Review Article

Journal of Applied Material Science & Engineering Research

# Superparamagnetic Iron Oxide Nanoparticles in Cancer Theranostics

**Mohammad Norouzi[1], Soroosh Derakhshanfar[2], Mohammad Reza Kazemian[1] and Rodrigo França[1,3]***

[1]*Graduate Program of Biomedical Engineering, University of Manitoba, Winnipeg, MB, Canada*

[2]*Department of Mechanical Engineering, University of Manitoba, Winnipeg, MB, Canada.*

[3]*Dental Biomaterials Research Lab, Department of Restorative Dentistry, University of Manitoba, Winnipeg, Canada.*

***Corresponding author**

Rodrigo França, Graduate Program of Biomedical Engineering, University of Manitoba, Winnipeg, MB, Canada, E-mail: Rodrigo.Franca@umanitoba.ca

**Submitted**: 15 Jan 2018; **Accepted**: 22 Jan 2018; **Published**: 05 Feb 2018

## Abstract

*Superparamagnetic iron oxide nanoparticles (SPIONs) have found extensive applications in both cancer therapy and diagnosis (theranostics) due to their intrinsic magnetic properties. SPIONs can be applied as contrast agents in magnetic resonance imaging while they are considered as efficacious drug carriers for targeted therapeutic systems as well as hyperthermia therapy. In this article, recent advances in application of SPIONs in the realm of cancer theranostics are reviewed. Moreover, biosafety issues arising from SPION application are briefly mentioned.*

**Keywords:** SPIONs, Cancer diagnosis, Cancer therapy, Drug delivery, Hyperthermia, Biosafety

## Introduction

Cancer is generally defined as an abnormal growth of cells that tend to proliferate uncontrollably. Cancer is one of the leading causes of morbidity and mortality worldwide. In 2012, 14.1 million new cases of cancer and 8.2 million cancer-related deaths were reported in the world. By 2025, 19.3 million new cancer cases are expected to be identified each year [1,2].

To alleviate this growing trend and to improve cancer patients' quality of life, new therapeutic approaches with high tumor targeting and drug delivery efficiency are being introduced. To this end, nanomedicines have attracted much attention due to their ability to amalgamate therapeutic and diagnostic (theranostic) approaches while providing targeted drug delivery and overcoming limitations of conventional cancer treatments such as systemic toxicity and low solubility of some chemotherapeutic drugs [3-6].

In cancer nanomedicine, various anticancer drugs and imaging agents can be encapsulated and/or embedded within nanoparticles to establish a multifunctional regime for both therapy and imaging. Among different types of nanoparticles, superparamagnetic iron-oxide nanoparticles (SPIONs) mostly magnetite (Fe3O4) and meghamite (Fe2O3) nanoparticles are of great interest owing to their desirable superparamagnetic behavior, biocompatibility and surface-modification features [3,7].

Generally, superparamagnetism is defined as a phenomenon that happens in magnetic materials whose sizes are under that of a single magnetic domain. In contrast to their paramagnetic counterparts, they do not show magnetization and become highly dispersed after removing an external magnetic field. SPIONs exhibit superparamagnetic features at room temperature with core diameter of 20 nm or less. This feature prevents nanoparticles from aggregation, otherwise, they may be eliminated (engulfed) by macrophages [3,8].

Currently, SPIONs are being utilized in theranostic applications such as magnetic resonance imaging (MRI), positron-emission tomography (PET), single-photon-emission computed tomography (SPECT), fluorescence imaging, in addition to the magnetic hyperthermia as well as drug carrier for chemotherapeutics. In fact, SPIONs can be functionalized with both contrast agents, fluorescence dyes, quantum dots, etc., for imaging and chemotherapeutic drugs, nucleic acids, monoclonal antibodies as well as targeting moieties for cancer treatment [9,10].

Drug delivery through magnetic drug-bearing nanostructures relies on external magnetic field guidance to reach target tissue, enhancing accumulation of magnetic nanoparticles in site of action. Furthermore, this approach can reduce the drug clearance, increase blood circulation time of the drug and enhance drug internalization efficiency within target cells, minimizing nonspecific cellular interactions (Drug exposure to the surrounding region). This consequently reduces associated side effects. Moreover, in terms of medical imaging, SPIONs as MRI contrast agents show some benefits over traditional ones such as high magnetic signal strength, relatively low cytotoxicity, longer lasting contrast enhancement as well as amended delineation of tumor edges [5,7-9,11,12].

In addition, high surface area of SPIONs provides the opportunity of increased covalent attachment with a variety of high-affinity ligands, molecular markers, receptors, peptides, antibodies, ligands and drugs,

making them a good candidate for both molecular imaging and targeted drug delivery to specific cells and organs [3,13]. Nowadays, some SPIONs are in clinical trial and some formulations have been clinically approved for both medical imaging and therapeutic applications, Lumiren, Gastromark, Feridex, Endorem, to name but a few [5,14].

SPIONs can be synthesized by a variety of methods, mostly classified into two approaches: c and non-hydrolytic. The hydrolytic methods involve reduction-precipitation, microemulsion and microfluidic techniques while among the non- hydrolytic methods, thermal decomposition of organometallic reagents is the most common technique. After synthesizing, SPIONs are typically coated with polymers to provide colloidal stability, biocompatibility and create functional groups. There are various natural and synthetic polymers can be utilized, such as dextran, chitosan, alginate, poly (D,L-lactide-coglycolide), polyethyleneimine (PEI) and poly(ethylene-glycol) (PEG) [8,13,15].

In this article, some advances in the fabrication and application of SPIONs for the purpose of cancer theranostics and biosafety issues arising from SPION utilization are reviewed.

## SPIONs for Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is generally an imaging tool to image soft tissue. This technique is based on alignment of hydrogen nucleuses in our body under external magnetic field. Then a radiofrequency signal with the same frequency is applied to realign the protons in the hydrogen nucleus. Since protons tend to align with the magnetic field and as they go back to their previous position, they release energy (relaxation energy) which is captured by the detectors in the machine [16].

Contrast agents are of great importance in diagnosis of cancer cells and MRI imaging. SPIONs are being used to detect cancer cells under external magnetic field. Common techniques have some drawbacks including inability to deliver high amounts of some contrast agents due to toxicity and inability to detect small size tumor cells. SPIONs have been shown to improve contrast as well as longitudinal and transverse relaxation time. Due to their ability to react strongly to external magnetic field and being coated with biocompatible polymers, images resolution is improved even at very low quantities of SPIONs [17].

SPIONs are highly sensitive to signal and can be detected swiftly by electron microscopy which has made them excellent candidates as MRI contrast agents. Many researchers have focused on relaxivity of SPIONs recently. Coating and nanoparticles' size are the main components affecting relaxivity values. For instance, in a recent investigation, Karagoz et al. altered the distribution of the particles in the encapsulants to attain a high r2 relaxivity value of 582 mM-1 s-1 at 9.4 T [18]. In another study, ultra-small SPIONs were coated with citrate and signal intensity was studied in rat liver. Also, Saraswathy et al. coated SPIONs with dextran and studied signal intensity in male Wistar rats [19]. A summary of a few commercial types of SPIONs as contrast agents for MRI in different organs is illustrated in Table 1.

**Table 1: Commercially available MNPs for use as contrast agents in MRI [5,20]**

| Number | Preclinical agent | Commercial name | Target | Status |
|---|---|---|---|---|
| 1 | AMI25 | FeridexW/ferumoxides, AMAG Pharma; Feridex I.V.W, Berlex Laboratories; EndoremW, Guerbet | Liver | Approved |
| 2 | SHU555A | Ferucarbotran, Schering AG; ResovistW, Bayer Healthcare | Liver | Approved |
| 3 | AMI227 | Ferumoxtran-10/CombidexW, AMAG Pharma; SineremTM, Guerbet | Lymph node metastases | Phase III |
| 4 | NC100150 | ClariscanTM, Nycomed Imaging (Part of GE Healthcare) | Tumor microvasculature | Discontinued owing to safety concern |
| 5 | AMI121 | FerumoxsilLumiremW, Guerbet; GastroMARKW, AMAG Pharma | Bowel | Approved |
| 6 | OMP | AbdoscanW, Nycomed Imaging | Bowel | Approved |
| 7 | Code 7228 | FerahemeW (ferumoxytol), AMAG Pharma | Vasculature | Phase II |

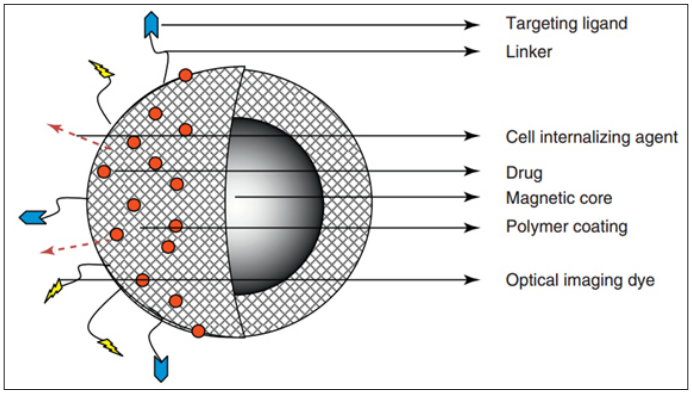


**Figure 1**: Different conjunction on the SPIONs Surface

Also, SPIONs play an important role in molecular imagining by providing through information on molecular changes in cells without interfering with their normal processes. In contrast to conventional methods, exploiting this technique, accurate information about molecular changes in advanced stages, before outbreak of cancer symptoms [21].

## SPIONs for Drug Delivery

Magnetic micro and nanoparticle has been used for drug delivery since 1970s. In 1976 magnetic erythrocytes was used for delivery of cytotoxic drugs. Magnetic albumin microspheres encapsulating an anticancer drug (doxorubicin) in animals was used as targeting model followed by development of this strategy to deliver different drugs using magnetic microcapsules and microspheres [22,23]. All these initial approaches were micro sized.

The first magnetic NPs used in animal model in 1996 and the first clinical use of these nanoparticle showed some advances and failure, about 50% of the magnetic nanoparticles ended up in liver in first trial use in human. Different companies now manufacture magnetic NPs. FeRx, Inc. (founded in 1997) use metallic Fe ground together with activated carbon as magnetic NPs and load doxorubicin on them. Clinical study of these magnetic NPs has been conducted on patients with primary liver cancer [24].

Chemicell commercialized Target MAG doxorubicin NPs involving a multidomain magnetite core and a cross-linked starch matrix with terminal cations and FluidMAG® for drug delivery applications [25]. Drug release can be done in different ways like simple diffusion, enzymatic activity or changes in physiological conditions such as pH, osmolality, or temperature [26].

Superparamagnetic iron oxide nanoparticlesare NPs which can have different cores like γ-Fe2O3 (maghemite), Fe3O4 (magnetite) or α-Fe2O3 (hematite). Even mixed transition metal oxides (copper, cobalt, nickel and manganese) with iron oxide also known as SPIONs [9]. SPIONs can bind to wide range of substances including antibodies, dyes, chemotherapeutic agents and nucleic acids; therefore, they can be used in different applications such as prevention, diagnosis, targeting and treatment of cancer. Irion-oxide nanoparticles are low cost, compatible and stable so it can be used as a good drug carrier. SPIONs can reach to the target through passive or active targeting and external magnetic field [12].

In passive targeting, nanoparticles use the leaky vasculature of tumor cells to easily enter the tumor cells [27]. In the other words, they use anatomical and physiological abnormalities of tumor tissues to distinguish them from healthy cells. This was the first techniques that were used for targeting tumor cells because they do not require the chemical conjugation of ligands to the SPIONs' surface [8]. Active targeting involves conducting vitamins, antibodies, and peptides on the SPIONs surface (Fig. 1) [28-30]. This allows SPIONs to accumulate efficiently at the target site and kill the cancer cells without harming other cells [10].

External magnetic force is also another approach to direct the iron-oxide nanoparticles towards the target tumor region. Conjunction of SPIONs with specified prostate cancer cell membranes antigens shows promising results in drug delivery to the cancer cells. SPIONs with PEG coating are good carrier for drugs like doxorubicin, this has efficient tumor targeting and drug-delivering capacities [31]. Positively charged SPIONs have indicated easier entry to the cells compare to negative ones [32].

In 1997, doxorubicin-loaded MNPs showing targeted accumulation by external magnetic force. To enhance the efficacy of magnetic nanoparticle accumulation, HER2-conjugated MNPs has been developed that contain anticancer drugs such as paclitaxel and rapamycin. This showed promising results in in vitro cell culture system [33]. Drug delivery to brain tumor has also been studied. Conjugation of chlorotoxin to the MNPs showed preferential accumulation and increased cytotoxicity in tumors in vitro and in vivo. In a study it has been indicated that chlorotoxin-decorated SPIONs can improve cellular uptake, and also an augmented invasion inhibition rate compering to free chlorotoxin (98% vs. 45%) [34]. In another study, injection of doxorubicin-loaded magnetic albumin intra-arterially proximal to the rat tumor site, increased the targeting yield by 200 times in comparison with intravenous injection [35].

Nanocapsules is one of the effective tools for increasing the functionality of magnetic NPs. Because of adjustable physiochemical properties of nanocapsules like size, surface charge, morphology, shell thickness, etc. this method is known as a promising candidates for biomedical applications. Moreover, this method can encapsulate drugs and protect them against degradation by pH and light, while minimizing tissue irritation and provide controlled release by external features such as temperature, reduction, and light radiation and pH changes [36]. Since chitosan is highly cationic, non-cytotoxic and biodegradable, it has been used as a gene nanocarrier. Encapsulation of iron oxide with pluronic/chitosan nanocapsules and using external magnetic force exhibited that iron oxide NPs preserved their magnetic property after encapsulation. The study revealed that after 2 h incubation, nanocapsules were not efficiently internalized by cells, but after using magnetic force they entered cytoplasm only after 30 min [37].

An animal study showed that chitosan coated magnetic NPs loaded with plasmid DNA-expressing enhanced green fluorescent protein can go directly to the heart and kidney of mice after injected to the tail. Drug release can also be controlled by manipulating the temperature of the polymeric shell like by swelling and de-swelling. Higher release rates could happens above the lower critical solution temperature (LCST) and lower rates below the LCST [38]. Iron oxide magnetic NPs can be encapsulated in thermoresponsive polymer like poly-N-isopropylacrylamide which has LCST about 37C so when alternating magnetic field applies to these nanoparticles, there will be higher release of loaded drug. Since tumor site is slightly more acidic than other parts, acid degradable polymers could be another approach for drug release at tumor site. In one study, SPIONs were encapsulated in hydrophobic polyglutamate polymer segments via an acid-cleavable hydrazone bond while doxorubicin was conjugated onto it [39].

## SPIONs for Hyperthermia

Hyperthermia is the use of powerful radio waves which can produce high temperature to destroy carcinomas. Since this method could be harmful to the surrounding cells, “magnetic hypothermia” has been proposed as a substitute method which will minimize harmful effect of the hyperthermia on the other cells [40]. After a strong magnetic field applied to the SPIONs and they reach to the target, magnetic direction alters repeatedly to produce heat at the tumor site. The heat could be controlled by adjusting the strength and exposure duration

of the magnetic field but 42-45 C is enough for initial apoptosis in cancer cells. This approach offers the possibility of taking a drug-free approach to the treatment of cancer. Transition of alternating magnetic field (AFM) to heat is also depends by Specific absorption rate (SAR) of SPIONs [7]. Frequency needed for inducing heat from SPIONs is ranged between less than 1 MHz up to 30 MHz Deoxy-chitosan polymer magnetic Nano flakes showed high SAR value at a frequency of 512 kHz than individual Nanocubes [41]. In the other study it has been indicated that multicore magnetic nanoparticles shows 2000W/g SAR at 520 kHz with increase in temperature by the rate of 1.04 C/s for iron concentration of 0.087M.

Hyperthermia activity of SPIONs could improve by doping with other metal atoms like manganese, but doping copper can have negative effect on SAR and decrease it. SPIONs showed good cancer cell killing rate. For example, 74% of cancer cells die by 14nm magnetic nanoclusters treatment with SAR value of 500Watt/g [42]. HeLa cells also reduced by 42% when they exposed to heat generated by silica coated iron oxide nanoparticles after applying an alternating magnetic field. If the concentration and the size of these nanoparticles increase, the apoptosis in cancer cells increase as well. For instance, by increasing sodium carbonate-stabilized-oleic acid-functionalized magnetic nanoparticles from 0.22 mg to 0.44 mg, the rate of cancer cell mortality increases to 80%. In the other study the effect of nanoparticle size has been shown when 16nm magnetic nanoparticles kills about 60% of the cancer cells while 12nm and 13nm nanoparticles kills only about 10% to 20% of the cancer cells [43]. Polymer stabilized-iron oxide-graphene nanocomposite can be heated up to 42C for a concentration of 2.5mg/ml in 15 minutes, after 4 and 8 hours about 40% and 76% of cell death observed respectively. Reduction in tumor size due to magnetic nanocluster has been reported in some studies. Exposure of magnetic nanoclusters to AMF intensity of 8 kA/m and frequency of 230 kHz can cause temperature increase by 6C and decrease tumor size by one-tenth times after 35 days of treatment [44].

In another study, SCCVII squamous cell carcinoma induced in mice reduced after applying 38kA/m of field at 980 kHz. Hyperthermia can be used as bond breaking phenomenon to release the drug which is linked to the MNPs via a heat labile linker that gets broken when exposed to heat. Derfus et al. successfully used this mechanism to release fluorescein-labeled DNA using Electromotive force (EMF) activation [45]. Thermosensitive polymers like poly (N-isopropylacrylamide)-based nanogels can use for therapeutic release in conjunction with MNPs. Hollow silica nanoparticle embedded with Fe3O4 has shown significant suppression of MT2 breast cancer cells and B16/BL6 mouse melanoma cells growth after in vitro radiofrequency-activation. In vivo mouse tumor penetration study using magnetic-guided nanocapsules shows 200 times higher magnetic nanocapsules trapped in the tumor cells [46]. Magnetite cationic liposome has been developed for treatment of osteosarcoma which uses localized hyperthermia. The animal study showed that tumor in hamster treated with this method became 1/1000 smaller than its initial size [47].

## SPION Toxicity

Toxicity issues are one of the factors limiting SPIONs application in biomedical fields. Contradictory results could be found in literature regarding toxicity of SPIONs. Gupta et al. reported bare SPIONs toxic via MTT assay while PEG-coated SPIONs were reported as nontoxic [48]. On the contrary, Muldoon et al. reported bare iron oxide nanoparticles nontoxic in rat brain cells [49]. In another study, it was demonstrated that PVA coated SPIONs depicted acceptable cell viability levels up to a concentration of 200mM but a higher concentration of 400mM interfered with the cell cycle [50]. Obviously, there is not a general toxicity response to SPIONs that could be applied to organs and cell types. An Important issue is the difference response to SPIONs in vitro studies compared to in vivo studies. Mahmoudi et al. argued that this difference could be due to changes made to culture medium by SPIONs which is not the case in the body since body maintains certain internal environment [51]. SPIONs also have been shown to cause stress. Production of reactive oxygen species by SPIONS affects cells and in turn causes stress. After penetration into the cells, SPIONs degrade mostly to iron ions and their reaction with hydrogen peroxide can potentially damage the cells by generating free hydroxyl radicals [52]. Other studies have shown that SPIONs have only caused very low toxicity in body.

Among all metal oxide nanoparticles, iron oxide is non-toxic in concentrations lower than 100 $\mu g/ml$ [53]. Also, another study on human glia, breast cancer and normal cell lines showed that SPIONs are only slightly toxic in doses above 100 $\mu g/ml$ and nontoxic below that [54]. The few available studies on humans show temporary and weak side effects caused by SPIONs coated with dextran [55]. It is also proposed that SPIONs degrade and exit the body through iron metabolic pathway. The biocompatibility of SPIONs is dependant on magnetic content, nanoparticle size and coating. Mahmoudi et al. investigated plain SPIONS, SPION-COOH and SPION-NH2 surface chemistries using DNA microarrays [56]. SPION-COOH was detected to affect the expression of the genes involved. In an important study, Fe3O4 with various diameters were studied for metabolic activity and DNA stability in normal fibroblasts versus fibrosarcoma cells [57]. In almost all cases, cytotoxicity or genotoxicity was below 5% at concentrations less than 500 $\mu g/ml$. However, positively charged magnetic nanoparticles coated with APTMS showed toxicity rates above 10% at the same concentration range. Concentration, diameter and suface charge were introduced as the main parameters participating in toxicity in this study. Smaller and positively charged particles coated with APTMS were found to be induce higher toxicity.

Since SPIONs are magnetic particles, they could potentially interfere with iron metabolic in body. Therefore alteration of serum and iron levels in tissue was studied for three weeks after injection of nanoparticles to rats [58]. Although gradual increment of iron was observed during the first week, iron levels start to decrease in the second week. Iron particles were observed to accumulate in the liver and spleen in comparison with other organs. Furthermore, Weissleder et al. studied tocicity of radioactive SPIONs in dogs and mice [59]. One hour after the injection, about 82.6% of the SPIONs were observed to accumulate in the liver and another 6.2% in spleen. These amounts started to decrease eventually by incorporation of iron particles into the hemoglobin of erythrocytes. Although anemia was successfully treated in a week with very high dose compared to regular dose used in liver MRI (about 150 times), no serious side effects were observed. Since free iron is generally considered toxic, the iron dose injected must be lower than iron levels present in body [60]. Normaly, injected iron needs to clean from body through iron metaboloism in body. Therfore, it is not approved by FDA to employ SPIONs for patients with iron metabolism problem. It is also argued by some researchers that toxicity of SPIONs could be linked to their ability to harm DNA by magnetite oxidation. Karlsson

et al. proposed (through comet assay) that low toxicity induced by magnetite nanoparticles could be due to SPIONs ability to cause oxidative DNA damage in human lung epithelial cell line [53].

## Conclusion

SPIONs show multifunctional potential in medical imaging, tumor targeting, drug delivery and cancer therapy. Sizes, shapes and surface properties of SPIONs can be engineered in order to improve their targeting efficiency, drug delivery, contrast in MRI, responses to external magnetic fields and reduce their toxicity as well as nonspecific cellular uptake. The success in MRI application and some clinical outcomes of SPIONs can pave the way for advanced theranostic utilization in clinical applications. However, further studies need to focus on improving the specific targeting features of SPIONs.

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