Applications of Magnetic Nanoparticles in Cancer Detection and Treatment

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Abstract: Cancer detection and treatment has been a challenge for medical science till the years. The ability of cancer cells to multiply quickly and invade other parts of the body by process of metastasis further complicates the situation. Hence prognosis of cancer and its proper treatment also becomes difficult, as invading cells are not easy to detect at initial stages of spreading of infection. This limitation of not being able to detect invading tumor cells can be overcome by applying nanotechnology based approaches. Nanomaterials being very small in size have remarkable properties which are absent in their bulk counterparts. These properties possessed by materials at the nanoscale make them very useful for cancer theranostics. Further nanomaterials are having large number of surface atoms, as well as high surface activity because of high surface area to volume ratio; therefore their surface functionalisation can be done so as to make them useful for diagnosis and treatment of cancer. This could prove to be very promising in the early detection and treatment of cancer.

Keywords: Embolotherapy, hyperthermia, magnetic separation, targeted delivery, thermoablation.

1. INTRODUCTION

Cancer being the leading cause of deaths in developed countries and second most leading cause of deaths in developing countries [1] has posed a great threat worldwide. According to a 2004 'WHO' report, regarding the deaths caused by various diseases, cancer alone caused about 13% of total deaths in the year with 7.9 million people killed worldwide alone with cancer [2]. According to another previous statistical data, about 7.6 million deaths due to cancer and 12.7 million cancer cases have been reported in 2008 also [3]. These numbers are sufficient to scare the entire human population and reflect the dark side of this disease. Further with the change in lifestyle and habits those humans are adopting today, one can easily expect these numbers to increase day by day. A few of those habits which have accounted for rise in these numbers include smoking, reduction in physical activity etc. Somehow food habits are also responsible for increase in these numbers.

The advancement in the field of nanotechnology can prove to be a boon for the early diagnosis and therapy of cancer because of the striking properties of the nanomaterials in contrast to their bulk counterparts. The small size of the nanomaterials in addition to their high surface area to volume ratio accounts for their high surface activity and their attractive properties. Because of the small size of the particles at the nanoscale, the *in vivo* targeting can be done in a more effective manner by exploiting the positivity offered by these nanomaterials. Hence nanomaterials can do wonders in the diagnosis and therapy of the cancer. But one of the difficulties that is commonly encountered, and limits their use for diagnosis and therapy, is the non specific accumulation of these nanoparticles in non target tissues. Due to this, the differentiation of the desired tissue from the rest becomes difficult and hence limiting their use '*in vivo*'. Another problem that commonly comes across in effectively using these nanoparticles '*in vivo*' is their stability. Because of their high surface area to volume ratio their tendency to undergo aggregation is very high and can lead to some clustering. This clustering results in increase in their overall size and negatively impacting their effective targeting leading to some reduction in their efficacy. In order to counteract the problem of clustering, different types of coatings have been / are being currently explored. These coatings not only prevent the clustering of the particles, by introducing steric repulsions among the neighbouring particles, but also increase their aqueous stability and solubility, in addition to providing the functional site for further modification, to which any desired agent can be coupled. By prior conjugation of a targeting agent to the possible site of modification of the nanoparticle, the problem of non specific uptake can be greatly reduced which leads to the better outcome in the diagnosis and therapy of the diseased site. The coatings which are being used for this purpose include oleic acid [4], oleylamine [5], polyethylene glycol [6], polyvinyl alcohol [7, 8], dextran [9] and its derivatives. As all these coatings contain long backbone chains of carbon

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atoms in addition to functional groups, out of which the former is easily adsorbed on the highly active surface of nanoparticles and the later are projected outwards, which helps in increasing the aqueous solubility and stability. In addition to these organic coatings some small molecules like silica [10], dopamine/catechol derivatives [11], amino acids [12], aminopropylsilanes [13] etc can also be used for this purpose. Although any of these molecules can be selected, but while exploiting these molecules for *in vivo* applications, an important aspect that needs to be considered carefully, is the toxicity level of these surface modified nanoparticles. Because for exploiting them for *in vivo* applications the particles applied should be biocompatible, so that they do not cause undue side effects to the rest of the body tissues. For satisfying this criterion polyethylene glycol and dextran are proving very useful as both of them are very much biocompatible with no reported toxicity, hence are currently being used enormously. However studies relating to the use of polyvinyl alcohol, oleic acid, oleylamine, amino acids and aminopropylsilanes are currently in progress but before using them for *in vivo* purposes their toxicity aspects should also be studied. Although few workers have tried to study their toxicity profiles but still a lot is needed to be explored in this direction for successfully exploiting them. Even after controlling their cytotoxicity/biocompatibility, another parameter that needs to be satisfied for their successful application in diagnosis and therapy is to conjugate target specific agents to their surface, so that their accumulation at the targeted site can be enhanced with minimal at the non targeted site. This can lead to better differentiation between the targeted and the non-targeted tissues in the diagnosis related operations, hence helps in identifying the diseased tissues from the healthy one. In addition to the usefulness of nanomaterials in the diagnosis, the therapeutic output of a treatment/therapeutic agent can be significantly enhanced by simultaneously tagging a therapeutic cum targeting agent which not only helps in the improved delivery of the therapeutic agent at the target site in sufficient concentrations, but also in very short times. This could be advantageous in the future, keeping in view the rapid diagnosis followed by rapid deployment of therapeutic strategy, producing much faster therapeutic effect. The rapid improvement in the diagnostic and therapeutic outcome of a diagnostic/therapeutic strategy, by the use of nanomaterials, could be owed to the ability of these nanomaterials to easily cross certain biological barriers which their bulk counterparts could not (because of their large size). This improvement in the outcome of a diagnostic/therapeutic modality by using nanomaterials is due to the passive targeting ability of nanomaterials combined with active targeting using a targeting agent. But here the objective, in which we are much interested in, is the benefit of the patient rather than how it is achieved. Although here it doesn't mean that we are neglecting the contribution of passive and active targeting. Rather we would prefer to say that this effectiveness of nanomaterials in improving the outcome of diagnosis/therapy rests on the backbone of passive and active targeting. Active targeting mentioned here, in addition to enhancing accumulation at the targeted site, also leads to reduction in the non specific delivery of the therapeutic agent elsewhere. This leads to reduction in side effects of the treatment, as is generally the case with conventional therapeutic procedures.

A few of the agents that have been studied for targeting purposes are folic acid [14, 15], RGD (Arginine-Glycine-Aspartic acid) peptides [16], chlorotoxin [17] and antibodies [18] etc. Although, all these agents are used in one step targeting, but in order to further enhance the targeting efficiency, the two or three stepped pretargeting approaches can also be used. Two or three stepped pretargeting approaches employ the use of well known affinity of biotin for avidin or streptavidin [19-21]. The earlier mentioned targeting agents can be used for targeting purposes because all these agents are having their receptors over-expressed on most of the tumors. These receptors help in the internalisation of the agents conjugated to these targeting moieties by process of receptor mediated endocytosis [22]. Folic acid a type of vitamin B found commonly in leafy vegetables, is a well known biomolecule for targeting folate receptors which are overexpressed in many types of cancers including lung, breast, cervical, colorectal, renal and ovarian cancers [23, 24] etc. RGD peptides which contain a sequence of different amino acids are also important ligands for targeting '$\alpha_v\beta_3$' integrin receptors [25, 26], which indicate onset of tumor angiogenesis. Chlorotoxin, a peptide having a long chain sequence of 36 amino acids is used to target matrix metalloproteinase-2 (MMP-2) receptors which are over-expressed on medulloblastoma, glioma, sarcoma, prostate cancer and intestinal cancer [27, 28]. Antibodies are also important vehicles for targeting various diagnostic and therapeutic agents to the desired site. But the main drawback of antibody mediated targeting is the very high molecular weight of the antibodies, due to which only 1 to 10 parts per 100,000 of intravenously administered monoclonal antibodies reach their targets *in vivo* [29].

In opposition to targeted nanoparticles, non targeted nanoparticles go to the non targeted organs in fairly high concentrations and get accumulated there. This unwanted accumulation of nanoparticles in non-targeted organs often lead to some ill effects, in addition to decreasing the target to non target contrast ratio and limiting the successful use of MR in theranostic applications, hence making the diagnosis of the diseased site difficult. So here lies the role of target specific nano-conjugates, which can lead to path breaking achievements in the early diagnosis and therapy of tumors.

2. APPLICATIONS OF NANOTECHNOLOGY IN CANCER DETECTION AND TREATMENT

The peculiarity of nanotechnology which is responsible for its extensive exploration in medical applications is the interesting properties possessed by materials at the nanoscale. These special properties inherited by the materials at the nanoscale make them useful over their bulk counterparts. This alteration in the properties of a material on size miniaturisation is due to the presence of large number of surface atoms as compared to bulk materials and also very high surface area to volume ratio, due to which their surface activity is very high. Furthermore, the ability to tailor these materials at such a minute level provides us a tool to explore the biological systems, as they can easily cross certain biological barriers within the body which bulk materials cannot overpower. Here is an overview of possible applications of nanoparticles in medical field.

3. MAGNETIC SEPARATION

Magnetic separation technique involves extraction of cells of interest from a group of cells by exploiting the inherent magnetic property of magnetic nanoparticles (MNPs). In this technique antibodies/ biomolecules modified MNPs are seeded with a group of cells which then bind with the antigens/biomarkers expressed on surface of desired cells. As antibodies/biomolecules modified MNPs can bind only to the cells which are having specific biomarkers and not to other cells, hence these biomarker specific MNPs coupled to surface of desired cells can be easily separated using a magnet. Berensmeier, 2006 has shown that MNPs can be used for separation and purification of nucleic acids from blood samples, cultivation media and tissue homogenates [30]. Although this technique has been used mainly for '*in vitro*' applications but exploitation of this technique for '*in vivo*' applications is being explored. It has also been found that some tumor cells after being detached from solid tumors diffuse into the blood stream and can spread to other body parts post surgical removal of solid tumors. Secondly, limitations of conventional methods in detecting these circulating tumor cells in the peripheral blood has led to interest in developing MNPs based techniques for detecting these circulating tumor cells. Therefore designing a treatment strategy would be easy if we can accurately detect and determine the number of circulating tumor cells [31, 32]. Sieben *et al.*, 2001 and other researchers have successfully shown that MNPs can be used for isolating circulating tumor cells [33-35]. Recently 'Yoo *et al.*, 2016' have successfully demonstrated the use of magnetic separation method for separating circulating tumor cells

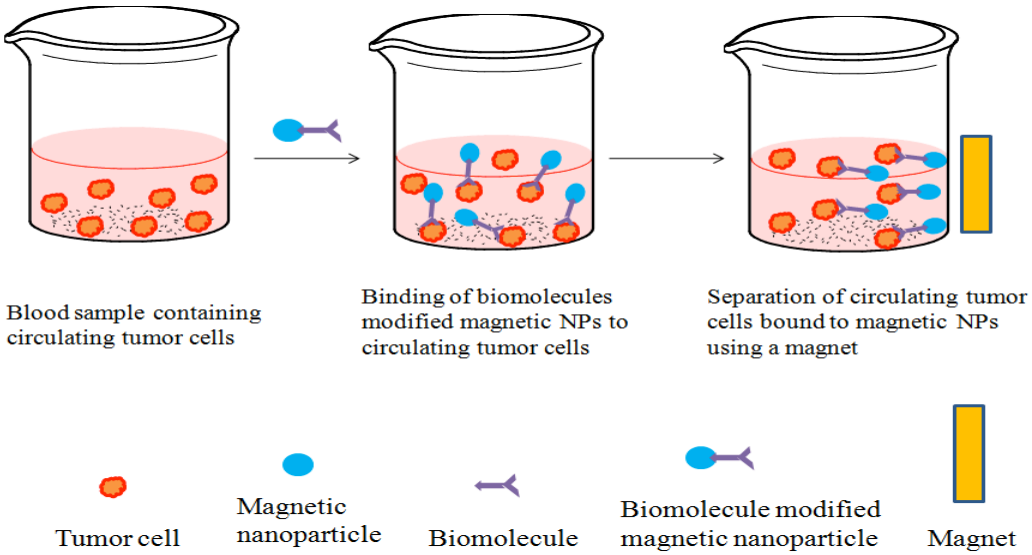

Figure 1: Diagrammatic depiction of separation of circulating tumor cells using MNPs.

from the blood of lung cancer patients by using antibodies modified magnetic beads [36]. One such compound that can be used clinically for detecting circulating tumor cells and has been approved by Food and Drug Administration (FDA) is Veridex [37]. The diagrammatic representation of strategy used for identification and separation of circulating tumors cells using MNPs is shown in Figure **1**. In this technique, the blood sample from a suspected patient is seeded with some specific MNPs which have been modified with some biomolecules or antibodies. As these biomolecules or antibodies can bind specifically to various receptors present on the surface of tumor cells, hence these MNPs get attached to the tumor cells. Later by using a simple magnet we can separate them from blood sample and test them for malignancy. This method could be very much useful in designing a chip/diagnostic kit, which can detect malignancy by simply using the blood sample from a suspected patient. Hence could prove to be a boon in the theranostics of cancer, which otherwise is marred by limitation of cancer not being able to be detected early.

2. HYPERTHERMIA AND THERMOABLATION

Hyperthermia is based on selectively increasing the temperature of the targeted site within the body slightly above the normal body temperature. This slight increase in temperature is generally considered to be therapeutically useful over much elevated temperatures, as very high temperatures can damage the healthy living cells. Hyperthermia is a new and advanced technique of killing tumor cells at their loci by targeting them with metal based nanoconjugates, followed by alteration in applied magnetic field which leads to generation of so sufficient amount of heat that tumor cells cannot bear and hence dies due to thermal shock. Two types of heating approaches are generally used in hyperthermia for raising the temperature. First one involves exposure of desired tissue at a temperature range of 42-45°C for few hours, which is actually termed as hyperthermia. Second approach involves the exposure of the tumor tissues to temperatures higher than 50°C for few minutes, later termed as thermoablation [38]. Metallic NPs of gold and iron oxide are generally used in this technique [39, 40].

Although there are some difficulties in the clinical use of hyperthermia, like difficulty in measuring the temperature of the tumor and its surrounding mass, and reproducibility of heat deposition into the tumor etc. Further the parameters like tumor size and positioning of electrodes sometimes also make it difficult to specifically heat tumors, as heating effects are regulated by these two parameters. The difficulty in attaining the required temperature only in the tumor region without damaging healthy tissues is also a limitation. Although these disadvantages, can be overpowered by using MNPs for hyperthermia treatment. *Johannsen et al*., 2005 and *Johannsen et al*., 2010 have highlighted the clinical use of MNPs for hyperthermia treatment of prostate cancer [41, 42]. Further when hyperthermia and radiation therapy are used in combination, then hyperthermia may make some cancer cells more sensitive to radiation or can even destroy them that radiation cannot damage alone. *Assogna et al*., 2013 demonstrated the use of hyperthermia in combination with chemotherapy in treatment of pancreatic cancer [43]. Superior therapeutic outcome was found by *Mandraveli et al*., 2015 in treatment of metastatic colorectal cancer when hyperthermia was used in combination with chemotherapy rather than using chemotherapy alone [44]. Recent trials in previous years in Germany and Holland have demonstrated that hyperthermia can prolong life when it was used in combination with radiotherapy and chemotherapy simultaneously [45].

Further, depending on the point of application, hyperthermia can be classified into various types like local hyperthermia, regional hyperthermia and whole-body hyperthermia etc. Local hyperthermia practice involves the treatment of tumors located within the body, at their loci, either by heating them by applying high frequency waves from outside or by heating them internally. On the other hand, regional hyperthermia includes a whole region or area within the body rather than a specific location. It may encompass the area around a tumor. While in whole body hyperthermia, the heat is applied to whole body of the organism rather than a specific location or region in the body. As in whole body hyperthermia, the heat is applied to whole of the organism therefore it is also known as systemic hyperthermia. But this technique is applied only in case of metastatic tumors that have proliferated throughout the body rather than limiting to a loci or a region.

Second approach used for destroying tumors by using heating approach is thermoablation in which still higher temperatures are used for destroying cells rather than that used in hyperthermia, but for shorter durations. Further thermoablation can be achieved by using different modes of heating like laser induced, radiofrequency induced and microwave induced thermo ablation. *Vogl et al*., 2016 recently have demonstrated

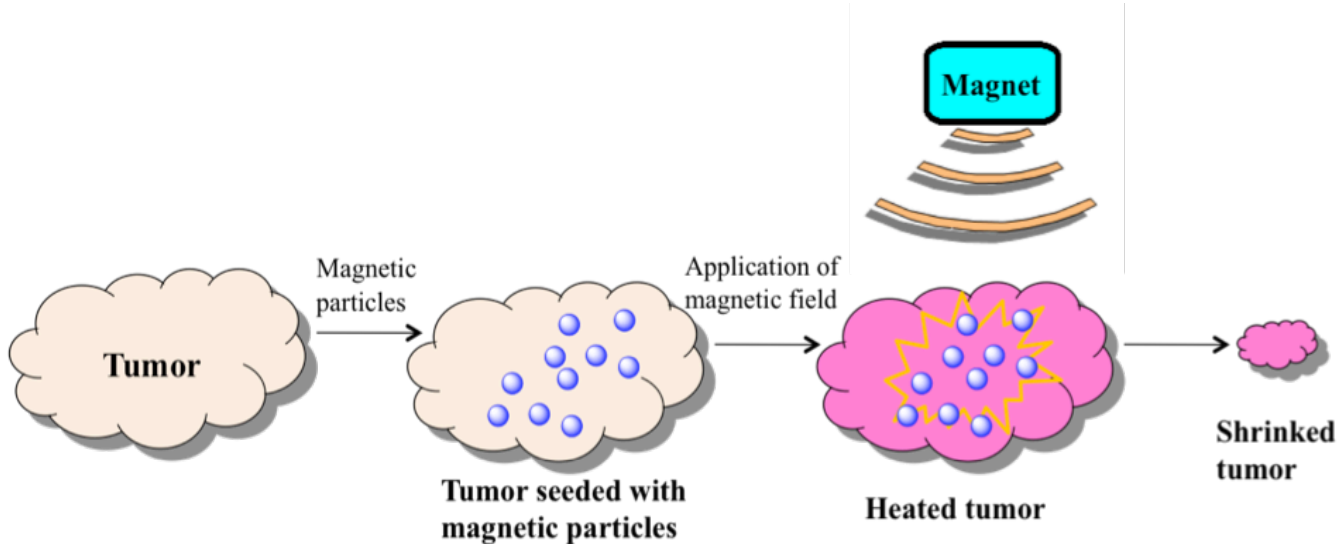

Figure 2: Diagrammatic representation of destruction of tumor due to heating effects produced by magnetic particles as is done in hyperthermia and thermoablation.

the use of three different thermo ablation techniques for studying lung metastasis resulting from colorectal cancer [46]. Treatment of tumors by using heating strategies, as employed in hyperthermia and thermoablation is shown in Figure **2**. In these methods the tumors are first seeded with some target specific MNPs. Once these MNPs are bound to tumors, a magnetic field is applied externally and then by altering magnetic field we can selectively heat the tumors at their loci without harming the healthy ones.

3. EMBOLOTHERAPY

Embolotherapy is a technique of destroying cancerous cells at their loci by starving them of the nutrients essential for their growth and survival. In this method the vessels supplying blood to the desired location is blocked by using some object. This may be done by inserting a thin catheter into the artery by making an incision in it. Then small particles are introduced into the artery to block its passage. This reduces the flow of blood supply or even may block it completely. By making the cells devoid of the essential nutrients, their normal growth and functions are halted and finally leading to their death. Embolotherapy is a good option in cases where tumors cannot be removed by surgery. This technique requires slightly larger particles, with size sufficient enough (i.e. neither to small nor to large) to get stuck in the vessels supplying to the tumors. Once these particles get stuck in the blood vessels they block the supply of nutrients to the tumor cells, hence curtail their growth and proliferation and finally leading to cell death. The different agents that can be used in embolotherapy are starch microspheres, collagen particles, polyvinyl alcohol beads and gelatin sponge etc. A study by *Bastian et al*., 1998 on rat model showed that the diameter of the particles for embolisation should be at least 40 μm [47]. Further the diameter of the particles being used for embolisation depends on the size of the artery being blocked. In addition to this, the shape and elasticity of the particles also play a role in embolisation. A study by *Siskin et al*., 2003 showed that particles having uneven surfaces tend to accumulate in vessels having larger diameter as compared to particles having regular surfaces. They also found that the particles having high degree of elasticity are more prone to reach smaller vessels as compared to those having lesser elasticity [48]. Wang, 2012 have shown the usefulness of embolotherapy in treatment of liver cancer [49]. His study showed the reduction in the size of hepatic tumor in a patient with hepatocellular carcinoma when anticancer drug is used in combination with embolotherapy. This reduction in size of the tumor was due to the combined effect of blocking of hepatic artery feeding the tumor, as well as the therapeutic effect of the drug. *Embolisation* is a good option for patients having tumors that cannot be removed by surgery. *Lee et al*., 2005 and Chung *et al*., 2012 embedded superparamagnetic iron oxide (SPIO) in chitosan shell for using them in MRI monitored embolotherapy [50, 51]. Further the efficacy of embolotherapy in treatment of tumors using SPIO nanoshells can be improved by loading an anticancer drug simultaneously onto them. The depiction of embolotherapy in controlling the growth of tumors is shown in Figure **3**. In this diagram the Figure (**A**) shows

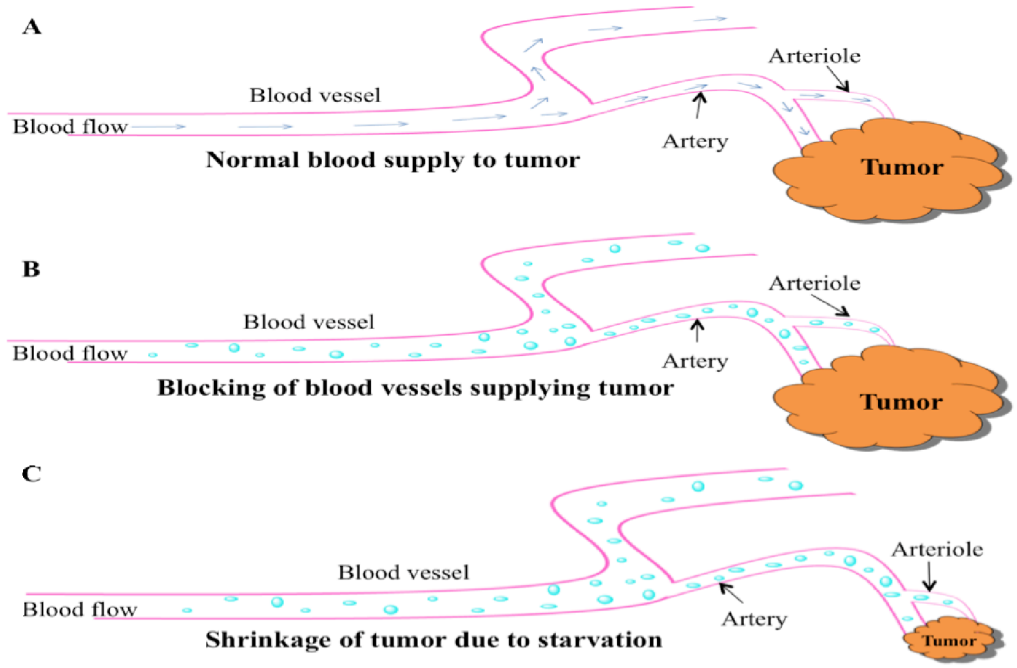

Figure 3: Diagrammatic representation of embolotherapy: Figure (**A**) showing normal blood supply to tumor, (**B**) Blocking of vessels supplying to tumor using magnetic particles and (**C**) Decrease in size of the tumor due to starvation.

the normal blood supply to a tumor. But when some magnetic spheres of appropriate size are introduced into the blood stream through an injection then they get stuck in the vessels supplying the tumor (**B**). Due to which the supply of nutrients to the tumor is prevented and hence its growth gets restricted (**C**).

4. TARGETED DRUG DELIVERY

Targeted drug delivery refers to the selective delivery of a therapeutic agent at the target site within the body. Although nanoparticles can be targeted passively to the tumors by using enhanced permeation and retention effect (EPR). The EPR effect shown by tumors is due to the reason that tumors rely on angiogenesis for their growth. Further the blood vessels in tumors have leaky vasculature due to large gaps between the adjacent cells. This gap lies in the range of 600-800 nm. Due to these large gaps in the tumor endothelium, the nanoparticles can easily cross them so as to enter in the tumor tissue. Also it is very well known that tumors have poor lymphatic drainage. Due to this permeation property of tumor microenvironment, nanoparticles can easily enter inside them. But as the drainage system is poorly developed so they are not able to escape from there hence leading to their accumulation in the tumor [52]. Further it has been found that when a drug is delivered by using a nanoparticle, then there is more than 10 times increase in tumor accumulation as compared to free drug alone [53]. In active targeting method, a target specific agent is usually coupled to a vehicle carrying the therapeutic agent (either conjugated or encapsulated) hence helps in delivering the therapeutic agent at the target site. This not only reduces its binding to non targeted locations within the body but simultaneously reduces the side effects of the coupled therapeutic agent. This targeted delivery of therapeutic agent can be further improved by using nano-systems as carriers for their transportation within the body. As these nano-systems are having very small size therefore their surface activity is very high which helps in their efficient functionalisation and further conjugation of any desired agent. Secondly due to their small size they can easily cross certain biological barriers in the body which bulk materials can't, hence helps in efficient transportation of the conjugated agent through body vasculature and delivering them at the target site maximally. The targeted NPs after being released into blood stream are taken up by specific receptors present on the surface of tumor cells followed by formation of endosome. The formed endosome after undergoing series of alterations like acidification and osmotic pressure changes, undergo rupture, finally leading to the release of the nanoparticle as well as the coupled therapeutic agent. Thus targeted drug delivery can be significantly enhanced/improved by the use of nano-vehicles. As targeted nano-delivery systems allow for a reduced systemic dosage along with providing relatively higher or more efficient dosing at the target site. One of the advantages of nanoparticle based delivery systems is that they result in increasing

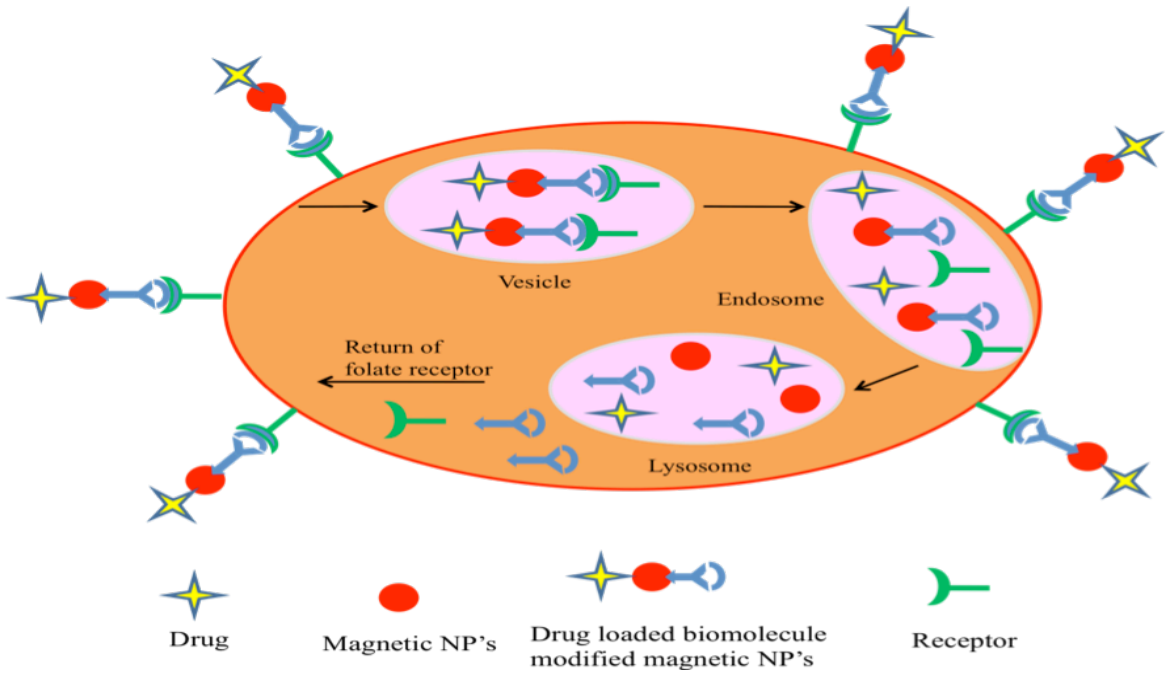

Figure 4: Diagram showing the utilisation of MNPs in targeted drug delivery.

the circulation time of drugs/diagnostic agents. The utilisation of MNPs in delivering a conjugated drug to a targeted site is shown in Figure **4**.

5. DIAGNOSIS AND THERAPY

One of the major drawbacks of conventional imaging methods is that they are unable to detect cancer at early stage. This limits the use of treatment strategy hence affecting the well being of the patient. If diagnosis of cancer is done at initial stages then the therapy can be very much fruitful. On contrary to this, where detection of cancer at early stages is not possible it is a big challenge. This limits the successful employment of treatment strategy for eradication of cancer. This limitation of not being able to detect cancer at early stages can be overcome by using nanotechnology based approaches for conventional imaging techniques. Among them most commonly explored are based on using metal nanoparticles (like gold and silver), metal oxide based nanoparticles (e.g. iron oxides like magnetite and maghemite), mixed metal oxides like metal ferrites based on manganese, cobalt and nickel etc and quantum dots [54] also. Among all of them iron oxide based NPs are of particular interest because of their biocompatibility and biodegradability. Further the metabolised iron is added to the iron store of the body and is utilised by erythrocytes in the synthesis of haemoglobin [55]. In addition to this, their associated superparamagnetic behaviour at the nanoscale size is useful for exploiting them as T_2 based contrast enhancement agent in MRI. Ability of these superparamagnetic NPs to shorten the transverse relaxation time of neighbouring protons could be useful in MRI. This remarkable shortening of transverse relaxation time of neighbouring protons by using superparamagnetic NPs results in loss of signal intensity, due to this the region appears dark hence can be easily distinguished from the surrounding tissue. Thus we can noninvasively diagnose the cells and tissues targeted by using MNPs.

Further by conjugating an anticancerous drug to a target specific nanocarrier we can deliver it to the target site selectively. This reduces excessive buildup of the drug elsewhere along with selectively concentrating it at the desired site and hence useful in treatment strategies. But before using iron oxide for diagnostic/therapeutic purpose we need to modify its magnetic core. This is achieved by coating them with a surface coating which should also be biocompatible in nature. This coating provides stability to MNPs against agglomeration, along with aqueous solubility as well as site for functionalisation. After this a targeting agent is coupled with the coating which helps in carrying the nanoconjugate to the target site and binding there to the specific receptors [56, 57]. Further, in addition to conjugating a targeting agent to the surface modified nanocore we can also conjugate simultaneously a therapeutic agent to its surface. This imparts the nanoconjugate a dual behaviour of diagnostic as well as therapeutic purpose. Hence could be useful in treatment strategies related to cancer. The utilisation of

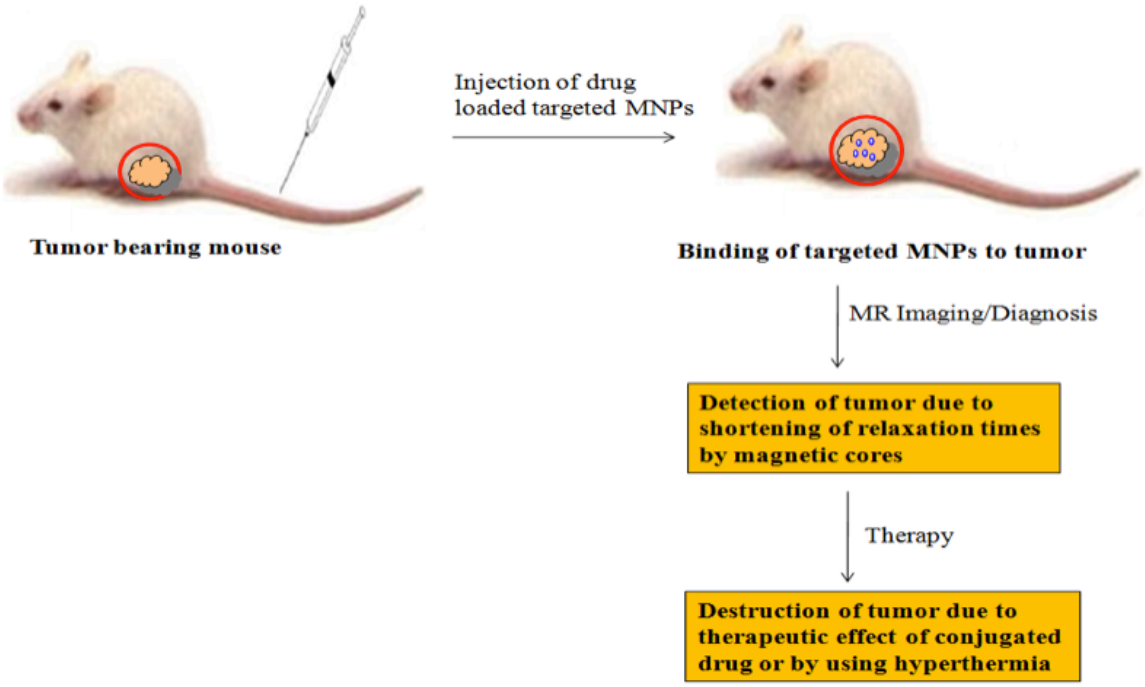

Figure 5: Diagram showing the utilisation of targeted MNPs in tumor diagnosis and therapy.

targeted MNPs in diagnosis and therapy of tumors has been demonstrated in Figure **5**.

CONCLUSIONS

All in all nanotechnology based approaches can be very useful in cancer eradication. By using specific nanoparticles that could bind to cells of desired interest we can isolate circulating tumor cells also, which is otherwise a difficult task. By doing so it would be easy to develop approaches to eradicate these circulating tumor cells *in vivo*, so that they do not invade other parts of the body. In addition to detecting and isolating circulating tumor cells, nanotechnology based tools can also be used for diagnosing localised tumor cells. After pinpointing the exact location of a tumor cell, it is quite easy to apply therapeutic approaches for destroying tumor cells at their loci by using hyperthermia, thermoablation, embolotherapy and most importantly by using target specific delivery of an anticancerous agent. Thus could be useful in theranostics of cancer.

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