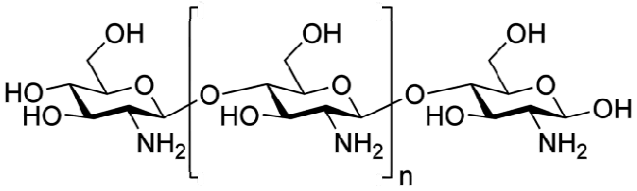
Synthesis of Chitosan-coated MNPs:

(i) *In-situ* precipitation of ferrous hydroxide by alkaline treatment along with chitosan. (ii) Co-precipitation and then cross linking.

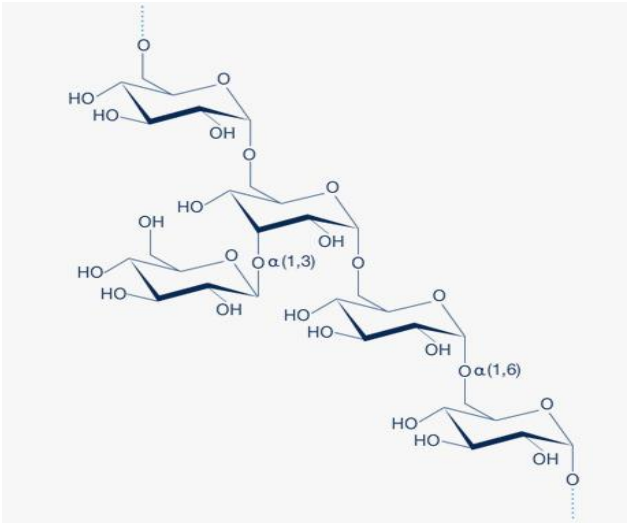


(I)

Chitosan-coated iron oxide nanoparticles shows high relaxivity which can be used as a potential MRI contrast agent for cell tracking [10]. Iron oxide nanoparticles coated with Polyethylene glycol (PEG)-grafted chitosan could be used as a nanoprobe which has the capability to cross the blood brain barrier, target a brain tumor after the coating was conjugated with chlorotoxin (tumor-targeting agent) and a near infra-red fluorophore [8].



(c) Dextran is a branched polysaccharide composed of glucose molecules with α-linked D-glucopyranosyl linear backbone. Dextran-coated SPIONs are commercial clinical contrast agents for MRI applied in the nodal staging of cancers. The coupling of dextran surface to the targeted ligands or labelled cells can be achieved using conjugation methods such as a robust bioorthogonal [4 + 2] cycloaddition reaction between 1,2,4,5-tetrazene (Tz) and trans-cyclooctene (TCO) [26]. To increase the stability and functionality of dextran-coated magnetic particles, various functional groups such as a carboxymethyl group cross-linked to epichlorohydrin The cross-linked dextran-coated iron oxide particles (CLIOs) obtained by the cross-linking of functional group such as carboxymethyl group to epichlorohydrin are found to be more stable than dextran-coated SPIONs of the same size. Dextran-stabilized monocrystalline iron oxide nanoparticles (MION) can be more effectively functionalized by conjugation with amine groups of biomaterials by epichlorohydrin treatment. The crosslinked iron oxide nanoparticles conjugated with HIV-Tat proteins (CLIO-Tat) shows efficient non-phagocytic cell labeling through activated macropinocytosis.

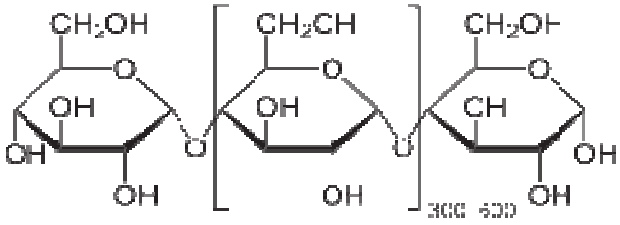


Kang et al. reported the use of antibody-conjugated CLIO in the *in-vitro* targeted MR imaging of E-Selectin in endothelial cells. The aminated cross-linked nanoparticles were made target specific by derivatizing the nanoparticles with a membrane translocation signal. The results proved that dual-labeled, dextran-coated MNPs can be used for the efficient labeling of hematopoietic and neural progenitor cells thus leading to the *in-vitro* single-cell visualization by MRI and can be applied in active molecular imaging which could effectively improve stem-cell based therapies [2,8]. Dextran-coated superparamagnetic iron oxide is a potential MR contrast agent for assessing lymph nodes in the head and neck [11]. A recent study has shown that the Dextran sulfate-coated superparamagnetic iron oxide nanoparticles can be applied as a contrast agent for atherosclerosis imaging [12]. Also, the non-invasive imaging of HER2/neu receptors using MRI can be efficiently aided with the help of targeted Herceptin-dextran iron oxide nanoparticles [13].

(d) Starch is a polysaccharide containing repeating units of glucose joined by glycosidic bonds. It consists of a functional

hydroxyl group with a neutral charge and widely preferred material for the surface coating of MNPs.

Synthesis of Starch-coated MNPs: Co-precipitation method followed by glutaraldehyde aided cross linking of thiolated starch [9].

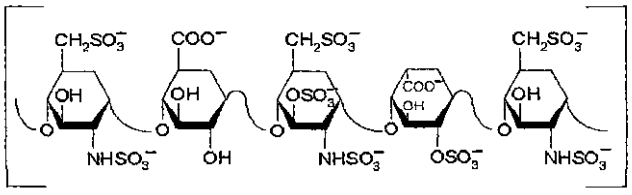


Polyethylene glycol modified, cross-linked starch coated iron oxide nanoparticles are widely used for the enhanced magnetic tumor targeting [14]. The 5-carboxyl-fluorescein (FAM-A54) coupled starch-coated iron oxide nanoparticles (SIONs) shows specific affinity to the tumor cells as it gets accumulated in the hepatocellular cancerous tissue with more efficiency than individual magnetic targeting or biomolecular targeting [15].

(e) Heparin is a polysaccharide composed of Glucuronic acid linked to N-acetyl glucosamine. It is an anti-coagulant agent and also has biological applications in MR imaging, drug delivery and tissue engineering. The surface coating of MNPs with heparin enhances the hydrophilic properties of MNPs and therefore, facilitates the cellular attachment to the surface of the nanoparticle. Also, the heparin-coated nanoparticles show higher stability and rapidinternalization.

Synthesis of Heparin-coated MNPs: Alkaline coprecipitation - In this method, Fe2+ and Fe3+ ions are precipitated in alkaline solutions (ammonium hydroxide, potassium hydroxide or sodium hydroxide). The synthesis is usually performed at 70–80°C or a higher temperature which is then followed by *in-situ* heparin surface coating.

Studies show that the Heparin-immobilised MNPs have been fabricated as tumor-targeting MRI agent due to their active interaction with the fibrinogen products in the tumor. Heparin-coated superparamagnetic iron oxide nanoparticles can be applied for *in vivo* MR imaging of human mesenchymal stem cells (MSCs) [16].



Heparin

Other mixed metal-ferrites are reported. For example, the polyethylene glycol (PEGylated) manganese ferrite nanoparticles studied showed excellent T_2 and r_2/r_1 values under low magnetic field. Also the polymer core shell of the PEGylated MNPs provides high stability in aqueous media with increased crystallinity and magnetization values with long blood circulation times and minimized cytotoxicity [17]. Also, the addition of Zinc ferrite into an inverse spinel structure such as Fe_3O_4 is found to increase the net magnetic moment of the resulting mixed spinel structure which in turn increases the T_2 relaxivity and improves the detection sensitivity of MRI [18].

4. ROLE OF GRAPHENE OXIDE-FERRITE NANOPARTICLES IN MR IMAGING

More importantly, the graphene oxide/ manganese ferrite nanohybrids obtained by the thermal decomposition method proves to be an effective T_2-contrast agent as they show negligible cytotoxicity and hemolytic activity in the *in vitro* and *in vivo* magnetic resonance imaging experiments [19]. Proton relaxivity value depends on the size, magnetic moment, spatial arrangement of nanoparticles especially in the infected tissue environment. The framework of the nanoparticle needs to be controlled as an optimal arrangement for a significant increase in the proton relaxivity value. Graphene oxide serves a suitable substrate for spatial distribution factor due to its hydrophilic, flexible and bio-compatible nature on which nanoparticles with super-paramagnetism could be organized in a controlled process. The structural and magnetic characterization of iron oxide nanoparticles containing different concentrations of graphene oxide studied using TEM analysis, XRD profile, XPS profile and Raman spectra revealed that the GO-Fe_3O_4 composite framework containing graphene oxide with least extent of carboxyl group reduction and with largest spacing between the graphene oxide sheets for yielding a very high transverse proton relaxivity value. Also, cytotoxicity analysis of GO-Fe_3O_4 showed that this nanomaterial is biocompatible with normal cells whereas producing considerably toxicity in breast tumor cells, thus proving its efficacy as a theranostic agent [20].

5. CONCLUSIONS AND FUTURE PERSPECTIVES

Polymer-coated magnetic nanoparticles facilitate the delivery of therapeutic agents as well as in the imaging of the tumor tissues, thereby aiding in theranosis. The presence of functional groups in the surface-coated SPIONs can be used for bioconjugation with cell-targeting agents. Moreover the polymer surface coatings of the magnetic nanoparticles enhance their biocompatibility, stability, and concentration in the *in vivo* circulation. Though polymer-coated magnetic particles appear to have significant potency for simultaneous imaging and therapeutic applications, there are many obstacles to overcome before applying this technique to the clinic. For

example, studies conducted in small animal models showed greater potential for particle targeting than studies in larger animals and humans. It is much more difficult to target sites located farther from the magnetic source.

There is an increasing need for the modulation and better functionalization of surface-engineered magnetic nanoparticles to modify or bypass the drawbacks posed by the SPIONS currently in use for MR imaging. Future research should be focused on upgrading the use of magnetic nanoparticles for nanoarticulation of the cells through cell-nanoparticle interaction.

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