Optimizing the characteristics of magnetic nanoparticles for their use in drug targeting

ABSTRACT

This review focusses on the characteristics of magnetic nanoparticles and how they can be optimised for their use in drug targeting. The size of the MNP should be large enough to be captured by the external magnetic field and to be loaded to sufficient therapeutic and targeting agents. However, there is an upper bound for the diameter of around 100nm in order for the particles to be superparamagnetic which prevents aggolmeration and rapid uptake by the immune system. The most common MNPs used *in vivo* experiments are iron oxide particles due to their good biocompatibility. They are also coated with a polymer or silicate coating to further prevent agglomeration and for agents to be bonded or adsorbed on. Using metallic or bimetallic MNPs would increase capture efficiency, but they will need a stronger coating (gold or carbon) in order to be biocompatible and there are currently few synthetic methods which make such mono-dispersed MNPs.

I. Introduction

I.1 Overview

The general use of drug delivery is non-specific. In chemotherapy, for example, cytotoxic drugs given intravenously lead to a general systemic distribution around the body. The drugs will attack healthy tissue as well as tumour cells, causing unwanted side effects.

The use of magnetic targeting localises the action of drugs to the tumour cells. Drugs are bonded to the magnetic carriers and are injected into the bloodstream. Then, by applying a high-gradient magnetic field from outside the body, the drug/carrier complexes are concentrated at the target tissue, where the drugs are released, usually by changing the magnetic field, enzymatic activity, or changes in physiological conditions such as pH, osmolality or temperature, leading to increased uptake of the drug at the target cells [1, 2].

Whilst the use of magnetic particles as drug carriers was first proposed in the late 1970s, it is only within the last two decades that the potential of using magnetic nanoparticles (MNPs) in a range of clinical applications has been widely recognised [1,2].

In this review, we shall look how the effectiveness of drug targeting can be optimised, specifically by looking at the properties of the MNPs used in experiments and pre-clinical trials. We will look at how size, shape, materials of the core and coating and surface chemistry affect how the drugs distribute (biodistribution) and move (pharmacokinetics) in the vasculature and uptake by the tumour cells. Ultimately, the magnetic targeting system devised must strive to do the following:

• To maximise the time in which the MNPs exist in the vasculature (blood circulation time) in order for them to reach and accumulate at the tumour.

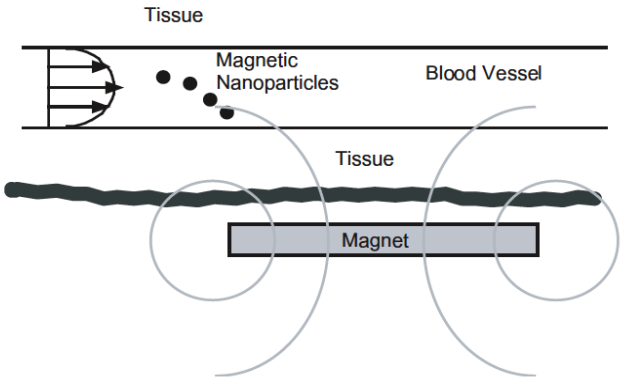


Fig 1. A hypothetical magnetic drug delivery system shown in cross-section: a magnet placed outside the body produces a high-gradient magnetic field which captures the MNPs flowing in the circulatory system [1].

• To maximise the ability to specifically target tumour cells and to permeate through their barriers

• To be biocompatible.

Finding an optimum combination is a challenge, and also depends on the target tissue However, there has been much progress with *in vitro* and *in vivo* experiments in determining these characteristics.

I.2 Biocompatibility

Magnetic carriers suitable for clinical applications need to be able to be biocompatible, meaning that they should not be toxic to the patient. We must therefore use materials which are inert in the blood within a range of conditions.

The particles must also be as isolated as possible; although agglomeration may increase flow stability, it will also increase the chance of being taken up by the reticuloendothelial system (RES). The RES, consisting of phagocytic cells which reside mainly in the liver, spleen, bone marrow and lymph nodes, engulfs foreign bodies in the vasculature before they agglomerate and

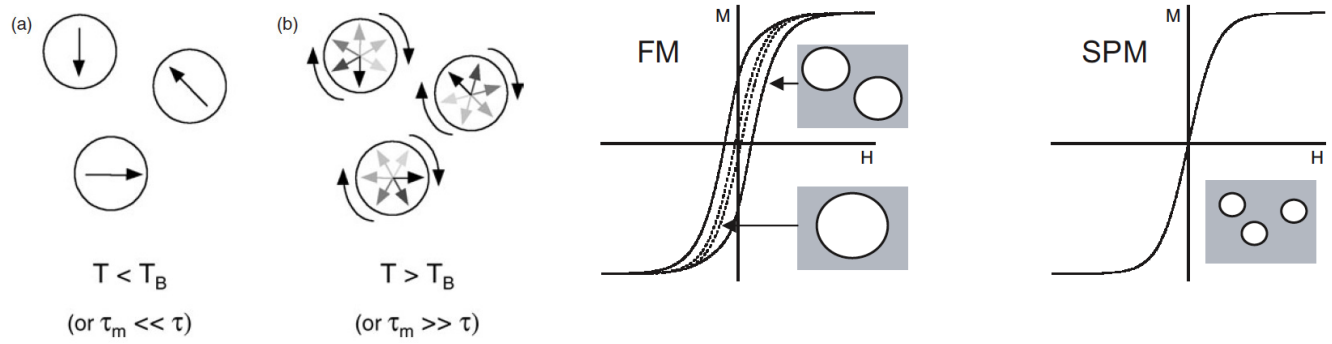


Fig. 2. Left: Diagram demonstrating the dynamics of the magnetic moments of single-domain MNPs at the different temperature regons. T_B is the blocking temperature which defines the boundary of the two regions. Right: Hysteresis loops of ferromagnetic and superparamagnetic particles. Ferromagnetic particles show hysteresis properties, where the width of the loop (coercivity) characterises the remnance of the magnetic moment. The coercivity increases as the particle size decreases. Superparamagnetic particles have zero hysteresis, and their magnetic moment rotates freely [1].

block capillary vessels. We must try to avoid this in order to maximize the blood circulation time of the MNPs [3,4].

Finally, the particles must also clear from the body after the drug has been released. This is possible through either biodegradation or intact excretion [5].

II. Size of MNPs

II.1 Transport

The force on a spherical MNP, F, in a non-uniform magnetic field, B, is given as:

$$\mathbf{F} = (\mathbf{m} \cdot \nabla)\mathbf{B} = V_{\mathrm{m}} \Delta\chi \nabla \left(\frac{B^2}{2\mu_0} \right), \qquad \text{(Eq. 1)}$$

where m is the magnetic dipole induced in the MNP, V_m is the volume of the MNP, $\Delta\chi = \chi_m - \chi_w$ is the effective magnetic susceptibility of the MNP relative to water, and μ_0 is the permeability of free space [1]. The magnetic force needs to be large enough to overcome the hydrodynamic drag forces that the MNP will experience in the bloodstream. Therefore, the parameters that increase this force, such as the particle size, need to be maximised in order to increase the capture efficiency of the MNPs [1]. However, there are other factors, as discussed below, which effect drug delivery and ultimately have their impact on the optimum size of the MNP for drug delivery.

II.2 Superparamagnetism

In order to maximize the susceptibility of the MNPs, they need to be made from a ferromagnetic material, such as iron, cobalt or nickel. These materials also display hysteresis properties and therefore a remnant magnetization after the external field has been removed. The magnetic field required to remove this is known as coercive field. This coercive field is inversely proportional to the diameter of the particle: smaller particles will have broader hysteresis curves [1,6].

However, if a particle of ferromagnetic material has a diameter smaller than a critical value, it will become a well-isolated single magnetic domain, maintaining one giant magnetic moment. At low temperatures, the magnetic moments are quasi-static and flip slowly relative to a reasonable experimental timeframe. At temperatures above the blocking temperature, T_B, the thermal energy is sufficient to induce free rotation of the magnetic moment and therefore the remnant magnetization that would be present in a ferromagnetic material is lost. This is known as superparamagnetism, and it enables the particles to maintain colloidal stability and avoid magnetic aggregation. The particles will also have much higher magnetic susceptibilities than normal ferromagnetic or paramagnetic materials due to the coupling interactions within the single domains [1,4,6].

It is therefore strongly preferred for clinical applications that the MNPs used are superparamagnetic (diameter, $D < 50$nm), or at least in the ferromagnetic-superparamagnetic limit ($D < 100$ nm) [5].

II.3 Surface effects

As the particle size decreases, the majority of the atoms in the nanoparticle are at the surface of the particle and therefore interfacial effects become more important. The large surface spins to bulk spins ratio leads to a local breaking of symmetry causing changes in magnetization. For example, decreasing the size of oxide particles decreases its magnetization, whereas for metallic nanoparticles, like cobalt, enhancement of the magnetic moment with decreasing size has been reported [6].

II.4 Circulation time

The size of the MNP has been shown to be a factor in its blood circulation time. It has been reported that small MNPs ($D < 5.5$nm) are rapidly excreted renally, whilst very large MNPs ($D > 200$nm) are rapidly taken up by the RES and are found in the liver and spleen [3,5].

Therefore, MNPs for drug targeting should aim

to be between these two extremes. Generally, larger MNPs are eliminated from the bloodstream faster than small ones. However, Berry *et al* demonstrated that for particles smaller than 40nm in diameter, both the circulation time and biodistribution of MNPs are determined by the coating material rather that the mean size [7].

II.5 Tumour Permeability

Nanoparticle size also affects the ability of NPs to extravasate from the vasculature. Most endothelial barriers allow NPs < 150nm in diameter to pass through, but there are more stringent barriers such as the blood-brain barrier (BBB), an essential barrier to penetrate in order to target brain tumours [3]. The exact mechanism of nanoparticle transport across the BBB is not fully understood, but most likely relies on endocytosis and/or passive leakage across defects in the BBB, where size is a factor. Pulfer and Gallo compared magnetic and non-magnetic nanoparticles of different sizes in targeting glioma-2 tumours. It was shown that 10-20nm magnetic particles are the most effective at targeting brain tumours [1,8]. In a similar experiment, Sonavane *et al* reported that gold NPs between 15-50nm were able to permeate across the BBB in rats, whereas larger NPs, specifically 100-200nm, could not [9]. It has been suggested, however, that BBB permeability is likely influenced by all physiochemical properties discussed here, particularly surface modification [5, 9].

III. Optimum Shape of MNPs

It has been suggested that anisotropically shaped MNPs can avoid bioelimination better than spherical MNPs, with a positive correlation between length-to-width aspect ratio and blood circulation time of the MNPs. Roca *et al* suggested that this is because anisotropic particles can align with the blood flow [10]. However, according to O. Veiseh *et al*, more studies are needed to identify exactly what aspect ratios yield most dramatic influence on MNP pharmacokinetics [3].

In the same review, the use of "nanoworm" MNP constructs (Fig. 3) have shown enhanced cell binding, since the elongated shape allows for more targeting molecules to be added [3].

IV. Surface Properties

IV.1 Surface Charge

Veiseh *et al* suggested that charged MNPs will cause proteins to adsorb the MNPs, which will be recognized by the RES and then removed from circulation [5]. Specifically, positive charged MNPs also bind to non-specific cells, and strong negative charged MNPs result in increased liver uptake [7]. Therefore, it would seem that MNPs with a neutral surface charge are preferred, unless the liver is the target organ [1-4,7]

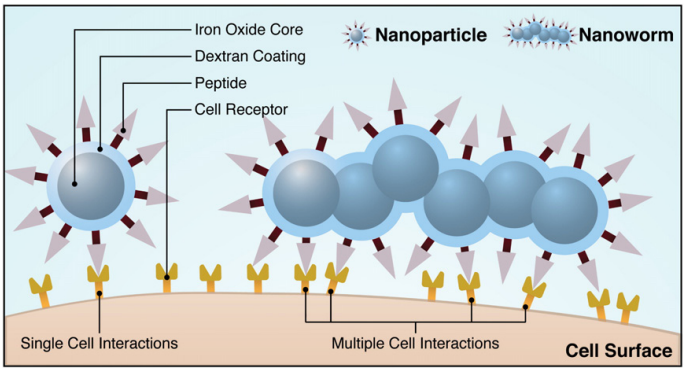


Fig 3. Diagram illustrating the multivalent interactions between receptors on a cell surface and targeting ligands on a nanoworm versus a nanosphere [3].

Durán *et al*, however, suggested that a small charge on the particle surface will give sufficient repulsion to reduce agglomeration, and particles in an aqueous electrolyte solution, such as blood, acquire a double-layered surface charge. This produces an electric potential distribution, which proves the basis of electrophoresis, the motion of dispersed colloids relative to a fluid upon application of an external electric field. Durán et al give examples on how this is useful for preparation and use of nanoparticles, such as coating and drug loading [5].

IV.2 Hydrophobicity

Like surface charge, hydrophobic MNPs will adsorb proteins and/or agglomerate and will be removed from circulation by the RES [3,5]. Therefore, zero hydrophobicity is preferred, but there may be a need to adsorb hydrophobic drugs to the MNP surface, which will decrease the MNP blood half-life [3, 11].

V. Materials - Core

In this section we shall look at the possible materials that can be used for the core and methods of their synthesis.

V.1 Iron Oxide

Iron oxide nanoparticles have been the most commonly investigated MNPs for biomedical applications due to their favourable biocompatibility, biodegradability and ease of use [4]. There are typically composed of magnetite (Fe_3O_4) or maghemite (γFe_2O_3), formed in a close-packed cubic lattice structure with the iron ions located at the interstices. In the case of Fe_3O_4, magnetisation arises from electron hopping between the Fe^{2+} and Fe^{3+} ions that co-exist at the octahedral sites. When the MNPs are metabolised, the iron ions are added to the body's iron stores, which are used to produce haemoglobin. These are all positive attributes for their safe use *in vivo* [4].

According to Roca *et al*, superparamagnetic iron oxide nanoparticles (SPIONs), 10-30nm in diameter,

are best for liver targeting, whereas ultra-small SPIONs (USPIONs), < 10nm in diameter, are best for tumour permeability. These particles are predominately synthesised by precipitation of iron salts in an aqueous medium, in the presence of a material that will coat the MNPs (see part VI).

Magnetisation varies vastly among synthesis methods even with particles of similar size The main problem with this method is that the saturation magnetization, M_s, for MNPs synthesized this way is lower (30 – 50 emu/g) than the expected bulk value (90 emu/g).

V.2 Metallic and Bimetallic

Iron, cobalt, nickel MNPs are attractive options for drug delivery, as they possess much larger magnet moments (nearly twice) and are able to maintain superparamagnetism at larger particle sizes than their iron oxide counterparts. This means that the magnetic force will be larger for a given particle size, and hence more effective capture of the MNP in the body. However, metallic MNPs will oxidise and corrode in the presence of water and oxygen, and oxidation products may even be toxic (e.g. cobalt oxides). These MNPs must then be protected with a coating. It is generally found that crystalline coatings, such as gold, carbon or iron oxide are capable of providing a robust coating that prevents oxidation, but amorphous coatings, such as polymeric coatings, cannot protect metallic cores from deep oxidation [4]. Another problem is that ferromagnetic materials in general are not biodegradable. Hence, such particles can only be completely cleared through intact excretion, where the coating plays an important role for avoiding exposure to the magnetic core and facilitating excretion through the kidneys [5].

Bimetallic (alloy) nanoparticles, like FePt and FeCo can also exhibit superparamagnetic properties, with even higher magnetization values. FeCo particles with 10-20 nm cores and 1-3 nm gold or silver shells, synthesised through a physical deposition processes, were measured to have a M_s value three times as high as that of comparable iron oxide nanoparticles [4]. The interactions between the two chemical species lead to greater stability in comparison to metallic MNPs. But like their metallic counterparts, bimetallic MNPs will oxidise or corrode if they are not coated with a sufficiently robust coating.

VI. Material – Coating

Surface coatings are an integral part of MNPs in biomedical applications. Even with their superparamagnetic properties, naked MNPs have a high surface energy, which will cause them to agglomerate and will become more readily taken up by the RES.

VI.1 Polymeric coating

Polymers are chemically anchored or physically adsorbed on MNPs, which creates a steric repulsion to balance the surface interactions and van der Waals attractive forces acting on the nanoparticles. The two that we shall discuss here are dextran and poly (ethylene glycol) (PEG).

Dextran, a branched polysaccharide compromised of glucose subunits, has been widely used for SPION coatings, due to its favourable biocompatibility and high affinity for iron oxide surfaces. Dextran-coated iron oxide MNPs are already being widely used clinically for cancer imaging. One of the main drawbacks of using dextran is that the coating is held to the MNP via hydrogen bonds and may detach if the interactions between the coating and the surroundings (blood) overcome the core-coating interactions. One solution to this is to crosslink the polymers after MNP attachment. The resulting particle, cross-linked iron oxide (CLIO), is much more stable, without change in size, circulation time or loss of coating in harsh conditions, and show no acute toxicity. However, there are unlikely to be used in a clinical setting, as the cross linking method involves the use of epichlorohydin, a moderately toxic carcinogen [4, 12].

PEG is another widely used polymer for nanoparticle coating in biomedical applications, and

Table 1. A summary of the most common MNP synthetic methods [3]. The details of the methods will not be dicussed here, but an extensive review on the subject can be found in the review from F. Schüth *et al* [6].

Synthetic method	Synthesis	Reaction temp. [°C]	Reaction period	Solvent	Surface-capping agents	Size distribution	Shape control	Yield
co-precipitation	very simple, ambient conditions	20–90	minutes	water	needed, added during or after reaction	relatively narrow	not good	high/ scalable
thermal decomposition	complicated, inert atmosphere	100–320	hours–days	organic compound	needed, added during reaction	very narrow	very good	high/ scalable
microemulsion	complicated, ambient conditions	20–50	hours	organic compound	needed, added during reaction	relatively narrow	good	low
hydrothermal synthesis	simple, high pressure	220	hours ca. days	water-ethanol	needed, added during reaction	very narrow	very good	medium

gives the MNPs a long circulation time as the coating makes them particularly resistant to protein adsorption, and thus not readily recognised by the RES. This makes them ideal for targeting specific cells after modification with targeting ligands [3].

As was mentioned in part V, polymeric coatings are not suitable to protect very reactive MNPs, such as metallic or bimetallic ones. Such MNPs were found not to be stable even in air, leaving the core easily leached by acidic solution, resulting in the loss of their magnetisation [6].

VI.2 Inorganic shells

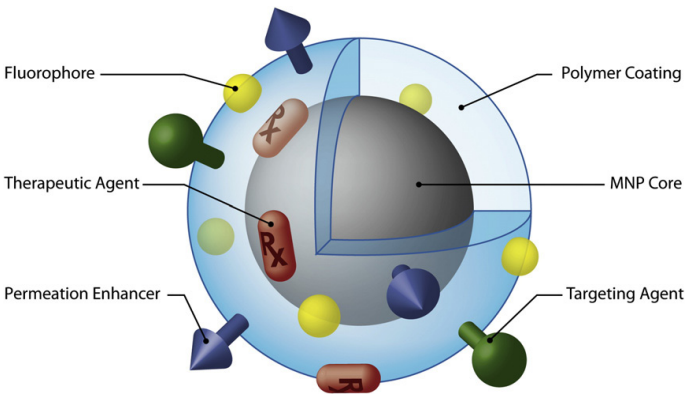
Gold seems to be an ideal coating due to its chemical inertness, but this also means that forming shells for direct coating is very difficult. Progess had been made though, through a variety of methods, including reversed microemulsion, combined wet chemical reduction and laser irradiation, which have all been reviewed by F. Schüth et al [6]. It is also possible to produce heterodimer MNPs, where ferromagnetic and gold NPs are fused together, with good biocompatibility and aqueous stability [9, 16].

Silica shell coated MNPs are also attractive for drug targeting as they have several advantages; they are stable in aqueous solution, relatively easy to synthesise and interparticle interactions are easy to control through variation of shell thickness. However, silica is not stable under alkaline conditions and may contain pores through which oxygen could diffuse [5].

V1.3 Carbon shells

Carbon coating is a more recent advancement and has shown many advantageous properties over polymers and silica. Carbon coated MNPs are very chemically and thermally stable, as well as non-toxic, making them biocompatible. Since the discovery of fullerenes, it has been demonstrated in numerous studies that, in the presence of metallic nanoparticles, graphitised carbon structures, such as carbon nanotubes and carbon onions, can been formed under arc-discharge, laser ablation and electron irradiation. The layers formed provide an effective barrier against oxidation and acid erosion.

Fig. 4. Schematic diagram of MNP possessing various ligands for effective targeting and detection (fluorophores) [4].



However, all of these methods produce agglomerated clusters, not mono-dispersed particles which are essential for surface modification and maximisation of blood circulation time [5].

VII. Surface modification

A vast majority of molecules can be conjugated to the organic/inorganic shell of the MNPs that can make the targeting mechanism more effective. In section II, we have seen how reducing the size of the MNPs can facilitate passive targeting (permeation through defects in the tumour cells). Attaching targeting moieties, such as antibodies, peptides, aptamers and small molecules to the MNP surface have been found to increase the concentration of MNPs at the target site. These moieties specifically bind to receptors at a tumour cell membrane and enter the cell via endocytosis. For example, chlorotoxin acts as a target to MMP-2 receptors which are found on brain tumor cells. Chlorotoxin also acts as a brain tumor therapeutic agent, making it ideal for drug delivery to brain tumours. In addition, permeation enhancers, such as the Tat peptide, can also be attached to MNPs to facilitate delivery through the cytoplasm. A table of targeting strategies can be found in the review by O. Veiseh *et al* [3].

The MNPs should also be loaded with therapeutic drugs. Several chemical drugs that have been integrated with MNPs specifically for chemotherapy include chlorotoxin as discussed above, paclitaxel and doxorubin. These drugs can be bonded to the surface by a covalent bond, adsorbed to a charged or hydrophobic surface or grafted on. The specifics of each of the methods are beyond the scope of this review, but they have been extensively reviewed by O. Veiseh *et al* [3]. After the MNPs have entered the cell, the loaded drugs are released via a change in conditions such as pH, or can be released by changing the external magnetic field [3,4].

VIII. Using magnetic implants

As denoted in part II, the magnetic force on the MNP is dependent on the size of the particle and the strength and gradient of the field. We have established that for effective drug delivery, MNPs should be smaller than 100nm in diameter in order to be superparamagnetic. Even though these particles have larger magnet moments, the small size of the particles makes it difficult to direct them to the target while withstanding the drag of blood flow. Targeting is likely to be most effective in regions of slower blood velocity, particularly if the magnetic field is close to the target tissue [1,14].

In most cases, the field gradient is generated by a superconducting permanent magnet, such as Nd-Fe-B,

fixed outside the body over the target site. Using such a magnet in combination with a SPION, the magnet can reach effective magnetic field depths up to 10-15 cm. The flux densities around the site must be the order of 0.2T, with field gradients of approximately 8 T m^{-1} for femoral arteries and greater than 100 T m^{-1} for carotid arteries [1, 15].

If the strength of the magnetic field were to increase, the particles will reach magnetic saturation and the magnetic force will only be proportional to the gradient of the field. Since the fields from such powerful magnets tend to be homogeneous over the target site, increasing the field strength will not necessarily produce larger forces. Instead, ferromagnetic implants can be inserted at the target site and, under the influence of the external magnetic field, the implant will be magnetized generating locally high gradient magnetic field, increasing the force on the particles and therefore, increasing the chance of capture. This allows for the use of smaller particles necessary for improving tissue permeability, blood circulation time and to produce superparamagnetic particles which will reduce their tendency to agglomerate [15,16]. Aviles *et al* enhanced targeting of a swine heart ventricle by implanting a ferromagnetic stent at the site. It was found that the capture efficiency increased by as much as 11-fold [17].

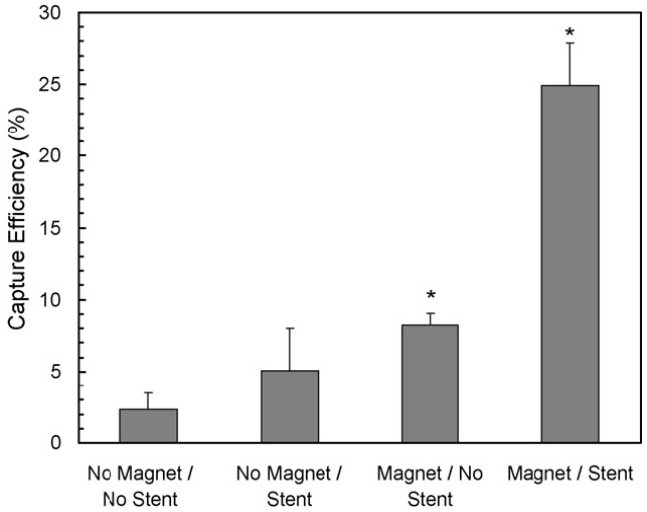


Fig. 6. Capture efficiency of MNPs (%) with different set-ups for drug targeting in the swine heart vetricle (External B-field =0.125 T, stent diameter = 0.5mm). The capture efficiency increases 11-fold with the use of the magnet and stent versus the control. From Aviles *et al* [17].

IX. Conclusions

We have looked at a range of variables for MNPs and we should be able to make some sort of judgement Larger particles have their advantage, as the capture efficiency is optimised and the targeting and therapeutic action increase too due to the increased loaded amount of the agents. However, this optimum size is capped to around 100nm in diameter in order to prevent agglomeration and rapid clearance via the RES. MNPs must be even smaller if they are to pass through the BBB.

Most of the literature studied suggests that the use of iron oxide MNPs with a polymer coating would probably be the most suitable carrier to use in drug delivery, because of its chemical stability and biocompatibility.

However, there has been motivation to make biocompatible inorganic, high-moment MNPs in order to increase the capture efficiency. Carbon and gold shells seem to be the most promising due to their high stability and lack of toxicity, but new synthetic methods are needed to easily synthesise mono-dispersed particles. More studies with anisotropic particles should also be considered.

The use of MNPs in medicine is not limited to drug delivery. There has been much progress in using MNPs for gene delivery (tissue engineering and regenitive medicine), MRI contrast enhancement and tumour cell ablation via hyperthermia, all of which are optimised with many of the topics discussed in this review. Several companies already synthesise MNPs for these uses as a commercial venture. For example, MagForce Nanotechnology AG synthesise Iron Oxide MNPs with an aminosilane coating to be used in therapeutic hyperthermia [14].

There is real potential in achieving the goal of an optimum drug targeting system and is motivated by the interdisciplinary work in the fields of physics, material science, pharmacology and medicine.

X. References

1. Q. Pankhurst *et al. J. Phys. D* **36** (2003) R167
2. J. Dobson *Drug Dev. Res.* **67** (2006) 55-60
3. O. Veiseh *et al. Adv. Drug Del. Rev.* **62** (2010) 204-304
4. C. Sun *et al. Adv. Drug Del. Rel.* **60** (2008) 1252-1265
5. Durán *et al. J. Pharm. Sci.* **97** (2008) 2948-2984
6. F. Schüth *et al. Angewandt Chemie Int. Ed.* **46** (2007) 1222-1244
7. C. Berry *J. Phys. D* **42** (2009) 224003
8. S. Pulfer, J. Gallo *J. Neuro-Oncol.* **41** (1999) 99-105
9. Y. Koo *et al. Adv. Drug. Del. Rev.* **58** (2006) 1556-1577
10. Roca *et al. J. Phys. D* **42** (2009) 224002
11. C. Xu, S. Sun *Dalton Trans.* **2009** 5583-5591
12. J. McCarthy, R. Weissleder *Adv. Drug. Del. Rev.* **60** (2008)
13. Choi, Jun, Yeon. *J. Amer. Chem. Soc.* **128** (2006) 15982
14. M. Arruebo *et al. Nano Today* **2** (2007) 3 p22-32 1241-1251
15. H. Kempe, M. Kempe *Biomaterials* **21** (2010) 9499-9510
16. Q. Pankhurst *et al. J. Phys. D* **42** (2009) 224001
17. M. Avilés *et al. Int. J. Pharm.* **361** (2008) 202-208