A new generation of magnetic nanoparticles as contrast agent (MRI) for biomedical applications

Contents

Abstract

Studies into magnetic nanoparticle are spreading out into different branches and is especially important within biomedical applications. Many nanoparticles are made up of different materials, all mostly being metal. Each material has its optimum use, but iron oxide has been the main material as a contrasting agent for MRI. MRI, in this age and time, is a very common method to diagnose internal problems including cancer. Although it is widely used, many improvements can still be made and one of these is the contrast agent used. Contrast agents are used to provide a more detailed image that helps make the diagnosis easier. Iron oxide has proven to be the most optimum choice due to a unique observation that is superparamagnetism. This allow the metal to have a "non-magnetic memory" and can be used to determine tissues and unwanted cells within the body through relaxation times when magnetised. However due to the weak magnetism, other materials are being researched. The most recent discovery is iron carbide. Little research has been made with this as it is a relatively new discovery, however so far most researchers have given praise to iron carbide and have said that there is a strong possibility that it will replace iron oxide. There have been many positive aspects with iron carbide such as a stronger magnetic susceptibility (therefore allowing the use of a lower concentration that can be used for the patient), however due to the lack of known effects on the human body in the long run, it is still under investigation for daily application. If further research was made into iron carbide, the possibility of it replacing iron oxide in biomedical application is very high.

1. Introduction

In this age and time, nanotechnology is beginning to dominate technology many in everyday uses. These involve from medical purposes to those of protective gear for motorcyclists and all the way to cosmetics and food packaging, as well as the manufacturing of strengthened materials e.g. scratchproof eyeglasses, self-cleaning windows and ceramic coatings for solar cells. For this review we are going to mainly focus on medical purposes, more specifically magnetic resonance imaging (MRI). Within MRI, magnetic nanoparticles are used as a contrasting agent within the biological environment. So the first thing is, what defines a nanoparticle (NP)? The IUPAC definition is that "a microscopic particle whose size is measured in nanometres" where specifically, the particle is less than 100nm. [1] Nanoparticles are increasing in importance due to their properties at such a small scale. Due to their small size,

NPs have their own unique structural, chemical, electrical and magnetic properties, which depend on the electron count but also somehow on the synthesis of the nanoparticles.

Another characteristic of NPs is the volume to surface area ratio. This point is better explained in Fig. 1. This figure represents atoms in a box, the green line represents the surface atoms and the colourless fill represents inner atoms. Since the atoms stay the same size, as the box decreases, the volume to surface area ratio increases. For nanoparticles, this is a very high number meaning that nanoparticles can be highly reactive. This is explained by the surface atoms experiencing different forces compared to the atom in the bulk. This makes NPs more reactive since the surface atoms want to become stable so will react with other atoms to achieve the correct stability. To explain this, imagine all the inner atoms are experiencing 4 forces from other atoms and they are in their stable state. However the surface atoms are experiencing 3 forces and are unstable, the corner surface atoms are experiencing even less force and are more unstable. At quantum size, an example of a change in properties is within the magnetic properties. This is altered due to the smaller size, exhibiting that of superparamagnetic properties. [2]

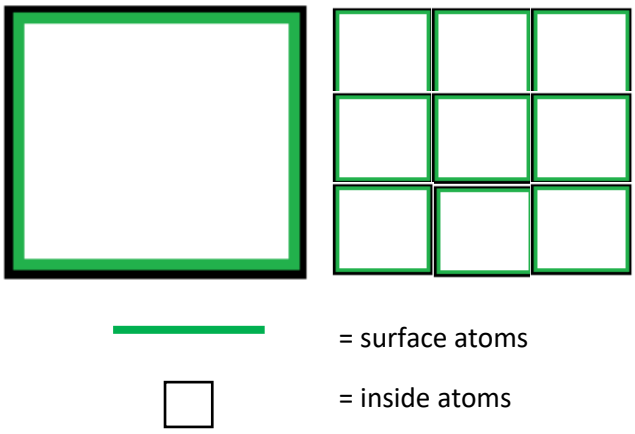


Fig. 1 Classic atoms in a box

As seen from the uses introduced in the first paragraph, one can begin to see where NPs are being constantly used in order to improve our quality of life. One area that uses nanoparticles is that in biomedicine, more specifically magnetic nanoparticles (MNP). Many biomedical applications require the use of MNP, especially that in the field of medicine. Inorganic MNPs are metallic allowing the magnetic properties occurred in normal sized particles to also be a property of the MNP. However this is not useful for medicine applications due to how reactive MNPs can be when bare. [3-4] Solutions and alternatives will be discussed further in this review. Medical applications can include drug delivery, MRI, tissue repair, cellular therapy, hyperthermia, magnetofection; etc. [2] As you can see many applications has been developed throughout a short period of time, that require the use of MNPs. So the importance of researching these within the human body is vital. MNPs are used in MRI as a contrasting agent. This would normally form up of a metallic core covered in a biocompatible coating. [2-6]

In this review, there will be an overview of MNPs and its uses towards biomedical application. Applications of MNPs as MRI contrast agents in terms of classical/ currently used NPs and possible new systems will also be discussed.

2. Synthesis of Nanoparticles

There are many ways to synthesise nanoparticles, but all methods fall into 2 categories: Top-down and Bottom-up approach, also known as physical and chemical approach respectively. Top-down is the decomposition of a bulk material into nanoparticles and this can achieved, e.g. through grinding or lithography. Bottom-up is the formation of nanoparticles from molecules or atoms. Here, there are two stages for crystal growth:

1) Nucleation: the major structural change and reorganisation of the molecules. At this stage, bonds are broken and reformed and particles travel across large distances to rearrange themselves. This requires a lot of energy so the process is normally done under high temperatures.
2) Growth: the process of which the crystal "grows" where ions travel to reaction interfaces. As the crystal "grows" larger, the ions travel further since the layer gets thicker. This step also requires energy.

The reaction rate depends on 3 factors:

1) Surface area of reactants: the smaller the reactant, the greater the surface area so more reaction interfaces will be made, therefore increasing the rate of reaction.
2) Rate of Nucleation: this can be increased when the crystal structure of the reactants is similar to that of the product.
3) Rate of Ion Diffusion: the less defects there are in the crystal structure (i.e pockets of air), the greater the rate.

Within Bottom-up routes (or the chemical methods), there is a classification system for the synthesis of the crystals, there can be Gas (vapour) phase fabrication or Liquid Phase Fabrication. The liquid phase fabrication or wet-chemical method, is when a solid phase reaction is carried out through the rapid mixing of reagents. This allows nucleation to occur and begin forming the crystal. Now growth was mentioned as one of the two stage, however for the formation of nanoparticles, the growing of the crystal has to be limited since if left to grow on its own, agglomeration would occur. In order to prevent that from happening, a capping agent is used in order to limit the rate of growth. Capping agents can be charged molecules, surfactants

or polymers. Examples of wet-chemical methods include sol-gel processing and solvothermal synthesis. Sol-gel processing is the most common method. This is a process where a stable sol solution is made and this slowly turns into a gel like substance through polycondensation. Once the gel is formed, the liquid phases are removed and decomposition of the gels at high temperatures drives out any remaining organic contaminants. The other system is the gas phase fabrication. Similar to that of liquid phase fabrication, however the only difference is that the precursor used is evaporated/ sublimated and a gas phased reaction is carried out for the nucleation stage. Some examples include pyrolysis and inert gas condensation. Inert gas condensation has two methods: physical vapour deposition (PVD) and chemical vapour deposition (CVD). The only difference between the two is that PVD uses physical processes and CVD primarily uses chemical processes. Inert gas condensation is when a metallic source is evaporated in a chamber that has been backfilled with inert gas and a cold finger is in the middle of the chamber. The evaporated gas particles collide with the cooler inert gas particles and allows the deposition onto the finger, due to the loss of kinetic energy. This is then collected and used for consolidation. Pyrolysis is when an organic material is thermochemically decomposed at high temperatures in the absence of oxygen and any halogens. One key step in the synthesis is homogenous preparation. NPs have to be the same size because as previously mentioned, different sizes will cause different magnetic properties to occur. This is better explained in the following chapter. [7]

3. Magnetic Nanoparticles

To further understand MNPs better, a small introduction to magnetism will be given. In order to classify a material's magnetic properties, the magnetic susceptibility (χ) is needed to be considered. This is defined by the ratio of induced magnetization (M) to applied magnetic field (H).

$$\chi = \frac{M}{H}$$

There are different types of magnetisms: paramagnetism, ferromagnetism and diamagnetism. To relate these magnetisms to M and H, paramagnetism occurs when magnetic moments are aligned parallel to H and susceptibilities are small and negative. Ferromagnetism also occur when magnetic moments are parallel to H, however susceptibilities depend on certain conditions such as temperature, atomic structure and external field.

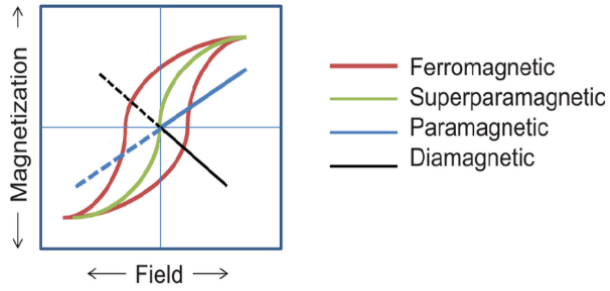


Fig. 2 M-H curves showing ferromagnetism, superparamagnetism, paramagnetism and diamagnetism

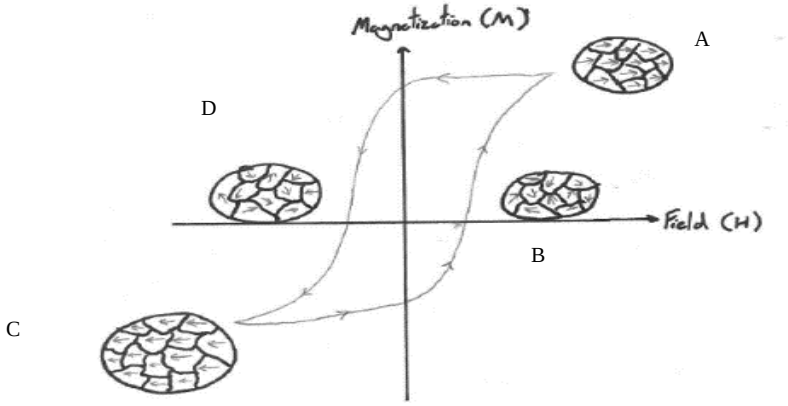


Fig. 3 M-H curve showing ferromagnetism with magnetic domains

At a quantum size, each crystal becomes a single magnetic domain and when multiple crystals form together, this produces one single large magnetic domain. However, one peculiar property is that at a certain temperature, the net magnetization decreases (presuming there is an absence of an external magnetic field). The temperature is known as the blocking temperature and if the amount of thermal energy is sufficiently enough, it provides enough energy to allow free rotation of the particle. This particular phenomena causes the material to exhibit superparamagnetism. Superparamagnetism is essentially the same as paramagnetism, except at much higher susceptibilities. [5-6] An easier way of understanding this would be shown by a graph (Figure 2). The graph shown in Figure 2 are known as M-H curves. As you can see ferromagnetism and superparamagnetism are very similar. However superparamagnetism does not show hysteresis loop. The loop is shown in the ferromagnetism curve, which shows the irreversible process of changes in the field. As H increases, M increases as well but as H reaches a large value, M becomes saturated and starts to plateau. This is the same when H decreases. The shape of the curve is dependent on the size of the particles, the larger the particle, the thinner the loop becomes. Superparamagnetism only occurs for very small particles, so the hysteresis does not exist therefore showing a single sigmoid curve. Hysteresis loop occurs through the lack of retraceability of the curve, which is from the property known as hysteresis.

This is related to magnetic domains within the material. As the domains are all oriented into one direction, energy is required to reorient them back. The domain orientation is shown in Fig. 3. At A, all the domains are oriented into the same direction. Once an opposing field is applied and field value becomes zero, the domain retains most of the magnetization as shown in point B. As the opposing field increases in strength, the domains are now magnetized in the opposite direction (C). This then brings us to the pathway back to A through the use of the original field but oppositely oriented.

Superparamagnetism is needed for biomedical applications, since if only paramagnetism was used, the water molecules that have been charged in the body will retain the magnetism and will forever be charged. Superparamagnetism does not retain the magnetism so will cause no future disturbances, e.g. going through airport security. Due to their magnetic property at such a small size, they can be used to be injected into a biological environment and not be hindered by the immune system. [6] This will be explained later on. The magnetism exhibited by this ultrafine particles can alter the magnetic field within the body during MRI and this will alter the relaxation times, as explained below. However the only problem is that this is all dependent on how the MNP is synthesised, since different synthesis routes will cause the MNP to have a different property.

4. How does MRI work?

MRI is one of the main techniques currently used to diagnose diseases, others include positron emission tomography and computed X-ray tomography. MRI is based on nuclear magnetic resonance, where an image can be produced through the signals of protons from water within the tissues and organs of the biological environment. Under a strong external magnetic field the protons align accordingly, and when switched off the protons relax. The process of switching the magnetic field on and off causes the proton to emit radio waves and this is used to produce the image. [8] This is

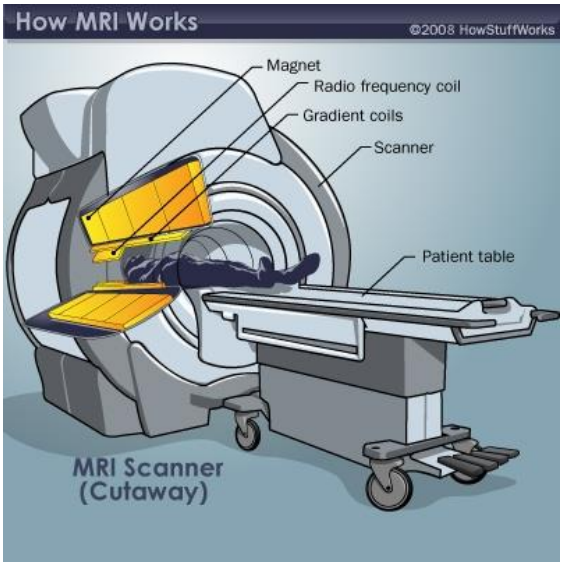


Fig. 4 MRI machine and components. Ref: http://science.howstuffworks.com/mri1.htm (04/03/2016, 16:51)

helpful since each organ and tissue within the body has different density and homogeneity, therefore the image produced will show the different organs and tissues from different radio waves. However, it is hard to distinguish between cancerous and normal tissues. This is where MNPs come in, they allow the enhancement of contrasts and can provide molecular imaging as it will now be discussed.

As mentioned before, MRI is based on nuclear magnetic resonance, with two types of relaxation times, T1 and T2. T1 is longitudinal relaxation and is known as T1-recovery (also referred to as 'spin-lattice' relaxation), T2 is transverse relaxation and known as T2-decay (also be referred to as 'spin-spin' relaxation). MNPs affects these differently. For T1, in order to shorten the process, it requires a close interaction between the proton and the contrasting agent. However this is strongly dependent on the thickness of the coating. [5] With reference to time constant, T1 is the time it takes for 63% of the longitudinal magnetization to recover in tissue. [11] But MNPs affect T2 the most and is sometimes referred to as "T2 agents". [6] T2 decay is the result of the exchange of energy between spinning protons, so it is the time it takes for transverse magnetization to decrease to 37% of the initial value. Shortening of T2 times is due to a large difference in magnetic susceptibility between the particles and the medium surrounding it, causing a microscopic magnetic field gradient. [5, 9] T2 is more important for superparamagnetic materials due to their high susceptibility, so there is a greater difference. [10] The effectiveness of a contrasting agent is described through its relaxivity over a range of concentrations which is

$$R_1 = \frac{1}{T_1} \text{ or } R_2 = \frac{1}{T_2}$$

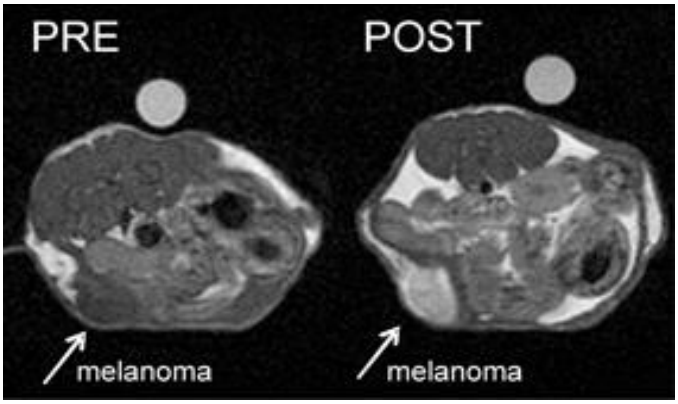


Fig. 4 MRI images of tumour-bearing mice before and after use of contrast agent. Ref: http://blogs.rsc.org/cc/2012/01/25/non-invasive-detection-of-melanin-formation/ (04/03/2016 13:53)

However, the relaxivity is also affected by a number of different variables such that they can be measured e.g. field strength, temperature, what medium the magnetic nanoparticles are in, etc.

Therefore, the 'contrast' in contrast agent is referring to the difference in signals for adjacent regions, such as 'tissue-tissue', 'tissue-vessel' and 'tissue-bone'. For MRI, the enhancement comes from the interaction between adjoining water protons and the contrasting agent itself. However this can be affected by lots of different factors, such as intrinsic and extrinsic factors, including proton density and MRI pulse sequences. [11] The protons spin under a specific frequency when in alignment, which also commonly known as the Larmor frequency. The alignment can either be parallel to that of the magnetic field or antiparallel to it. When a frequency that causes the proton to resonate is interacting with the proton, this provides sufficient energy for the proton to absorb and jump to the antiparallel state. When the frequency disappears, the proton relaxes back to the lower state and this is what gives off the radio waves in which the machine detects and provides the image. The contrast agent is classified depending on the relaxation process as follows, T1 and T2 agents. T1 agents are normally paramagnetic complexes and T2 agents are usually based on iron oxide nanoparticle agents. [12]

6. Examples of MNPs for MRI

MRI is a technique known since 1971 however the process is still has room for improvement. Research is still currently being done in order to improve the process so the risk to the patient is reduced to as close to zero as possible. One main problem is the contrast agent used. Although yes, MRI is being used today, problems could still occur due to the unknown of how the agent reacts in the long term.

Since MNPs have first been suggested to medical applications, researchers have proposed different types of MNPs as carriers for contrast agent. These can be split into classical and modern MNPs as well as T1 contrast agents and T2 contrast agents. A few examples from all types will be discussed, together with their advantages, disadvantages and possible room for improvements.

6.1 Classical Examples

6.1.1 Gadolinium-based

The most commonly used nanoparticles for biomedical diagnosis are mainly gadolinium based (Gd^{3+}) or manganese based (Mn^{2+}).[13] Examples of these metals used as contrast agents

in biomedical application include blood-pool agents, hepatobiliary and renal agents and Molecular agents.[14] These are paired with metal ion complexes that have a high thermodynamic and kinetic stability in vivo to prevent harm. However currently researchers have been experimenting with Gd^{3+} and reported that it has been causing changes, both qualitative and quantitative, within that of the brains of an adult patient. [15-17] Since Gd^{3+} is a T1 type of contrast agent, researchers noticed that there was a correlation between the MRI signal intensity ratio changes in the dentate nucleus and globus pallidus of the brain and the number of gadolinium-based MRI contrast agent administrations. This is still being researched to see if any solution may be found. Now the two mentioned metal ions are both T1 contrasting agents presently used.

Now as mentioned before, there are 2 groups of contrast agent, T_1 and T_2. These are determined by how the relaxation time is affected. The common ones that are used are gadolinium and iron based. [14] Gadolinium is used in its ionic state, Gd^{3+}, however this cannot be used by itself since it is highly toxic and has an undesirable biodistribution. This was initially

Table 1. Examples of commercial contrast agents. This shows the different ligands that can be used to indicate the same region of the body. [7a]

Type	Name of Compound	Indication
Ionic	Gadopentate dimeglumine, Gd-DTPA	Neuro/whole body
	Gadoterate meglumine, Gd-DOTA	Neuro/whole body
Neutral	Gadodiamide, Gd-DTPA-BMA	Neuro/whole body
	Gadobutrol, Gd-BT-DO3A	Neuro/whole body

seen when the first Gd-based ($[Gd\text{-}DTPA(H_2O)]^{2-}$) contrast agent was used in 1981. It also undergoes hydrolysis at physiological pH so produces the insoluble compound, $Gd(OH)_3$. Through the promising discovery that Gd^{3+} Is a good candidate as a contrast agent, research was made on it to discover that chelates can be used to form a complex that minimizes the toxicity to a tolerable level. However, since chelates are very big in size, even if they do have a high relaxivity compared to the more traditional one, it prevents effective extravasation from the vasculature. [13] Other examples can be seen by Chan. [14] Chan reports about the examples and compares each Gd compound with different ligands, describing how each one has its own unique property and their optimal uses. To summarise, there are many MNPs used today, each specific to its uses, so many that it would take too long to talk about.

6.1.2 Iron Oxide

Currently the most common metals used for MNPs are cobalt, nickel and especially iron. These are not used in their solid metal forms but rather in their oxidised form. The most popular core used is iron oxide. Iron oxide has been referred to as ferrites ($Fe_2M_xO_4$) and magnetites (Fe_3O_4) in the pharmaceutical field. [6] When used in application, iron oxide is chemically altered into a colloid and still maintain the magnetic property. This is done since when in its normal state, iron oxide is water insoluble and therefore cannot be clinically used. Another problem is that it is unstable in a colloid solution, so a coating is applied in order to provide the stability within the colloid as well as in vivo; providing solubility within the biological media. [6, 11] Iron oxide is now very popular amongst biomedical application, ever since its first use in 1989, it greatly reduces the T2 relaxation times within the liver, spleen, and bone marrow via selective uptake and accumulation in the cells of the reticuloendothelial system (part of the immune system that consists of phagocytic cells). [18-19]

Another classical example is iron oxide, a T_2 contrast agent. As always, the story of how it is made would be a good start to tell the tale. As with any other compound/ material, there are many different ways of making it. Throughout time many researchers have developed methods to formulate the most efficient way to produce the product with the highest yield. However, since there are many synthetic routes, this review will only mention the classical methods known. There are many methods to produce nanoparticles. These include microemulsion, [20] sol-gel syntheses, [21] sonochemical reactions, [22] hydrothermal reactions, [23] hydrolysis and thermolysis of precursors, [24] flow injection syntheses, [25] and electrospray syntheses. [26] References have been provided for further detail for each methods. There were two main challenges when it came to the production of superparamagnetic nanoparticles (SPNO) due to their colloidal nature. Firstly, the overall product all have to be grains of suitable size so finding experimental conditions for this proved difficult. Secondly, the process had to be able to be industrialised without the use of any complex purification procedure. [27] The method that was devised and is now used commonly for the production of MNPs is the chemical coprecipitation of iron salts. Coprecipitation is the simultaneous formation of two or more precipitates. This tends to be unwanted but in some cases can be exploited. The first controlled preparation of superparamagnetic iron oxide (SPIO) particles was in 1979 by Massart through the coprecipitation of $FeCl_3$ and $FeCl_2$ and added to an alkaline solution, ammonia. [28] Massart demonstrated that although the isoelectric point was near pH 7, he attempted to add the iron solutions with ammonia and through experimentation, parameters were found that could

produce particles ranging from 16.6 to 4.2 nm; parameters including: influence of base, pH value, added cations and Fe^{2+}/Fe^{3+} ratio from the yield of the coprecipitation reaction. [29] So he demonstrated that SPIO can be formulated in either acidic or alkaline condition.

MNPs are formed of a toxic metallic core with a shell around it. Without a shell, iron oxide is a toxic substance within biological environment. SPIO particles are relatively large in size and can be prone to have a fast clearance rate due to opsonization by plasma proteins and phagocytic cells. One example of a material used as a shell is dextran (figure 5). Dextran provides the stability of the SPIO in order to prevent aggregation. [30] Dextran is also mainly used since it provides biochemical manipulation (i.e. changes within the biological environment). For example, studies show that with oxidation with periodate, the number of hydroxyl groups increased and allowed more amino groups from proteins to bind on. [31] However due to the fact that dextran can come in different sizes (i.e. chain length), it is important that the choice of dextran is considered. Using a favourable sized chain length enables optimum polar interactions with iron oxide surfaces, interactions mainly chelation and hydrogen bonding. Poly(ethylene glycol) was also investigated for its compatibility with iron oxide and it has been reported that the polymer increases the iron oxide dispersion and blood circulation times. [32]

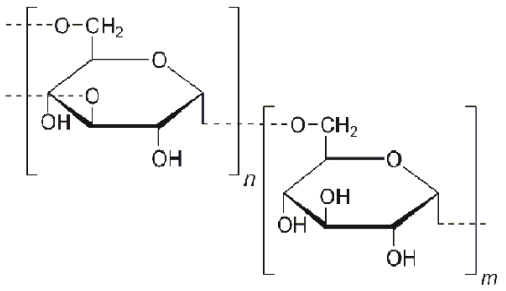


Fig. 5 Molecular structure of dextran

Iron oxide is currently one of the most commonly used MNP for MRI, however it is not necessarily the most optimum material that can be used. So what limitations are there? During imaging, a loss of signal is seen when using iron oxide particles, known as ‘black holes’. This proves as a disadvantage seen it prevents direct evaluation of the tissue (so will need to compare pre- and post-images) and will make it difficult to discriminate between targeted molecules and cells and image artefacts. So further research will be required to increase the specificity of detection of iron oxides. Also, as it can be seen from Table 2, the difference in magnetic susceptibility is big. Metallic iron has the greatest susceptibility however due to it being too poisonous for biological use, it is rarely used for in vivo applications. This leads us to iron

nitride and iron carbide to compare. Although not as great as iron in terms of magnetization, they both are greater than that of magnetite.

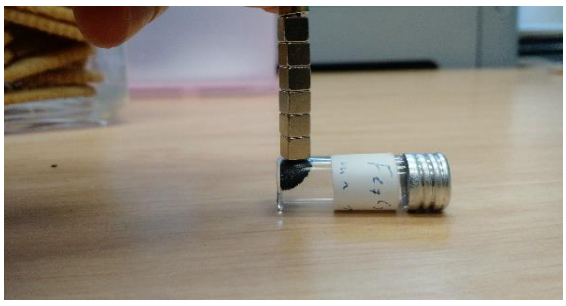
Table 2. A table comparing the magnetic susceptibility of different MNPs. As courtesy of Dr. Giordano

Ref: https://publishup.uni-potsdam.de/opus4-ubp/files/7537/giordano_habil.pdf

Compound	Molecular Formula	Magnetic Saturation (emu/g)
Metallic iron	Fe^0	200
Magnetite Iron(II, III) Oxide	Fe_3O_4	90
Iron Nitride	Fe_3N	123
Iron Carbide	Fe_7C_3	120
Cementite (Iron Carbide)	Fe_3C	140

6.2 Recent Examples

6.2.1 Iron Carbide and iron nitride



Pic 2. Picture showing iron carbide nanoparticle attached to an external magnet. Courtesy of Dr. Giordano

Pic 1. Iron carbide and iron nitride nanoparticles. Courtesy of Dr. Giordano

There are two recent examples that will be presented in this section, iron carbide and iron nitride. These materials are appealing to use due to its many different useful properties, including good mechanical properties (durability), thermal chemical resistance, conductivity, catalytic activities and of course magnetic properties. Due to the high energy required to form the metal-non-metal bond, the only way to form the compound synthetically would be to apply high temperatures, high pressures and strong nitrification/ reducing agents as the conditions. However due to new pathways being found, interest in these compounds have picked up again. [33]

Little is known about these non-oxidic nanoparticles as research is still currently being done. However, papers that have been published have shown that iron carbide has proven to be a promising core material. Hermann *et al.* [4] in his paper shows a method that is low cost and has a good production rate, one that exceeds 10g/h. The method involved using the conventional flame spray synthesis. Iron oxide can be made with the combustion of iron-2-ethylhexanoate. However if the synthesis was done in a reducing environment, oxygen becomes the limiting reagent and the product produced were iron-based nanoparticles that had varying carbon content ranging from 1.8 to 8.05 wt%.

Hermann also published another paper describing the use of metal nanomagnets for in vitro applications mainly for drug delivery. [3] The focus of the paper was on carbon-coated metal nanomagnets and the stable carbon shell protects the core, which is oxygen sensitive, from oxidation and prevents loss in magnetism. It was suggested that when applied in vivo, no major issues such as clotting and complement activation, would arise. The experiment had proved that the carbon-coated nanomagnet were "well-tolerated" by the blood as well as endothelial cells. However, the lack of results on long term interactions needs to be clarified so further studies would need to be made.

Bircher published a paper describing the possibilities of carbon coated nanoparticles within a biological environment, more specifically within the blood. [34] The main purpose was to see if the nanoparticles were possible candidates to be used in magnetic drug delivery and blood purification. Results proved that there were some minor blood coagulation, however this was very minor when put into comparison of the observed effect. But as mentioned in Hermann's paper, this requires more study since the outcome of long term exposure is still unknown. This is important since if blood coagulation is discovered late stage, this will put the subjects at high risk. The earlier this is found, then corrections can be made now rather than later on preventing amplifying effects. Also this study can be translated to other researches as blood coagulation is an important risk that needs to be addressed.

7. Biocompatibility

Biocompatibility is an important factor when using MNPs in vivo. Now all common metal cores used are toxic to the internal biological environment. [3, 4, 18-19, 35] Biocompatibility is allowing the substance, in this case a MNP, to become safe to use within a biological environment without harming the environment itself. Biocompatibility is an important issue since a lot of useful medical compounds are hydrophobic and so are not stable in aqueous matter.

For example iron oxide is toxic within the human body and this has been studied in detail. [36,37] However due to its perks (explained later), iron oxide is a very useful and reliable compound as a MNP.

In order to make it biocompatible, a shell is enveloped around the core, providing both protection against chemical degradation of the core and prevents the release of any potential toxic components. Currently research is being made into using ligands as the shell. Ligands allows surface modification of the nanoparticle to impart additional functions to it. Recently researchers have developed methods of various surface modification. Here two main methods: ligand exchange with water-dispersible ligands and encapsulation with biocompatible shells, will be talked about. NPs can be prepared in different ways, a common way to prepare NPs are in organic solvents, NPs are stabilized by surfactants with hydrophilic heads attached to the surface of the NP and the hydrophobic tails facing the solvent. This allows phase transfer of the NP from organic to aqueous media due to the atoms on the surface having an affinity with the functional groups. What this allows is a simple procedure of making the MNP more stable, a thin protective coating and overall a small size. [9] For example, silanes were used to terminate amino-, carboxylic- and poly (ethylene glycol) - ligands as experimented by Palma and et. The silanes allowed the ferrite MNPs to become stable and water-dispersible. This change was due to the electrostatic and/ or steric repulsion. Further experiments showed that a protective layer was formed that protected against mild acid and alkaline environments. Further details are reported in Palma et al. [38]

The second method, encapsulation with biocompatible shells, is the formation of biocompatible shell around NP(s). There are many varieties of methods for shell formation and can be classified by the material used and the process, but the main common materials are silica and polymers. Oxide nanoparticles can be stabilized by crosslinked amorphous silica shells. However the use of silica brings about the question of the toxicity of nano sized silica, but reports have shown that silica-encapsulated NPs have proven non cytotoxic. Yi and colleagues reports that silica-coated nanoparticles were produced via base-catalysed silica formation in a reverse microemulsion. [39] The process is simple and different kinds of NPs can be contained, as well as multiple NPs can be encapsulated at the same time in one shell. But one problem with silica shells is that they are pH sensitive, so depending on the pH of the surrounding medium, precipitation and gel formation will occur. Polymers have already been widely used throughout biomedical applications, both natural and synthetic polymers. These polymers (that can be attached to the previously mentioned silica shells) have reduced concerns on safety and

toxicology. They can also be multifunctional such that simultaneously, the capabilities of drug-delivery and imaging can be applied through alteration to the polymers. [40] Common examples include poly(lactide-co-glycolide) (PLGA), poly(lactide) (PLA) and poly(glycolide) (PGA). [41] However there is one polymer that has received a lot of attention throughout the past years, polyethylene glycol (PEG). PEG is a hydrophilic and water soluble polymer, and due to its nonfouling property, low protein adsorption; almost can be classified as resistance to protein adsorption, and the ability to pass through the reticuloendotherial system (RES), part of the immune system, and other natural barriers, [42] it has been investigated upon a lot to further discover the biocompatibility with MNPs. Due to its inert properties, materials that can strongly bind to the surface of the NP are combined to encapsulate the NP and this is one of the main reason why the polymer is extensively used as stabilizing material in biomedical application. More details can be found in LaConte et al. [43-45]

8. Related Biomedical Applications

Beside their application in MRI, MNPs can be used for other biomedical purposes. A brief overview will be given here.

8.1 Drug Delivery

Alongside contrast imaging, another important and promising field application of MNPs is drug delivery i.e. MNPs can be used as carriers for drugs that are site-specific. The ideal scenario would be for the drug to attach on the surface of the MNP or in bulk and then be driven to the target organ and released there. Therefore the size, charge and surface chemistry of the magnetic particles are important and will affect the blood circulation and bioavailability of the particles in the body. [46] So talking about size first, this is important since particles with diameters greater than 200 nm are removed by the phagocyte system so will decrease blood circulation time. However if the particles have diameters less than 10 nm, they are removed through extravasations and renal clearance. Reports have shown that particles with size ranging from ~10 to 100 nm are optimal since it is the easiest to inject through the vein and has the most pro-longed blood circulation times. The size range is enough are to evade RES of the body as well as enter into the capillaries, therefore providing the most effective distribution. [47]

The advantages of using MNP is that it can be actively shuttled to the specific target tissue with the help of a magnetic field. That way, no side effects will occur through the attack of healthy cells since the drug will only attack cells that are unhealthy. The realisation of this led researchers in the late 1970s to research the use of magnetic carriers targeting specific sites

within the body. [48] Overall the objectives for them was to be able to reduce the systemic distribution of drugs in the body and reducing the dosage of the drug. The way it worked was for the drug to be attached to a biocompatible MNP and this would then be injected into the patient in the form of a ferrofluid. An external, high-gradient magnetic field would then be used to concentrate the fluid in the target area and through either enzyme activity or changes in physiological conditions (e.g. pH or temperature), the drug would be released and take effect on the unwanted cells. The effectiveness of this depends on several parameters, including field strength, gradient and volumetric and magnetic properties of the MNP used.

8.2 Tissue Repair

The MNPs allow tissue repair through the wielding of two tissue surfaces and heating it to join them together. Also before the heating, polymer coated nanoparticles are placed between the tissue in order to enhance the joining. It has been reported that to induce tissue union, temperatures greater than 50˚C is required. It is believed that the joining happens due to the denaturation of proteins followed by the entanglement of adjacent chains. [49] It has also been reported that nanoparticles that have light absorbing properties with the output of the laser are useful for tissue-repairing procedures. [2] Examples of this include gold- or silica-coated iron oxide nanoparticles. The reason for this is that it would minimize tissue damage through the use of the least harmful wavelengths of light.

Other uses include the targeting of damaged tissues with stem cells. Stem cells are the body's "master" cells and have the ability to specialise into other specific cells. The possibility would be to coat the stem cells in MNPs and then with the help of a magnetic field, the cells are guided to the damaged tissue site and the process of healing would proceed. [2] Another addition that could be added would be the addition of various proteins on the MNP that can aid the tissue development.

8.3 Cellular Therapy

Cellular therapy is the process of which cellular materials are injected into a patient, normally those of living, healthy cells. This process helps the human cells from rejecting outside cells. There are many different ways for cellular therapy, but here we will only touch on some methods briefly.

Cellular labelling is now an increasingly common method for in vivo separation. This can be done via two ways: 1) attaching magnetic particles to the cell surface, 2) internalizing

biocompatible magnetic particles by endocytosis. This allows the cells to be detected by MRI. The most efficient and specific cell labelling is the attachment of a ligand, to the surface of the MNP, which is taken up by the target cells via endocytosis. [2]

8.4 Hyperthermia

Hyperthermia is a therapeutic treatment where heat is used to destroy malignant tumours. In current times, many different methods were used to produce heat and destroy the tumours. One such technique is magnetic induction. Cancer tissues are exposed to alternating magnetic fields. Living tissue does not absorb the field so the field can penetrate through the living body to target location even if it is deep within the body. The use of magnetic particles is when a magnetic field is applied, the particles generate heat due to magnetic hysteresis loss. The amount of heat generated depended on the material used and magnetic field parameters. Magnetic particles are embedded around the site and when an oscillating field is applied, the particles heat up and destroy the tumour cells. Cancer cells are destroyed at 43˚C whereas normal cells can survive at higher temperatures. [2]

So the choice of a high-power magnetic particle and with the appropriate magnetic field, a very tiny amount of particles would be required in order to heat the tissue to acquire cell necrosis. Many reports have shown that hyperthermia treatments increases the cytotoxicity of radiation and drug treatment allowing the quicker process of destroying tumour cells. [2, 26]

Use of MNPs are promising however, due to synthesis problems; producing the correct size and industrialising it, it still requires more research and development.

8.5 Magnetofection

This is a method where MNPs that are associated with vector DNA where the nucleic acids are introduced with the help of an external magnetic field. The magnetic particles that are used are positively charged due to a coated shell, normally polethylenimine a polycation. So these are associated with negatively charged DNA. Magnetofection has been reported that, regardless of the vectors being viral or non-viral, it has increased the efficiency of the vectors several times. [50] It has enhanced the delivery of these processes since it is a very quick process, simple and in vitro, provides saturation at low doses. However since this is a relatively new application, research is still being carried out. [2]

Conclusion and Outlook

Throughout this research, I have discovered many different compounds that are being used today. Even as a chemist, there are many compounds still unknown to me. I was aware of NPs before starting this literature however I never fully understood their uses until now.

The aim of this research was to provide insight on what magnetic nanoparticles are and how they are used in MRI and other biological applications. The main MNPs used as contrast agents are gadolinium-based and iron oxide. Iron oxide is a well-known and studied compound, yet is not the most efficient. As shown in this review, the magnetization of other compounds are higher. Iron carbide in particular has better magnetization than iron oxide. As described by Hermann *et al.*, a little tweak in the synthesis of iron oxide can allow the synthetic route to produce iron carbide. Problems with the synthesis is that a secondary metal ion is required and a lot of energy is needed for the high temperature treatment. Iron oxide also seems to have problems when imaging. The "black holes" that can be seen may cause problems in the future. So iron carbide may produce better quality imaging, however more research would be needed to prove it. This is a good starting point for the research into iron carbide and if proven successful, could be a possible replacement for iron oxide.

Once the synthesis is refined, other modifications can made in order to improve the procedure according to requirements and needs. Investigations into the system is still required, such as the mechanics and structure of iron carbide especially at a nanoscale level. Other problems that will rise is the issue of biocompatibility. Iron carbide is toxic and will cause complications within a biological environment, so a "shell" is required to be researched in order for it be of use. As the same with gadolinium, many shells may be discovered that can be of use, and each combination will be used for different target indications. Overall the biocompatibility still needs to be researched upon in order to find the most compatible shell, one that prevents the core from oxidising and be able to provide surface engineering.

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