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Diagnostics and Treatment of Metastatic Cancers with Magnetic Nanoparticles

Sunitha Abdeen and Praseetha PK*

Department of Nanotechnology, Noorul Islam University, Kumaracoil, Tamil Nadu, India

Abstract

Cancer is the leading cause of death among people younger than 85 years. One of the most important factors in effective cancer treatment is the detection of cancerous tumor cells in an early stage. Magnetic nanoparticles have the unique ability to specifically target tumor tissue leaving healthy cells intact. These superparamagnetic particles offer great potential in variety of applications in their bare form or with a surface coating and functional group chosen for a specific application. Upon functionalization, MNPs can be used in *in vivo* and *in vitro* by application of magnetic field. Magnetic nanoparticles are less toxic, biocompatible and have a shorter relaxation time. Magnetic nanoparticles have been an important class of biomaterials. Magnetic nanoparticles are globular, iron oxide containing particles having large surface to volume ratio, quantum size effect, superparamagnetic character and functional groups for conjugating to multiple diagnostic and therapeutic agents. Magnetic nanoparticles have shown clinical utility in cancer imaging, biomolecular profiling of cancer biomarkers and drug delivery. The knowledge about magnetic nanoparticles has increased tremendously providing great opportunities for improving the management of cancer patients by enhancing the efficiency of detection and efficacy of treatment. The prospects of magnetic nanoparticles in cancer imaging and treatment are reviewed here. These particles are sure to create wonders in the future of nanomedicine.

Keywords: Cancer; Magnetic nanoparticles; Cancer biomarker; MRI; Drug delivery; Nanomedicine

Introduction

Cancer is one of the major causes of illness and death worldwide [1]. Human cancer is a complex disease caused by genetic instability and accumulation of multiple molecular alterations causing the cells to present uncontrolled proliferation, genomic and chromosomal instability. This results in the transformation of normal cells into cancerous cells. The uncontrolled proliferation, further mutations allows the development of the primary tumour [2]. Infiltration of cancerous cells from the primary tumour to other organs in the body is governed by their ability to leave the primary tumour, invade through membranes and tissues, survive in circulation by avoiding immune attack and establishing themselves in surrounding tissues [3]. Furthermore, to maintain growth, cancer cells require the capacity to initiate the formation of new blood vessels [4], providing a constant supply of nutrients. Once tumour cells have metastasized and taken over distant organs, they become difficult to treat and often results in the patient mortality [5]. Cancerous cells show unique expression or over-expression of certain antigens and receptors making them attractive targets for targeted therapies. These include: HER2, (EGFR), EGFR1, CEA, VEGF, CD20, CD22 and CD52.

Early detection of cancer biomarkers, pathological characterization, and individualized treatments are recognized as important aspects for improving the survival of cancer patients [6]. Current anticancer agents do not greatly differentiate between cancerous and normal cells, leading to systemic toxicity and adverse effects. In addition, cancer is often diagnosed and treated too late, when the cancer cells have already invaded and metastasized into other parts of the body. At the time of clinical presentation more than 60% of patients with breast, lung, colon, prostate, and ovarian cancer have hidden or overt metastatic colonies [7]. Current cancer treatment regimens consist of doses of compounds that are non-specific and highly toxic. The inability of conventional diagnosis tools to detect cancer in an early and potentially curable stage further hinders effective treatment options, and thus by the time cancer is detected it may be too late to prevent metastasis to other organs in the body. Two key problems presently preventing effective cancer cures are: a) early detection of cancer before it metastasizes; and b) specific treatment of malignant cancer cells without affecting surrounding, normal tissues.

During the last decade, significant scientific research efforts have led to a significant growth in understanding of cancer at the genetic, molecular, and cellular levels providing great opportunities for diagnosis and treatment of cancer diseases. The hopes for fast cancer diagnosis and treatment were significantly increased by the entrance of nanoparticles to the medical sciences. Nanomaterials are the most advanced of the three major levels nanomaterials, nanodevices and nanosystem. The nanoparticles that are used to determine cancer detection and treatment include quantum dots, nanoshells, nanocrystals, nanocells, dendrimers and carbon nanotubes. Magnetic Nanoparticles being a sub-family of nanomaterials show remarkable new phenomena such as superparamagnetism, high saturation field, extra anisotropy contributions or shifted loops after field cooling. These phenomena arise from finite size and surface effects that dominate the magnetic behaviour of individual nanoparticles. Small size gives effective surface area, low sedimentation rate, tissular diffusion and reduces dipole-dipole moment [8]. Nanoparticles possessing magnetic properties offer great advantages in that they can provide selective

***Corresponding author:** Praseetha PK, Department of Nanotechnology, Noorul Islam University, Kumaracoil, Tamil Nadu, India, Tel: +919486855091; E-mail: crkpkp@gmail.com

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attachment to a functional molecule, confer magnetic properties to the target, and allow manipulation and transportation to a desired location through the control of a magnetic field produced by an electromagnet or permanent magnet. MNPs can be easily controlled by external magnetic field gradients. This helps to transport the MNPs into human tissue and be directed and concentrated within the target tissue by means of external magnetic field. Thus, opens up many potential applications that they can be made to deliver drugs to tumor [9-11]. Nanoparticles coupled with cancer specific targeting ligands can be used to image tumours and detect peripheral metastases [12]. MNPs can be heated up by subjecting them to varying magnetic fields. This leads to their use as hyperthermia agents. Thermal energy is delivered to the targeted tumors by MNPs resulting in cell destruction [13]. MNPs are found to have bioapplication such as magnetic bioseparation, gene therapy, immunoassay, magnetic biosensing etc.

Biomedical application of MNPs can be classified on the basis of their application inside or outside the body. *In vivo* applications can be further classified into Therapeutic (Hyperthermia and drug-targeting) and Diagnosis (NMR, Imaging) whereas *in vitro* applications are mainly used in diagnostics (separation/selection and magneto relaxometry. In biomedical applications, MNPs are used in the form of magnetic beads. Magnetic beads are made by embedding magnetic nanoparticles in suitable matrix. Biocompatibility, biodegradability and stability, shape and size of the particles should be taken into full considerations for the fabrication of magnetic beads. The content of iron oxide and bead surface modification on magnetic beads need to be carefully designed. For biological applications, MNPs must be coated with a biocompatible polymer before or after synthesis in order to prevent agglomeration [14,15]. For *in vivo* applications MNPs must be made non-toxic, non-immunogenic and should have a high magnetization so that they can be easily immobilized to the targeted tissue. Because of smaller sizes, effective surface area, low sedimentation rates, easy tissue diffusion and reduced dipole-dipole interaction, iron oxide and its oxidized forms are commonly used in biological applications [16,17]. Each application depends upon the relationship between the external magnetic field and the biological system. One of the means by which MNPs are transported into the targeted region is by injecting intravenously or via blood circulation. Alternate means is by using MNPs suspension for injection. A stable uniform magnetic medium is required to prevent nanoparticle aggregation [18]. Ultra small dimension and surface chemistry are two parameter required for the stability of the magnetic colloidal suspension. Selection of proper MNPs is the first crucial step for bioapplication. For applications in biology and medicine, the MNPs require to be stable in water at neutral pH, depends on the size, charge and surface chemistry [19]. Highly magnetic material such as cobalt and nickel are not used in biomedical application due to their toxic properties and susceptibility to oxidation. So iron oxides are employed in biomedical application. Industrial applications of MNPs are magnetic recording media for data storage, magnetic seals in motors, magnetic inks etc.

Efforts have been stimulated for the usage of MNPs in the field of biomedicine due to their comparable dimension to biological entities coupled with their unique magnetic behavior [9]. In summary, magnetic iron nanoparticles can serve as both a diagnostic and therapeutic agent.

Applications of Magnetic Nanoparticles in Cancer

The biomedical applications of magnetic nanoparticles include several concepts from electromagnetic radiation, solid state magnetism, surface chemistry and fluid rheology. The applications of MNPs are based on the following physical principles: a) the application of controlled magnetic field gradients around the desired target location for remotely positioning MNPs in organs or tissues. b) The utilization of the magnetic moment of the MNPs as a disturbance of the proton nuclear resonance. c) The magnetic losses of nanometric particles in colloids for heating purposes. Biomedical applications of magnetic nanoparticles can be classified according to their application inside (*in vivo*) or outside (*in vitro*) the body. *In vivo* applications could be further separated in therapeutic (hyperthermia and drug-targeting) and diagnostic applications (nuclear magnetic resonance (NMR) imaging), while for *in vitro* applications the main use is in diagnostic (separation/selection, and magnetorelaxometry).

Diagnostic Applications

Magnetic resonance imaging: Magnetic Resonance imaging (MRI) is used mainly for brain and spinal cord imaging. The MRI contrast seen from the different tissues is due to the concentration of hydrogen nuclei within the tissues. All tissues have a different concentration of hydrogen nuclei, causing relaxivity rates to differ, allowing a contrast to be seen. Using contrast agents, MR images can be enhanced. The most common contrast agent used is paramagnetic gadolinium ion complexed with diethylenetriaminepentaacetic acid (DTPA) [20]. Magnetic nanoparticles are the most commonly used magnetic nanoparticle-based contrast agents for MRI. An iron oxide particle with this property is known as superparamagnetic iron oxide (SPIONs). They use a highly superparamagnetic iron oxide (SPIONs) as a core material, and biocompatible polymers such as dextran as a coating. Large magnetic moments of SPIONs nanoparticles make them appropriate contrast agents in MRI. The most attractive SPIONs for medical applications is magnetite (Fe_3O_4), which has mixed oxidation state of iron. The most widely used methods to synthesize Fe_3O_4 particles is co-precipitation of $Fe(OH)_2$ and $Fe(OH)_3$ suspensions or the use of microemulsion technique. They have been so used because of their chemical stability, lack of toxicity and biodegradability. These particles are also known to exhibit an MRI contrast enhancement potential. SPIONs cause a shortening in the T2 signals, which leads to a negative contrast [21]. The transport of most of the contrast agents is by intravenous administration, which determines the size of the nanoparticles. For successful delivery, the particles have to pass through the vascular capillary wall. Depending on their size, charge and the configuration of the coating these particles are metabolized by the Reticuloendothelial System (RES) consisting of monocytes and macrophages. These cells accumulate in lymph nodes and the spleen as well as in the liver (Kupfer cells) and are favored for the uptake of SPIONs and influence the delivery time and the diffusion to certain tissues. Smaller particles generally circulate longer and are taken up by cells of the lymphatic system and bone marrow while particles >50 nm are taken up by liver cells [22]. If particles are not entirely captured by the liver and spleen, they are widely evaluated as potential marker of inflammation for the diagnosis of inflammatory and degenerative disorders associated with high macrophage phagocytic activity like for plaque imaging or brain ischemia [23].

For many years, supermagnetic nanoparticles have been used in diagnostics as contrast agent in Magnetic Resonance Imaging (MRI) and Magnetic Resonance Angiography (MRA) [24]. To fulfill the requirements of improved enhancement, the substances used must be magnetically active.

Magnetic nanoparticles provide several unique features and capabilities in comparison with traditional *in vivo* imaging probes. First,

their size-dependent optical and electronic properties can be tuned continuously by changing the particle size. This size effect provides a broad range of nanoparticles for simultaneous detection of multiple cancer biomarkers. The *r*2 and *r*2* relaxivity of Fe nanoparticles is significantly higher than that of iron oxide at a comparable particle size [25]. Secondly, nanoparticles have more surface area to accommodate a large number or different types of functional groups that can be linked with multiple diagnostic (e.g. radioisotopic or magnetic) and therapeutic (e.g. anticancer) agents. Third, studies has shown that nanoparticles in the size range of 10-100 nm are accumulated at tumor sites through an effect called enhanced permeability and retention (EPR). This effect is believed to arise from two factors: (a) growing tumors produce vascular endothelial growth factors (VEGFs) that promote angiogenesis and (b) many tumors lack an effective lymphatic drainage system, which leads to subsequent macromolecule or nanoparticle accumulation. This causes tumor-associated neo vasculatures to be highly permeable, allowing the leakage of circulating macromolecules and nanoparticles into the tumor interstitium [26-29].

Superparamagnetic materials have a higher magnetic susceptibility compared to paramagnetic material. Upon application of a magnetic field the magnetic moments within the SPIONs align in the direction of the field, this gives rise to a large net magnetic moment, in comparison, paramagnetic material exhibit only a small net magnetic moment [30]. The large magnetic moment generated by SPIONs leads to a disturbance in the local magnetic field, causing a shortening of the hydrogen nuclei relaxation times. This shortening in proton relaxation times leads to a detectible change in the T2 MRI signal. Due to their larger magnetic moment, only nanomolar concentrations of SPIONs are needed compared to millimolar concentrations of Gd complexes to be effective as MRI contrast agents [31].

Currently there are two FDA approved SPION contrast enhancement agents, Endorem EU (Ferridex USA; Guerbet/Berlex lab) and Resovist (Schering AG), both used for liver and spleen imaging. Sinerem EU (Combidex USA;Guerbet/Advance Magnetics) is another SPION contrast agent currently in phase III trial for application in lymph node imaging. Endorem and Resovist are stabilized in dextran, the particles are only partially coated, increasing the likelihood of agglomeration. The application of these SPIONs is for liver specific imaging; therefore it is a requirement for the particles to be taken up by the body's RES. The SPIONs accumulate in the organs of the RES, with 80% taken by Kupffer cells within liver and 5-10% in the spleen. Sinerem consist of a single 5 nm iron oxide core crystal covered more completely in dextran and has a hydrodynamic diameter of 20-40 nm [32]. Due to Sinerem's hydrodynamic radii and its thicker dextran coat, opsonisation by plasma proteins and recognition by RES is reduced. This allows the particles to remain in the blood longer; increasing its blood half-life [33]. While in the blood, a percentage of SPIONs can leak into the interstitium where clearance is via macrophages of the lymphatic system. The accumulation of SPIONs within the lymph nodes in-turn allows for MRI imaging of lymph node metastases [34]. SPION contrast agents work by passive accumulation within healthy macrophages within organs of the RES. However, in areas of organs of the RES that are cancerous the macrophages function is altered, preventing uptake of the SPIONs. Cancerous tissues therefore, appear brighter (positive contrast) as they contain no SPIONs, in comparison the healthy tissues appear darker due to the T2 negative contrast caused by the uptake of SPIONs. SPIONs are used in tumor detection, as the tumor lesions exclude the uptake of particles (tissue in MRI remains bright) while the macrophages in the normal liver tissue will take up the particles and darken the image of the tissue [35]. Other applications in MRI are the use of such particles to track stem cells transplanted into organs such as brain or to demonstrate macrophage activity within atherosclerotic plaque [36].

Atherosclerosis imaging: Macrophages such as monocytes are in highly active state of phagocytosis, promote atherosclerosis by secreting mediators and secretion of cytokines and chemokines. Atherosclerosis lesions show accumulation of macrophages. Research on the use of SPIONs for the detection of atherosclerotic plaques by MRI showed SPIONS as a potential marker of inflammation for plaque imaging. In this study, MAC-1 expressing Chinese Hamster Ovary (CHO) cells, expressing MAC-1 either in a native, low affinity state (wildtype, WT) or a high affinity state (GFFKR-deleted cells, DEL) were used to simulate the type of activated macrophages found in atherosclerotic plaques. CHO-cells not expressing MAC-1 were used as controls (NCHO) The studies identified the integrin MAC-1 (CD11b/CD18) as a mediator for the superparamagnetic iron oxide nanoparticles to endocytose into monocytes/macrophages and showed that MAC-1 is also a central mediator of inflammation. Dextran-coated particles, which are mostly used as contrast agents, are commonly attached to cells but not taken up by them, while SPIONs coated with amino-functionalized polyvinyl alcohols interact with different cells [37] and therefore underline a receptor based uptake of SPIONs by cells.

Diagnosis of cancer cells

Cancer is a difficult disease to treat and identify. There are many different ways for treating cancer such as surgery, chemotherapy, radiation and many others. These methods are effective if the cancer tumor is caught soon enough. However, these treatments are not effective enough because they do not only target the affected cells, they also affect healthy cells. Nanotechnology has found many new ways in detecting cancer cells and how far the disease has spread throughout the body. A couple of these new cancer detecting nanoparticles are gold nanoparticles and magnetic iron oxide nanoparticles encased in a biocompatible material. Magnetic iron oxide nanoparticles encased in a biocompatible material can make detecting cancer cells easier, even if the cancer cells are small and clearer so there is less mistakes in the detecting process. These particles stick to the tumor cells turning them into little magnets which are then attracted to the tip of a biopsy needle [38]. Instead of using biopsies, MRI's can be used to distinguish malignant lymph nodes which can help in telling how far cancer has spread.

Magnetic separation: This includes techniques and devices for using SPIONs for use in targeting and magnetic extraction of cellular compounds and their chemical, optical, and physical analysis including proteomics. This is a very convenient way to separate cell from blood samples, bone marrow, tissue grinds or culture media. Magnetic separation involves no interference of iron or charged solvents in the sample under the static magnetic field. Another advantage is less damage to the separated cells. One way of separation of targeted cells is by normal phase separation. The cells are separated directly from the mixed solution. Another way is by negative phase separation. The unrelated cells from the mixed solution are separated using an external magnetic field resulting in purified and concentrated targeted cells. For cell separation, a suspension of magnetic nanoparticles with specific antibody is added directly to a biological fluid sample. After 10-20 minutes of incubation the solution is placed in a magnetic separator, where the desired magnetically labeled cells are retained on the magnet while the supernatant is removed. Magnetic separation of cells finds application in clinical diagnosis. These magnetic methods have reached high efficiency levels for cell separation, as compared with electric and

centrifugal, and also standard methods based on fluid-fluid interface separation. Magnetic separation has been successfully tested for precise separation of specific cells in blood [39], gram positive pathogens [40] and protein purification [41].

Magnetic immunoassay: This use of functional nanoparticles enables identification of their specific interaction partners in a physiological environment, such as the entire intact cell. Proteomic analysis of diseases, where the immobilized interaction partner reacts with the target in physiological condition is possible. MNP can capture free floating cancer cells, which can then be carried out of the body. Moreover, they can be used for the detection of cancer by coating with antibodies specific to the targeted cancer cells or Proteins. The development of magnetic immunoassay [42] is another most prospective technology based on the use of magnetic beads either at the capture antibody stage or at the detection antibody stage. The capture antibody-bound magnetic beads can lead to rapid immunoassay as they can be instantaneously bound to the substrate using the magnet but the detection of antibody-bound magnetic beads in sandwich immunoassay can be done using a magnetometer.

Magnetorelaxometry: Magnetorelaxometry was introduced as a method for the evaluation of immunoassays [43]. Magnetorelaxometry measures the magnetic viscosity, i.e., the relaxation of the net magnetic moment of a system of magnetic nanoparticles after removal of a magnetic field. There are two different relaxation mechanisms. First, the internal magnetization vector of a nanoparticle relaxes in the direction of the easy axis inside the core; this is called Neel relaxation. Second, particles accomplish rotational diffusion in a carrier liquid, called Brownian relaxation. Neel and Brownian relaxation can be distinguished by their different relaxation times [44]. Furthermore, Brownian relaxation can take place only in liquids, whereas Neel relaxation does not depend on the dispersion of the ´nanoparticles. Magnetorelaxometry depends on the core size, the hydrodynamic size and the anisotropy allows this technique to distinguish between free and bound conjugates by their different magnetic behaviour, and therefore can be used as an analytical tool for the evaluation of immunoassays.

Solid-phase extraction: Solid-phase extraction (SPE) is a way to isolate and preconcentrate desired components from a sample matrix. SPE offers an excellent alternative to the conventional sample concentration methods, such as liquid–liquid extraction [45]. The separation and preconcentration of the substance from large volumes of solution can be highly time consuming when using standard column SPE, and is in this field where the use of magnetic or magnetizable adsorbents called magnetic solid-phase extraction (MSPE) gains importance. In this procedure, the magnetic adsorbent is added to a solution or suspension containing the target. This is adsorbed onto the magnetic adsorbent and then the adsorbent with the adsorbed target is recovered from the suspension using an appropriate magnetic separator. For separation and selection the advantage of using magnetic nanoparticles instead magnetic microparticles is that we can prepare suspensions that are stable against sedimentation in absence of an applied magnetic field.

Nanomagnetic molecular sensing

Highly sensitive detection and accurate analysis of biomarker molecules in human fluid samples are essential for the early detection, treatment, and management of diseases [46]. Biosensors are widely used in medicine to monitor or detect biological molecules for applications ranging from diabetes to cancer. In a biosensor, a ligand and a receptor bind together in a reaction that is collected as a signal to a transductor using different methods, including optical, magnetic, electrochemical, radioactive, piezoelectric, mechanical, mass spectrometric, and so forth. Like any sensor, a biosensor should be cheap, compact, selective, sensitive, portable, reusable, and have a fast readout. The introduction of nanoparticles in the molecular diagnosis field has represented an advantage in many cases towell-established detection techniques based on fluorophores, such as Polymerase Chain Reaction (PCR) and Enzyme-Linked Immuno Sorbent Assay (ELISA). Nanoparticles offer their physical properties to the biosensor. In some cases, nanoparticles are used simply as carriers of antibodies to recognize them by association in biosensors. The absence of magnetic material in biological samples allows a controlled application of magnetic fields. Super-paramagnetic particles are therefore powerful because they can be easily manipulated and reliably detected inside complex biological fluids.

Magnetic bead biosensor: In the case of the biomedical applications, the medium may be a fluid like, blood, the cerebrospinal fluid or a culture medium or it may be organic tissue. In any case there will be an interaction between elements, the magnetic material and the organic medium, which is undesirable. The organic media may be harmed since the most widely used magnetic materials, (transition metals and their alloys), are not biocompatible, except for iron which is very biocompatible. Human organs and tissues have a great affinity for iron. The above mentioned organic fluids have Cl^-, Ca^+, Na^+, K^+ ions and several free organic radicals which are, in general, corrosive for magnetic materials. The solution to this problem consists in coating the magnetic material with a thin layer (100 to 200 nm) of another biocompatible material, like gold, platinum, titanium oxide, silica or alumina. The most widely used coating methods are evaporation, sputtering, electrolysis and ionic implantation. The non metallic parts of the sensor also have to be made of biocompatible polymers or ceramics such as Teflon, medical silicones, and silica. Magnetic sensors and actuators offer a great advantage in that is the magnetic field is itself a wireless transmission.

Magnetic beads are small, mainly globular, iron oxide containing particles. They are available at diameter sizes of nanometers up to hundreds of micrometers. The normal magnetic bead organization contains an iron oxide (magnetite) core, which provides the paramagnetic attraction of the particles to a magnet. Normal magnetite contents are 50-60% of the whole bead. This core is usually encased by an organic polymer, e.g. Poly Vinyl Alcohol (PVA). The core shelling allows different surface modifications of the beads, from which many different types are already commercially available. A frequently used bead type is streptavidin-coated; their main application area is labelling and separation of biotinylated DNA. These approaches use the magnetic bead as binding platform or linker between analyte and label, which also allows an easy but state of the art separation of analytes from a sample by simply using a magnet. These properties are exploited in magnetic-label biosensors, which employ the magnetic particles as labels in order to measure the concentration of target molecules in a biological sample. Furthermore the biological fluids are hardly magnetic, so magnetic fields can be reliably applied and the magnetic particles can easily be detected, which allows for a low detection limit. Detection of magnetic labels has been demonstrated using magneto-resistive sensors [47-50]. Hall sensors [51,52], field coils [53] and by optical detection [54]. The magnetic particles offer another particularly interesting option: by moving the labels coated with antibodies through the fluid, the labels can efficiently catch the antigen from the fluid. Once the antigen is bound, the labels can be pulled down towards the sensor surface where they bind due to the formation of the sandwich format. Finally the unbound labels are pulled away by magnetic forces and the

number of magnetic particles is measured. Using these concepts, the assay can be integrated and accelerated and picomolar detection limits are achievable for fast (<10 min) POC sensor applications [55-57].

In situ measurement of the mass evolution of cell culture: Cell cultures constitute one of the most frequently used assays in biology and also one of the diagnostic methods most used in Medicine. The monitoring of the progress of a cell culture is conventionally done by direct observation of the evolution that takes place on a culture plate. It is essential to have perfect control over the microenvironment in which the culture is developing. The cultures are carried out inside incubation chambers where it is possible to control factors such as humidity, pH and temperature. When the evolution of the cells is observed using a microscope, it is necessary to extract the cells from the chamber, submitting to changes in temperature, aside from the mechanical damages that take place in each measurement. Sensor based on the magneto-elastic resonance of ferromagnetic amorphous ribbons. a system that makes it possible to continuously quantify the mass evolution of a cellular culture "*in situ*", that is, without extraction from the incubation chamber would be very useful. This sensor system consists of the following elements : a) A culture plate that has been designed with two separate baths, each one containing an amorphous magnetoelastic ribbon with an iron based composition of area 40×4 mm^2 and 15 microns in thickness, coated with 250 nm of TiO_2 immersed in a culture medium. Only one bath is seeded with cells. b) An arrangement of two coils and a permanent magnet under the plate to apply the bias and the alternating field. c) A scanner impedance meter connected to the coils, which measures the coils impedance around the resonance frequency of the ribbons. d) A software program developed to extract the ribbons resonance frequency from the impedance measurements. Inside the culture plate designed for the device two ribbons of an amorphous magnetostrictive material with the same dimensions are placed in the compartments designed for them immersed in the culture environment. One of the ribbons acts as a reference, in order to evaluate the changes on the magnetoelastic resonance frequency due to possible changes of temperature or other factors that are not directly related to the evolution of the cell culture. So, it is immersed in one of the baths containing the culture environment but without cells. A seed of the cells, whose evolution and growth is being studied, is placed in the bath containing the other ribbon. By means of the coils system (excitation-pick up system) an electromagnetic field of frequency is applied over the whole set-up and the variations in the magnetic flux density, created by the sensor system, are collected. The designed electronic system makes it possible to change the frequency of the applied electromagnetic field as well as receiving the answer of the sensor in function of the frequency [58].

Test of prothrombin time for leukemia: The Time of Prothrombine (TP), and the values that derive from it, the International Normalized Ratio (INR) are used for determining the tendency of the blood to coagulate in the presence of possible biological disorders like hepatic failure or K vitamin deficiency. A sensor, based on a magnetoelastic material, can be used to determine the TP and the INR in patients under anticoagulation treatment, without the need for specialized staff or installations. The method is based on the variation in the magnetic permeability of a magnetoelastic microwire induced by the change on the blood viscosity when it coagulates. When the blood coagulates, the viscosity force applied to the immersed wire dissipates, as heat, a portion of the magnetic energy supplied by the magnetic field. Therefore the apparent magnetic permeability of the microwire decreases due to the magnetoelastic coupling. The sensor consists of two identical microwires, with an iron base, that are placed into two capillaries with tenths of mm. inner diameter and around 5 cm in length. These capillaries are surrounded by one coil each, which are fed by two power amplifiers driven by a signal generator. The capillaries are filled with blood and with a fluid of reference, respectively. The difference between both signals increases when the blood coagulation process begins and its absolute value tends to a maximum when the blood is fully clotted. The experimental set-up compares this permeability with that of a reference wire immersed in an inalterable fluid [59]. The absolute value of the measured signal tends to a maximum when the blood coagulates. The time to raise this maximum enables the TP and the INR to be calculated.

Therapeutic Applications

Hyperthermic therapy

Magnetic induced thermal therapy also known as Magnetic Fluid Hyperthermia (MFH) was first reported by Gilchrist in 1957. The heat-induced cell death with Magnetic Nanoparticles (MNPs) generates numerous cellular changes, leading to morphological changes, cell detachment, and death. Cellular alterations include changes in the membrane, nuclear and cytoskeletal structures, cellular metabolism, macromolecular synthesis etc. Its use is based on the fact that tumor cells are more sensitive to temperature in the range of 42-45°C which yields necrosis, coagulation, or carbonization than normal tissue cells. This temperature range has become critical for cancer treatment due to damaging the cancerous cells without altering the healthy cells by selective heating (up to 45°C) and controlling heating rate and time.

This process not only enhances the effectiveness of other cancer treatments, but it also kills tumor cells that are resistant to other forms of cancer. Increased tumor tissue perfusion facilitates the absorption of chemotherapeutic drugs through cell membrane without being more toxic [60,61]. As a result, the action of combination of hyperthermia with radiotherapy or chemotherapy becomes more efficient. Consequently, hyperthermia allows reducing of tumors resistant to various chemotherapeutic drugs such as doxorubicin, cisplatin, bleomycin, nitrosoureas, and cyclophosphamide. It has been demonstrated that hyperthermia also has an anti-angiogenic action and an immunotherapeutic role, due to thermal shock proteins, which are produced by stressed tumor cells [62,63]. Thus, the magnetic materials with Curie temperature ~45°C, having sufficient biocompatibility are the best candidates for effective cancer hyperthermia treatment to avoid overheating. Because of unique capability of turning on and off the magnetic properties depending on temperature, the tumors will be continuously heated at a self-controlled temperature equal to the Curie temperature of the magnetic nanoparticles. This approach will allow to heat the tumor cells and vasculature selectively and to prevent overheating with subsequent damage to neighboring healthy tissues. Tumour cells have shown a greater sensitivity to heat treatments compared to healthy cells [64]. This has led to the use of thermo-ablation and hyperthermic therapies in the clinic, often in combination with other treatments.

M**agnetic hyperthermia:** There exist at least four different mechanisms by which magnetic materials can generate heat in an alternating field [65]: a) generation of eddy currents in magnetic particles with size >1 μ. b) hysteresis losses in magnetic particles >1 μ and multidomain magnetic particles. c) relaxation losses in 'superparamagnetic' single-domain magnetic particles. d) frictional losses in viscous suspensions.

Hyperthermia is classed into two categories; themo-ablation and mild hyperthermia. Thermo-ablation is where a temperature rise exceeds 46°C and causes cell necrosis [66]. Mild hyperthermia is where the temperature increase is between 41-46°C [67]. This temperature rise is high enough to cause partial cell kill and to damage and sensitize cancer cells to chemotherapy and radiotherapy [68]. Studies has shown to cause increase blood flow into radio-resistant, hypoxic, low pH areas, causing oxygenation of the tissues, in turn increasing the cells radio-sensitivity [69]. Furthermore, thermal treatment can enhance the toxicity of chemotherapy. By exposing magnetic nanoparticles to an Alternating Magnetic Field (AMF), energy is created in the form of heat, this thermal energy dissipates into the surrounding tissues and if the temperature is high enough can destroy or weaken cancerous cells. Using MNPs to generate heat can overcome the issue of non-specific tissue heating, as the application of the alternating magnetic field can be localised to the area of the body where the MNPs have collected. Through varying the frequency, current parameter of the AMF and the size and composition of the MNPs, the temperature generated from the MNPs can be controlled. MNPs are thought to generate heat through Neel relaxation and Brownian motion. In the case of Neel relaxation, the AMF causes the magnetic moments within the MNPs to rotate, generating internal friction. When the field is off the moments return to equilibrium, this is where energy is released in the form of heat [70,71]. Brownian motion requires the rotation of the MNPs as a whole therefore; the heat is generated through frictional movement in its surroundings. However, MNPs can become trapped within biological tissues, this in-turn blocks free rotation of the particles, preventing the generation of frictional heat [72].

Magnetic hyperthermia and simultaneous drug delivery: One of the problems with magnetic targeted hyperthermia is that a limited dose of nanoparticles reached the tumor tissue resulting in insufficient temperature enhancement in the cancerous sites; thus there is a risk of proliferation of cancer cells that survived during thermotherapy. In order to overcome the problem, several specific tumor receptor targeting moieties together with anticancer drugs attached to the surface of particles were employed. For example, β-cyclodextrin (CD) was used as a drug container for hydrophilic (paclitaxel) or lipophilic (doxorubicin) structures. Drugs incorporated in the CD can thus be released through the use of induction heating, or hyperthermic effects, by applying a high frequency magnetic field. In this case, folic acid (FA) and CD-functionalized magnetic nanoparticles were synthesized and it was found that by induction of heating, drug release was triggered from the CD cavity on the particle - a behavior that was controlled by switching the high frequency magnetic field on and off. Another drug delivery system, based on covalently attaching genistein onto SPIONs coated by cross-linked Carboxymethylated Chitosan (CMCH), has been developed [73] and the results confirmed that the nanosystem could significantly enhanced cancer cell apoptosis

Magnetic gene therapy

Gene therapy is an experimental technique that uses genes to treat or prevent diseases. The most common form of gene therapy involves inserting a normal gene to replace an abnormal gene. Other approaches include swapping an abnormal gene for a normal one, repairing an abnormal gene or altering the degree to which a gene is turned on or off. There are two types of gene therapy: somatic gene therapy and germ line gene therapy. In somatic gene therapy, the therapeutic genes are transferred into the somatic cells, or body of a patient. Any modifications will not be inherited by the offsprings of patients or later generation. In germ line gene therapy, germ cell is sperm or eggs modified by the introduction of functional genes. This are inherited and passed on to later generation [74]. There are three primary gene delivery systems that employ viral vectors (retroviruses and adenoviruses), nucleic acid electroporation, and nucleic acid transfection. Gene delivery by viral vectors can be highly efficient (80-90%) but may insert viral vector nucleic acid sequences into the host genome, causing inappropriate expression of deleterious genes. Electroporation is also a highly efficient technique for introducing foreign genes into a host (50-70%); however, half of the recipient cells die due to the electrical stimulation. Transfection reagents do not efficiently deliver nucleic acids into cells (20-30%); however, cell viability is largely preserved and the method is safe enough for clinical use. Therefore, this method holds relatively more promise for medical applications, provided that its efficiency can be improved. MNPs are already in use by basic researchers to increase transfection efficiencies of cultured cells.

Magnetofection: Magnetic nanoparticle based gene therapy methods are based on the principles of magnetically targeted drug delivery. Magnetofection involves coupling of genetic materials to magnetic nanoparticles to express homogeneous gene products. The gene is attached directly to the magnetic nanoparticle [75]. This technique is based on the attractive force exerted on magnetic nanoparticles by a field source. The mechanism of magnetofection is similar to transfection reagents (Lipofectamine 2000, FuGENE HD, and PEI). The only difference is that the plasmids form complexes with cationic polymer-coated MNPs (called as "Magnetoplex") [76,77]. The behavior of magnetoplex is readily controlled by magnetic force. Upon binding to the cell surface they are taken up by endocytosis [78]. The magnetic field must have a gradient. For this reason, high gradient, rare-earth magnets matrix are used for magnetofection which improves the transfection efficiency.

The particles carrying the gene consist of a magnetic iron-oxide either dispersed within a polymer matrix or encapsulated within a polymer. The surface of iron oxide-based MNPs is modified to increase transfection efficiency and reduce cytotoxicity. To achieve this, coating agents such as anionic surfactants (oleic acid, lauroylsarcosinate), a non-ionic water-soluble surfactant (Pluronic F-127), fluorinated surfactant (lithium 3-[2-(perfluoroalkyl) ethylthio]propionate), a polymer (polyethylene glycol, poly-L-lysine, poly(propyleneimine) dendrimers), carbohydrates (Chitosan, Heparan sulfate), silica particles (MCM48), proteins (serum albumin, streptavidin), hydroxyapatite, phospholipids, a cationic cell penetrating peptide (TAT peptide), non-activated virus envelope (HVJ-E), a transfection reagent (Lipofectamine 2000), and viruses(adenovirus, retrovirus) were used [79]. These coating agents are often used in conjunction with PEI. PEI is a well-known cationic gene carrier with high transfection efficiency. Thus, the PEI was modified to increase transfection efficiency, and decrease cytotoxicity [80]. PEI-coated MNPs are stable in water, bind nucleic acids, and control MNP behavior by magnetic force.

In vivo, the particles are coated with polyethylinimine which binds DNA to the particles surface via charge interactions [81,82]. SPIONs are strongly magnetic when placed in a magnetic field and hence are attracted along the field gradient. The strong magnetization arises from the spin of unpaired electron within the crystal lattice. They have fewer tendencies to agglomerate which may lead to embolism. In *in vitro* magnetic nanoparticle based transfection, the particle-DNA is introduced in the cell culture. The field gradient produced by electromagnets placed below the cell culture increase the sedimentation of complex and thereby increasing the speed of transfection. *In vivo*

magnetic nanoparticle based transfection, the magnetic nanoparticles under the influence of magnetic field have the potential to enhance transfection and target the therapeutic gene to a specific organ or site within the body. Generally, the particles carrying the therapeutic genes are injected intraveneously. Particles passing through the blood stream are captured by strong, high-gradient external magnets. Once captured, they are taken up by the tissue. These genes can be released through enzymatic cleavage of the cross-linking molecules. If the DNA is embedded within the matrix alternating fields may be applied to heat the particles and releases the genes from the magnetic carrier.

In a study, Green Fluorescent Protein (GFP) gene were introduced into a mouse embryonic carcinoma cell line, P19CL6 using PEI max-MNPs, and were succeeded in establishing a highly efficient and low cytotoxic gene delivery system [10]. Magnetic nanoparticle based gene therapy has been clearly demonstrated *in vitro*. It finds many potential application in non-viral *in vitro* transfection, repairs errors in mRNA and has the potential to treat the blood disorders like thalassemia, cystic fibrosis and cancer.

Magnetic drug targeting

Magnetic iron oxide nanoparticles could enter into the human body through administration with an arterial duct, intravenous or oral administration, or direct injection. Nanoparticles were distributed in specific tumor areas under magnetic field with enough strength, so that loaded drugs were efficiently and directionally delivered into tumor tissues. The released drugs exerted therapeutic effects at tissue, cell or subcellular levels, and no significant influences on normal tissues were found. This is magnetic drug targeting [11].

In therapeutic application such as cancer treatment, controlled drug delivery is of great importance. One of the important applications of magnetic nano particles in targeted drug delivery is the encapsulation of magnetic materials to make it bio compatible. Bio medical application of MNPs in targeted drug delivery depends on number of factors such as field strength, geometry, depth of the target tissue, rate of blood flow and physico chemical properties of the drug-loaded MNPs [83]. Small particle size favors the biological absorption, excretion of the particles by the body and avoids agglomeration. The most attractive characteristic of using nanoparticles is their ability of controlled delivery to a specific area by applying an external magnetic field. Selectively delivering drug molecule to the diseased site without damaging healthy tissues is important in drug delivery for cancer treatment. MNPs have the ability to get directed and concentrated within the target tissue and ability to hold until the therapy is completed. MNPs can be removed from the body once the therapy is completed. In a study, Zhang [84] injected MMADR into rats with hepatic tumor implantation, and found that MMADR aggregated in the cancerization site of rats. After 7 days, most tumor cells were inhibited, and lump of tumor disappeared. Moreover, magnetic iron oxide nanoparticles could carry more adriamycin, and the release rate of adriamycin slowed down so that the release time was extended to more than one week. Consequently, the damage of chemotherapeutic drugs on liver can be avoided. Magnetic iron oxide nanoparticles could enter into the main supply arteries in target tissues after injection, and were subjected to sufficient uptake and adsorption by target tissues. Because the diameter of these nanoparticles was less than 1 nm, they could enter into microvessels in target organs prior to systemic clearance. Subsequently, these nanoparticles could be retained in arterioles and capillary vessels of target organs under magnetic field. The retained nanoparticles were absorbed through extravascular routes, which finally led to intracellular absorption of cells (tumor cells), exerting their therapeutic effects. The advantages of drug delivery system based on nanoparticles is the ability to target specific location in the body, the reduction of the drug quantity, reduction of the concentration of the drug at non target sites minimizing side effects and visualization of the nanoparticles using magnetic resonance imaging. Superparamagnetic particles are used as carrier for targeted drug delivery because they do not aggregate under a magnetic field.

Before synthesis, the fundamental requirement of magnetic nanoparticles is that it must navigate the body in search of its target, should be non-toxic and stable. Drug loaded nanoparticles can be separated in two groups according to their structures: core-shell and matrix like nanoparticles. Core-shell nanoparticles are based on the use of a magnet to locate them in a tumor. These nanoparticles have a magnetic core encapsulated in an organic or inorganic shell. At the target site nanoparticles release the conjugated drug which is associated to the magnetic core or an alternative magnetic field to heat the magnetic nanoparticles.

There are two possibilities of localizing drug to the target tissue: passive and active. Passive targeting implies using characteristic properties of the tumor to locate in its proximity as an encapsulated, bonded or adsorbed drug on nanoparticles or using affinity or physicochemical properties of the tumor tissue. Active targeting is based on inducing external properties to target nanoparticles to specific tumor tissues and uses light, magnetism or specific recognition mechanisms. To avoid non-specific binding, MNPs have been tailored to have an affinity for target tissue through passive, active and magnetic targeting approaches.

Coated nanoparticles are widely used as magnetic carries of medical drugs. Functionalization of magnetic nano particles with amino groups, silica, polymer is usually used in order to achieve physical and chemical properties. Core/shell structure of magnetic nano particles offers good dispersion and high stability [85]. After synthesis, modified MNPs are stable in high and low PH solutions. *In vivo* techniques require the MNPs to be coated for stability and biodegradability. This can be achieved by coating the MNPs with a suitable material which prevents oxidation and offers the possibility of attaching them to antibody, protein, medical drug etc. Coating modifies the particle surface by attaching bioactive components. Coating can be done via in situ coating, post synthesis adsorption and post synthesis end grafting [86]. *In situ* and post synthetic modification with polysaccharide and copolymers lead to uniformly encapsulated cores. Coating materials influence the magnetic properties of MNPs in many ways. The magnetic core is composed of 3D metals. The shell is composed of polymeric materials or inorganic materials. Outer coating surface of the particles can be functionalized to allow the binding of drugs. PEG is a linear biocompatible linear structure. They are neutral, hydrophilic molecules in biological fluids which help to improve the dispersity and blood circulation time of MNPs [87]. Dextran coatings are based on hydrogen bondings making the polymer susceptible to detachment. They have been cross linked using epichlohydrin and ammonia to form CLIO [88]. Chitosan, due to its large abundance, biocompatibility and ease of functionalization is an efficient material for drug delivery application. Chitosan is a cationic, hydrophilic polymer that is non toxic, biocompatible and bio absorbance. Another organic surface coating is Polyethyleneimine (PEI), can take both linear and branched forms. It is a water soluble cationic polymer [89]. MNPs coatings with liposomal or micellar structures provides simple and easy surface modifications, convenient encapsulation, sequestration and protection of pharmaceuticals from the body. Copolymers have been developed that can attach to MNPs surface. Polysaccharides (dextran) or polymer

(polyvinyl alcohol) are used as typical coatings [90,91]. Coating inorganic materials include silica, metal, nonmetal, metal oxides and sulfides. Covalent linkage strategies and physical interactions have been used for the conjugation of targeting, therapeutic and imaging target molecules with nanoparticle surfaces. Targeting agents have been used in MNPs which enables binding specificity

The importance of targeted drug delivery is to transport a drug directly to the targeted tissue under various conditions and to treat it with no side effects. The particles are loaded with drugs that are concentrated at the targeted organ with the help of external magnet. The drugs are then released. The process of drug localization using magnetic delivery systems is based on the competition between forces exerted on the particles by blood compartment, and magnetic forces generated from the magnet, i.e., applied field. When the magnetic forces exceed the linear blood flow rates in arteries (10 cm s^{-1}) or capillaries (0.05 cm s^{-1}), the magnetic particles are retained at the target site and maybe internalized by the endothelial cells of the target tissue. Magnetic nano particles smaller than 4 μm are eliminated by cells in the liver and spleen. The rate of release can be controlled by modulating the magnetic field. Chemotherapeutic approaches to cancer treatment are non-specific. Drugs are administered intravenously leading to systemic distribution. Magnetic nano particles can be used to overcome this disadvantage. MNPs act as efficient drug delivery system offering non-specific cell interactions, controlled therapeutic release, flexible drug loading and delivery. In the future, magnetic nanoparticles based drug delivery system can help in targeted and triggered drug releases, image guided therapy, surface functionalization and in hyperthermia.

Conclusion

The present review summarizes the potential application of magnetic nanoparticles in diagnostics and cancer therapy. Magnetic nanoparticles loaded with chemotherapeutic drug targeting the tumor site can not only eliminate adverse side effects, but may also pave the way for bringing a more effective, specific, and personalized medicine for eradicating cancer and many other complex diseases. Magnetic nanoparticles in cancer detection and treatment have the potential to replace highly invasive conventional cancer detection and treatment, which often includes biopsies, irradiation, and painful therapies.

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