Wu Y, et al., J Nanotechnol Nanomed Nanobiotechnol 2015, 2: 003
DOI: 10.24966/NTMB-2044/100003

HSOA Journal of
Nanotechnology: Nanomedicine & Nanobiotechnology

Review Article

Magnetic Nanoparticle for Biomedicine Applications

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Abstract

Magnetic Nanoparticles (MNPs) have been widely applied in the area of biomedicine in recent years due to their broad versatility, such as good biological compatibility, unique physicochemical properties and easy guidance by external magnetic fields. In this review, the recent progress in the synthesis and surface modification of MNPs, as well as their applications in biomedicine including bioseparation, molecular detection, drug delivery, hyperthermia and Magnetic Resonance Imaging (MRI) and etc., have been summarized.

Keywords: Bioseparation; Drug delivery; Hyperthermia; Molecular detection; MNPs; MRI

Introduction

As a subject of the worldwide research activity, Nano-biotechnology is currently largely driven by the rapid development of the nanotechnology and their application in molecular biology and biomedicine. Concurrently, the research on the Magnetic Nanoparticles (MNPs) with the dimensions ranging from the nanometer to micrometer scales is extremely active thanks to their unique physicochemical properties [1-4]. First, these particles are liable to be guided by external magnetic field. Second, MNPs are possible to be coated by functional groups to tailor their physicochemical properties and realize versatility. Besides, the synthesis of MNPs is rather facile and their size is easily controlled by the experimental conditions, making them possible to satisfy different research requirements. Therefore, functionalized MNPs, by allowing the biomolecules, be tagged, detected and controlled magnetically, enable new promising approaches to bioseparation, biodetection and drug delivery [5-9].

The aim of this review article is to summarize the recent progress in the synthesis and functionalization of magnetic nanoparticles and their applications in biomedicine, such as bioseparation, molecular detection, drug delivery, Magnetic Resonance Imaging (MRI) and hyperthermia. With the development in the synthesis and functionalization of the MNPS, the above applications are demonstrating great vitality and have been or may be used in clinical practice.

Synthesis of Magnetic Nanoparticles

The methods of synthesis influence the properties of MNPs on the dimension, particle-size distribution and morphology [10]. Physical methods including gas phase deposition and electron beam lithography are elaborate procedures that cannot control the size of particles [5,11]. The wet chemical routes to MNPs, which provide a convenient way for the size control and composition modulation, are simpler, more efficient and wildly adopted for the preparation of MNPs [12,13].

The most common wet chemical method used for the preparation of MNPs is co-precipitation. This approach offers a wide range of advantages: 1) It is low-cost because of the use of inexpensive chemicals and mild reaction conditions, 2) It is environmentally friendly because the MNPs can be directly synthesized in water, 3) The method is extremely flexible when it comes to the modulation of the core and surface properties by controlling the experimental parameters such as the reaction temperature, pH value and ionic strength of the media; 4) It is extremely repeatable if the experimental parameters are fixed [14]. For example, by using this method, size-controllable iron oxides (either Fe_3O_4 or γ-Fe_2O_3) nanoparticles can be efficiently synthesized from aqueous Fe^{2+}/Fe^{3+} salt solutions by the addition of a base at room temperature [15].

Other approaches including microemulsions [16], sol-gel synthesis [17], sonochemical reactions [18], hydrothermal reactions [19], hydrolysis and thermolysis of precursors [20], flow injection synthesis [21], electrospray synthesis [22], and graft copolymer method [23] are reported in the literature as well. For instance, in order to increase the loading amount of the reactive groups on the magnetic particles and to improve the stability and dispersibility of magnetic particles, several magnetic particles have been synthesized by the graft copolymer method, with the flexibility and diversity to control the chemical composition and functional groups on the surface of nanoparticles. XF Sun et al., reported the synthesis of a novel hemicelluloses based magnetic hydrogel by using such a graft

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Citation: Wu Y, Yang X, Yi X, Liu Y, Chen Y, et al. (2015) Magnetic Nanoparticle for Biomedicine Applications. J Nanotechnol Nanomed Nanobiotechnol 2: 003.

Received: December 24, 2014; **Accepted:** February 09, 2015; **Published:** February 24, 2015

copolymer method, showing that the Fe_3O_4 particles can be well dispersed in the hydrogel matrix and the as-prepared hydrogels had promising paramagnetic properties for potential biomedical application. A brief summary of various MNPs synthesis methods is shown in table 1.

Synthetic method	Synthesis condition	Size distribution	Shape control
Co-Precipitation	Very simple, ambient conditions	relatively narrow	not good
Thermal decomposition	complicated, inert atmosphere	very narrow	very good
Microemulsion	complicated, ambient conditions	relatively narrow	good
Hydrothermal synthesis	simple, high pressure	very narrow	very good

Table 1: Summary comparison of various magnetic nanoparticle synthesis methods [14].

Functionalization of Magnetic Nanoparticles

The functionalization of MNPs is critical to their performance and applications. Functionalized MNPs with excellent biological compatibility and specificity enable plenty of exciting new approaches to applications for biomedicine. For instance, MNPs which are attached to functional molecules, such as antibodies, aptamers, DNA or RNA, can be applied in bioseparation [24]. And these attached to paclitaxel, doxorubicin, etc., can be used in the field of drug delivery and cancer treatment [25]. These functionalization are generally achieved by employing the molecules containing anchor groups based on carboxyl, thiol or amine groups to attach to the surface of MNPs through covalent linkage [26].

Other ways of functionalization of MNPs, such as combining them with other functional nanostructures by sequential growth or coating, have also been reported. For example, MNPs combined with quantum dots or metallic species lead to a promising candidate for molecular imaging [27-29]. The encapsulation of a potential anticancer drug by iron oxide nanoshells affords the yolk-shell nanostructures, which is potentially promising for controlled drug delivery (Figure 1). In addition, owing to the large surface area to volume ratios and strong dipole-dipole interactions, MNPs tend to agglomerate and form larger clusters with limited application [5,30]. By functionalizing MNPs, it is possible to avoid this phenomenon and achieve desirable dispersibility in the liquid state as well (Figure 1,Table 2) [31,32].

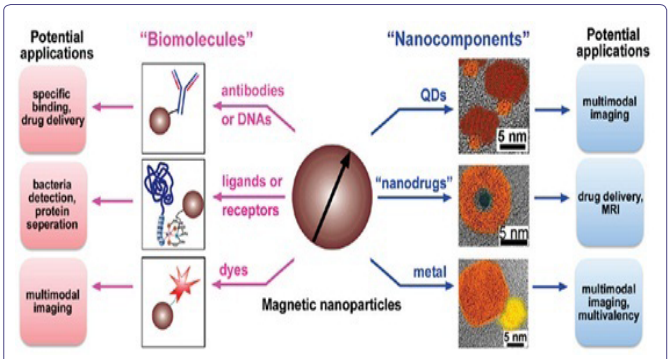

Figure 1: The scheme illustrates strategies to fabricate multifunctional MNPs and their potential applications [33].

Biomolecule coating agent	Applications
Adenoviral vectors Antibodies	1. MRI and gene delivery. 2. Recognition processes, cell capturing and immune- magnetic separation. 3. Detection of *in-vivo* enzyme activity. 4. Intelligent drug delivery.
Aptamers	Recognition processes.
Dextran	Stabilizer agent. Enhances blood circulation.
DNA or RNA	Recognition processes. Drug delivery.
Doxorubicin	Drug for cancer treatment.
Enzymes or proteins	1. Biomarkers of process such cancer, apoptosis, inflammatory reactions. 2. Protein separation, purification, detection and analysis. 3. Drug delivery and hyperthermia
Folic acid	Effective tumor targeting agent.
Nitrilotriacetic acid	Protein separation.
Oligonucleotides	Probes for detecting or separating DNA or RNA. Nanoswitches.
Paclitaxel	Drug for cancer treatment.
Polyethylene glycol	Widely used for protein conjugation.
Silica	Stabilizer agent that can be loaded (within the pores). Useful in the fabrication of multifunctional MNPs.

Table 2: Examples of different coating agents and biomolecules used in the fabrication of magnetic biocomposites [32].

Biomedical Applications of Magnetic Nanoparticles

Bioseparation

In the field of biomedicine, magnetic separation based on MNPs, which is inexpensive and efficient in nature, is an excellent solution to the problems encountered by the conventional tedious and time-consuming biomedicine separation methods including centrifugation and filtration [34,35]. Upon the conjugation of the target biomolecules and MNPs functionalized with specific receptors, the as-formed complexes can be easily attracted by the applied magnetic field and extracted from the pristine mixture, thus providing a convenient and time-saving approach for bioseparation [36]. For instance, MNPs bearing immobilized single stranded DNAs are used to purify complementary DNAs [37]. After a suitable incubation time, the complementary DNAs are allowed to tightly bind to the single stranded DNAs anchored to the MNPs. Through magnetic decantation and washing procedure, the purified DNAs are easily isolated from and recovered by displacement from the MNPs by proper elution procedures (Figure 2).

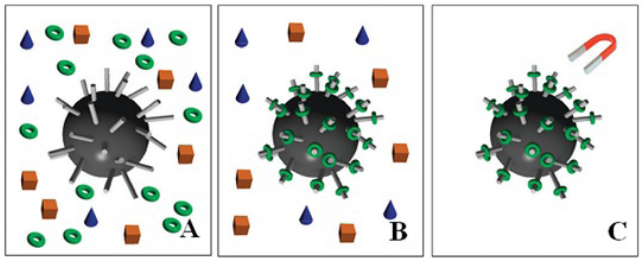

Figure 2: Schematic representation of magnetic separation of DNA or proteins in solution.

The grey rods on the surface of the spherical MNP represent the immobilized tag which will tightly bind the DNA strand or protein of interest. The separation process follows the steps: (A) MNPs and the solution with different components are mixed, (B) particular proteins or DNA (green rings) bind to the MNPs and (C) magnetic field is applied to trigger magnetic decantation, followed by further washing steps and collecting the molecule of interest [37].

Depending on the specific functionalization of the MNPs for different targets, this technique has been already applied in the field of the separation and purification of cells [38], proteins [39], bacterial [40] and virus as well. Some work is summarized in table 3. In the study by Yi-Ran Cui et al., Anti-CD 3 monoclonal antibody bioconjugated to core/shell Fe_3O_4@Au magnetic nanoparticles were synthesized for cell separation (Figure 3). It is showed that the functional Fe_3O_4@Au MNPs successfully pulled down $CD3^+$ T cells from the whole splenocytes. This method with high efficiency of up to 98.4% demonstrates a more effective cell-capture nanostructure than that obtained by non-oriented strategy [41]. Zhen Liu et al., reported a multifunctional magnetic mesoporous core/shell hetero-nanostructure (designated as Fe_3O_4@$NiSiO_3$), which combined the capacity of effective protein purification from protein mixture and selective low molecule weight biomolecule enrichment. The hetero-nanoparticles were employed to selectively bind to and magnetically separate of His-tagged proteins from a cell lysate of *Escherichia coli* [42].

Biomolecules	MNPs
Cell	core/shell Fe3O4@Au @Au [43]
Protein	Fe3O4@NiSiO3/Au/γ-Fe2O3/Fe3O4 [44]
Bacterial	Fe3O4@Au [45]
Virus	Fe3O4@polymer [46]
Gene	Fe3O4-SiO2-Au [47]

Table 3: Examples of bioseparation by using MNPs.

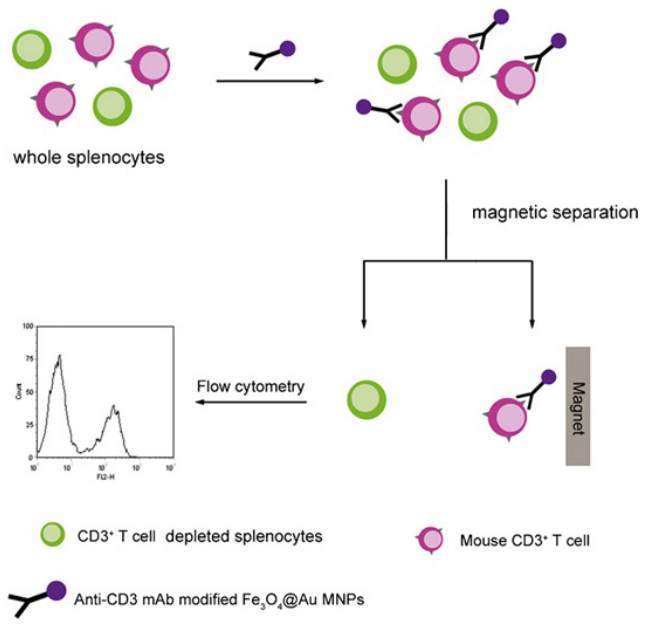

Figure 3: Procedure of magnetic cell separation for whole splenocytes of mouse using anti-CD3 mAb modified Fe_3O_4@Au MNPs [41].

Biosensing

The functional unit of the magnetioresistive sensors based on MNPs for biosensing application consists of the sensor and MNPs binding to the sensors. The working principle of this kind of magnetoresistive sensors is that the magnetic fields generated by the magnetic particles which alter the magnetic fields of the sensor result in electrical current or resistance changes within the sensor [48]. Compared to colorimetric, radioactive or electrochemical methods [49], the magnetic sensors technique has shown superior advantages for biomarker detection and qualifications, providing high sensitivity (negligible interference from the sample background), long-term stability (no photo bleaching, such as in fluorescent labeling) and possibility of the equipment miniaturization [50]. Thus the technique has broad applications in clinical diagnosis, food industry and environmental control [51].

The modes in which magnetic particles bind to the sensor surface include direct labeling and indirect labeling. The direct labeling method is that MNPs bind to the sensor surface by streptavidin-biotin interaction or complementary DNA sequence recognition. As shown in figure 4, single-stranded DNA receptors are immobilized on the surface of magnetic sensors as a probe. Oligonucleotides of unknown sequence are selectively captured by these complementary probes. Streptavidin-coated MNPs which are capable to bind to the biotin of the hybridized DNA are then introduced into the system. Consequently, magnetic field disturbances because of these MNPs are sensed by the magnetic sensors. The principle of indirect labeling is the sandwich immunoassay such as the Enzyme-Linked Immuno Sorbent Assay (ELISA). The antibodies for the target protein are immobilized on the surface. Then the sample solution containing the target proteins and second biotinylated antibodies are added to the system. Finally Streptavidin-coated magnetic particles are applied for tagging the biotinylated antibodies.

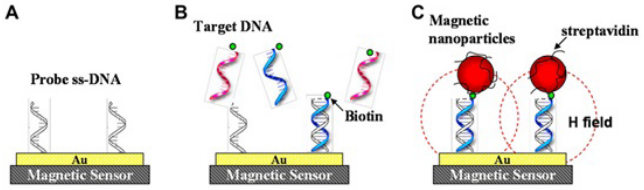

Figure 4: Principle of using magnetic biosensor and magnetic nanotags.

(A) Immobilization of single-stranded DNA with known sequence.

(B) Hybridization of complementary target DNA.

(C) Capture of magnetic NPs via streptavidine-biotin binding. The resistance of the sensor is altered by the magnetic field generated from attached NPs [50].

MNP-based Giant Magnetoresistance (GMR), Spin Valve (SV), Magnetic Tunnel Junction (MTJ), Hall biosensor, and Giant Magneto Impedance (GMI) sensor [52-54] have been successfully developed for biosensing. Steven M Hira et al., has shown the successful detection of a 35-base pathogenic DNA target by Hall-based magnetic transduction. The detection platform has low background noise, large signal amplification ratio following target binding, and can discriminate low concentration of DNA labeled by 350 nm superparamagnetic bead [55]. Another example is the combination of magnetoresistive (spin valve) sensors and magnetic labeling of bioanalytes. By using this technique, a powerful quantitative immunological assays is attained. By reading out the magnetic signals of bio-functionalized magnetic nanoparticles through spin valves sensor, it is shown that the detection sensitivity of human chorionic gonadotropin hormone was well below 5.5 ng/mL (the visual inspection limit) [53].

Drug delivery

During cancer chemotherapeutic treatment, therapeutic compounds with high cytotoxic activities need to be delivered into individual tumor cells to damage or kill tumor cells. In the conventional methods, the accumulation of these drugs in the tumor and healthy tissue is often equivalent due to the non-specific nature

of drugs injected into the blood systems [56]. This phenomenon gives rise to the well known side-effects such as that normal healthy cells are attacked in the procedure of treatment.

Targeted drug delivery of molecules with MNPs can improve the drug specificity and reduce this side-effect [57]. Therapeutic agents are attached to, or encapsulated within MNPs to form the MNPs/therapeutic agent co-complex. These magnetic carriers are injected into the blood-stream and guided to focus over the tumor location through external applied inhomogeneous magnetic fields [58]. The therapeutic agents packed in the NMPs are then released to destroy the tumor cell effectively (Figure 5). Targeted delivery of drug molecules with MNPs can improve the biodistribution and protect the drugs from the microenvironment, exhibiting higher internalization by cancer cells than healthy cells and permitting the use of the therapeutic agents at low enough doses to reduce the toxicity of chemotherapy [24].

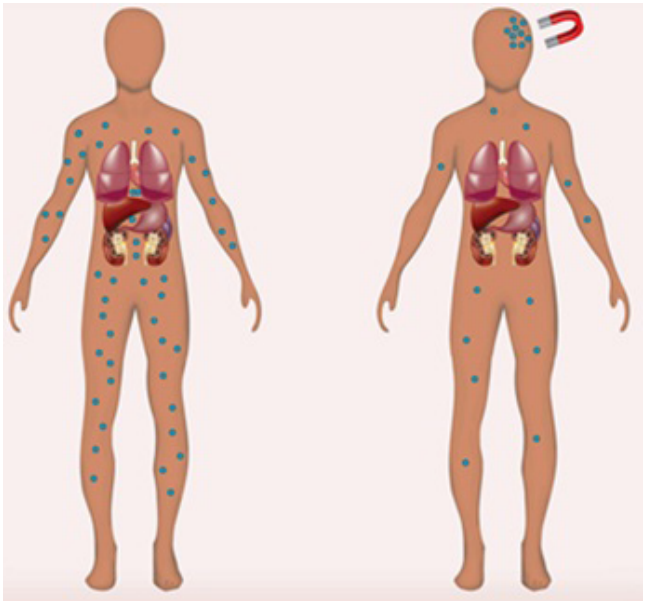

Figure 5: Magnetic targeting.

No accumulation of Magnetic Nanoparticles (MNPs) occurs in the absence of a magnetic field, whereas under the influence of this field, MNPs alone or in combination with therapeutic cargo accumulate at a destined site [59].

A number of studies have demonstrated the advantages of using MNPs for drug delivery. Jon Dobson demonstrated that targeted delivery technique using magnetic nanoparticles was a major breakthrough to the treatment of many diseases in the clinical practice in recent years. Therapeutic compounds are attached to biocompatible magnetic nanoparticles and the applied magnetic fields are focused on specific targets *in-vivo*. The fields capture the particle complex and result in enhanced delivery to the target site [8].

WK Oh et al., reports that the Carbonized Polypyrrole (CPyNs) nanoparticles show low toxicity at low concentrations via cell viability test. The magnetic property of CPyNs provides selective separation and CPyNs sustains *in-vitro* drug release properties. A new platform for the drug delivery using carbonized polypyrrole nanoparticles was established [60].

Magnetic resonance imaging

Magnetic Resonance Imaging (MRI) is a noninvasive technique that uses magnetic fields to produce high resolution and high-contrast images of tissue structure and function. It is based on the response of proton spin in the presence of an applied magnetic field. Under the applied magnetic field, protons align in one direction. When the Radio Frequency (RF) pulse is applied, aligned protons are perturbed and subsequently relaxed to their original state [61]. Two independent relaxation processes, longitudinal relaxation (T_1-recovery) and transverse relaxation (T_2-decay), can be utilized to generate an MR image. Conventional Gd^{3+} or Mn^{2+} complexes, belonging to the T_1 contrast agents, may display toxicity due to the existence of metal ions and raise new environmental issues. On the other side, MNPs with improved efficiency, dispensability, and biocompatibility, belonging to the T_2 contrast agents, have thus been the subject of numerous investigations as potential MRI contrast agents [62].

Many efforts have been undertaken to improve the current contrast agents, aiming to increase the circulation time and to reduce toxicity. Manuel Pernia Leal et al., showed that water soluble MNPs display a high dense Polyethylene Glycol (PEG) core shell and allow long blood circulation times up to 24 h. The combination of low toxicity, excellent T_2 relaxation times, as well as excellent r_2/r_1 values under a low magnetic field, together with the long circulation times, makes these nanomaterials very promising contrast agents for MRI-based molecular imaging in clinical practice (Figure 6) [63]. Ji-Ho Park et al., demonstrated that the geometry of the nanoparticles can enhance their magnetic relaxivity in MRI. The elongated shape of the magnetic iron oxide nanoparticle, with its larger surface area, multiple attachment points, and long blood half-life, improves the T_2 relaxivity for MR imaging [64].

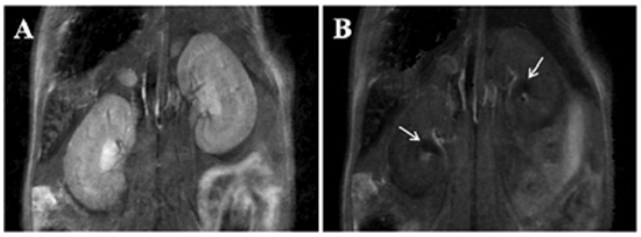

Figure 6: Renal clearance of MNP-GA-PEG-OH.

Kidneys before injection of the MNPs (A) and 12 minutes after injection (B), where accumulation of MNPs can be observed in the renal pelvis (white arrows) [63].

In a study by Hyeon et al., Polyethylene Glycol (PEG) coated MNPs with a diameter of 3 nm have been explored. These MR-active nanoparticles enable clear observation of various blood vessels. Thus they can be used for imaging the vasculature, liver and other organs, as well as molecular imaging, cell tracking and theranostics [65]. Yan Wang et al., indicated a system combination of Poly(Lactide-co-Glycolide)-methoxy poly(Ethylene Glycol) (PLGA-mPEG) nanoparticles and MNPs for Magnetic Resonance Imaging (MRI) can improve the imaging with reduced side effects [66]. KG Neoh and ET Kang also have the similar work about MNPs application in MRI [67,68].

Hyperthermia

Hyperthermia is a medical treatment that selectively kill the tumor cells over healthy cells by heating a tumor at temperatures between 42°C-46°C, and includes full body hyperthermia and localized hyperthermia [69,70]. The full body hyperthermia involves heating the entire body and requires careful control of the temperature to treat metastatic cancer cells when spread throughout the body. Localized hyperthermia involves only heating a small area of interest such as a

tumor. Currently available techniques for induction of hyperthermia are ultrasound, radiofrequency, microwaves, infrared radiation, magnetically excitable thermoseeds, and tubes with hot water [71]. The challenges in traditional hyperthermia treatment are either healthy tissue being damaged by heating, or limited penetration of heat into body tissues.

In the MNPs-based hyperthermia treatment, ferromagnetic or superparamagnetic particles are employed to generate heat into the tumor tissue, followed by irradiation using an Alternating Current (AC) magnetic field [72]. Compared with conventional hyperthermia treatment, MNPs can offer several advantages: 1) Increasing the effectiveness of hyperthermia. MNPs used for hyperthermia are in the range of few tens of nanometer in size, thus allowing easy reach into tumors after intravenous injection with the aid of the applied magnetic field [73]; 2) Improving the specificity of hyperthermia. The MNPs tailored with cancer-specific binding agents allow them being targeted toward specific tumor tissues [74-76]; 3) Ensuring the safety of hyperthermia. The heating of MNPs by the externally alternating magnetic field allows the heat action only killing the tumor cells with minimum damage to normal tissue [77]. Figure 7, illustrates the hyperthermia experimental scheme, where the mouse is placed inside the solenoid coil (blue color) with the tumor positioned at the coil center in order to expose the tumor to the strongest magnetic field generated by the coil [78].

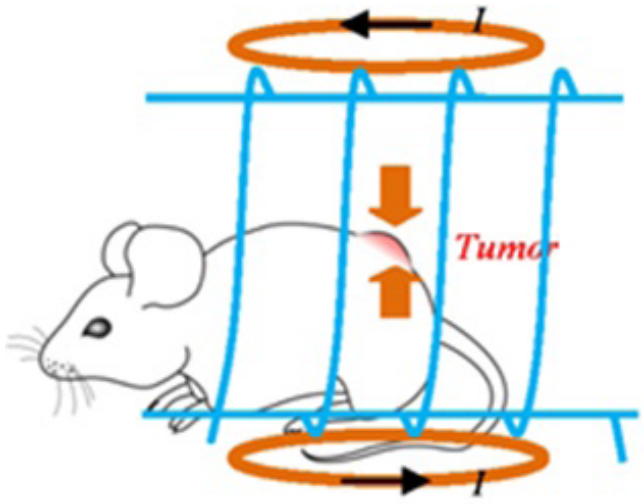

Figure 7: Illustration of the hyperthermia experimental scheme [78].

Chun-Han Hou et al., investigated the effect of magnetic hydroxyapatite nanoparticles injected around the tumor of mice. The mice were placed into an inductive heater with high frequency and alternating magnetic field. Laboratory results showed that the tumor volume of mice was reduced with injected magnetic hydroxyapatite nanoparticles, which demonstrated its therapeutic effect in the mice [79]. In the study by Y Krupskaya, the potential of iron containing Carbon Nanotubes (Fe-CNT) for contactless magnetic heating in hyperthermia cancer treatment was presented. In this paper, the heating mechanism also has been studied [80].

Conclusion and Perspectives

In this review, we have summarized the synthesis, function and biomedicine applications of magnetic nanoparticles. The concept of employing magnetic nanoparticles on the biomedicine applications greatly benefits from the rapid development of the nanotechnology. On one hand, the magnetic nanoparticles with controlled and narrowly distributed sizes can be synthesized by delicately selecting the experimental conditions, while on the other hand, surface functionalization of the magnetic nanoparticles with various biomolecules, which is highly essential for the biomedicine application, has been developed and demonstrated by the rational design of both the composition of the magnetic nanoparticles and the molecular structure of the biomolecules. These functionalized magnetic nanoparticles with high selectivity, high sensitivity and unique magnetic properties exhibit the superiority not only over the traditional methods or agents used in the bioseparation, biosensing and magnetic resonance imaging by increasing the efficiency and accuracy, but also in the drug delivery and hyperthermia by increasing the specificity and reducing the damage of the health human tissues during the *in-vivo* treatment.

Although, there are many promising and exciting accomplishment of the magnetic nanoparticles in biological applications, as described in this review, researches on these magnetic nanoparticles for biomedicine application is just on the beginning of the road with vast challenges and issues remaining to be resolved. For instance, the clinical trials are very restricted since toxicity and biocompatibility are of major concerns for biomedical use. Although so many magnetic nanoparticles documented in the references have shown the potential to be applied in biomedicine systems, *in-vitro* and *in-vivo* studies are still very rare at the moment. Another challenge is that, though the functional molecules attached on the surface of the magnetic nanoparticles, such as antibodies, aptamers, DNA or RNA, offer the required high sensitivity, these biomolecules are easily denatured by the surrounding environment. New strategy is thus necessary to increase their environmental stability. Fortunately, Young-Wook Choi et al., showed good long-term stability of particles and a narrow particle distribution using poly-electrolytes with a negatively charged functional group [81]. In addition, developing and designing multifunctional magnetic nanoparticles which, for example, is capable to integrate optical imaging with magnetic resonance imaging or to deliver the drug while performing the hyperthermia treatment, is of great interest in the current research activity and is considered as the one of future directions of designing smart materials. In these concerns, the development of multifunctional magnetic nanoparticles with the distinguished features of broad versatility, biocompatibility and unique multifunctional properties will play a key role in the future biomedicine areas.

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