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Review Article

Current investigations into magnetic nanoparticles for biomedical applications

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Abstract: It is generally recognized that nanoparticles possess unique physicochemical properties that are largely different from those of conventional materials, specifically the electromagnetic properties of magnetic nanoparticles (MNPs). These properties have attracted many researchers to launch investigations into their potential biomedical applications, which have been reviewed in this article. First, common types of MNPs were briefly introduced. Then, the biomedical applications of MNPs were reviewed in seven parts: magnetic resonance imaging (MRI), cancer therapy, the delivery of drugs and genes, bone and dental repair, tissue engineering, biosensors, and in other aspects, which indicated that MNPs possess great potentials for many kinds of biomedical applications due to their unique properties. Although lots of achievements have been obtained, there is still a lot of work to do. New synthesis techniques and methods are still needed to develop the MNPs with satisfactory biocompatibility. More effective methods need to be exploited to prepare MNPs-based composites with fine microstructures and high biomedical performances. Other promising research points include the development of more appropriate techniques of experiments both *in vitro* and *in vivo* to detect and analyze the biocompatibility and cytotoxicity of MNPs and understand the possible influencing mechanism of the two properties. More comprehensive investigations into the diagnostic and therapeutic applications of composites containing MNPs with "core-shell" structure and deeper understanding and further study into the properties of MNPs to reveal their new biomedical applications, are also described in the conclusion and perspectives part. © 2016 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 104A: 1285–1296, 2016.

Key Words: magnetic nanoparticle, magnetic resonance imaging, drug delivery, tissue engineering, biosensor

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INTRODUCTION

Magnetic nanoparticles (MNPs) have been widely researched for biomedical applications recently.[1] In the early 20th century, the magnetism of particles has been paid close attention since the magnetic domain theory were developed. The theory shows that the size of magntic particles significantly influence their behaviors in magnetic field. When their size is smaller than the exchange interaction critical size (typically 10–15 nm), they will exhibit superparamagnetic property at a certain temperature range. Due to the superparamagnetic property, the low-temperature magnetic properties of Ni nanoparticles were studied since the 1950s. Then, they were

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successfully prepared into magnetic fluid in the late 1960s. Since the 1980s, MNPs have captured close attention of researchers, and have been extensively studied for biomedical applications.[2–5]

Generally, MNPs can be divided into metal oxides, pure metals and magnetic nanocomposite. The most commonly used MNPs in biomedical field are Fe, Co, Ti, Ni metal alloys and iron oxide, ferrite ($BaFe_{12}O_{19}$, $CoFe_2O_4$). Among them, iron oxide nanoparticles (usually Fe_3O_4 or Fe_2O_3) are the most extensively studied because of their lower toxicity profile.[3,6–10]

As one kind of nanoparticles, MNPs possess large surface to volume ratio to adsorb proteins or load drugs. Moreover, they have special superparamagnetic property, which can respond to an external magnetic field.[11,12] With the help of magnets, MNPs can be easily separated from components of the mixture. Thus, they can be applied for separation techniques (e.g. cell separation).[13–15] Due to the superparamagnetic property, MNPs can be accumulated accurately in a desired site by exerting an external field, which is thereby very suitable for the target delivery of genes and drugs.[16–18] Also, MNPs can be applied in heat-triggered drug release or in hyperthermia therapy by being heated in high-frequency magnetic field.[19] Specially, MNPs do not exhibit any magnetization after removing the external magnetic field. Thus, their aggregation *in vivo* can be avoided.[20] This clear advantage has made MNPs more and more attractive for *in vivo* applications, such as magnetic resonance imaging (MRI),[21,22] drugs and genes delivery, cancer treatment, hard tissue repair,[23,24] tissue engineering, biosensors[25] and other aspects,[26–30] which are shown in Figure 1.

The review article firstly describes different types of MNPs, especially iron oxide nanoparticles and magnetic nanocomposites. Then, the biomedical applications of MNPs were reviewed in seven applications: magnetic resonance imaging (MRI), cancer therapy, the delivery of drugs and genes, bone and dental repair, tissue engineering, biosensors, and other aspects.

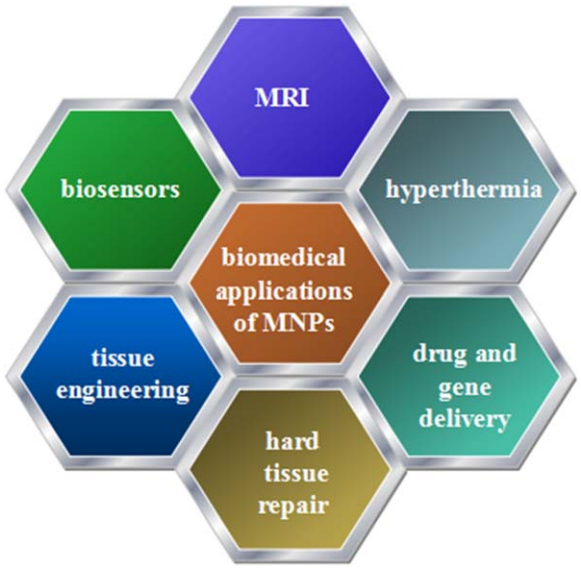

FIGURE 1. MNPs used in biomedical applications.

MNPS

MNPs have good magnetic orientation, the small size effect, biodegradability and reactive functional groups. However, there are negative reports that points that several nanoparticles applied in clinical applications induced cardiac toxicity, especially for the patients with cardiac ailments. Thus the overall pathophysiological state of the patient must also be considered before using of nanoparticles.[31] Nanocarriers have been demonstrated that they could deliver drugs or therapeutics from the implant surface directly to the lesioned sections, circumventing the risks of systemic toxicities. In addition, the biocompatibility of MNPs can be improved by combining them with a variety of functional molecules such as enzymes, antibodies, cells, DNA or RNA. It has been shown that coating other materials such as polyethylene glycol, chitosan, lipid, protein on the MNPs enhances their stability and expands applications via integrating more functionalities.[32] Coating materials with good biocompatibility can stabilize MNPs in physiologic fluids and provide chemical functionality for additional modifications. In addition, varying the sizes of nanoparticles, adjusting the concentrations of soft and hard magnetic phases in the materials with MNPs can improve their suitability for boimedical applications.[33] For example, superparamagnetic iron oxide nanoparticles with gold coating on their surface have good biocompatibility. The presence of gold helps to immobilize affinity ligands on the surface of the superparamagnetic iron oxide nanoparticles.[34,35] Prasad et al. used three different fatty acid chlorides (hexanoyl, octanoyl, and myristoyl chloride) to preform chemical modification of natural chitosan and subsequently used them to stabilize iron oxide nanoparticles.[36] Among the nanoparticles, they selected the hexanoyl chloride modified chitosan stabilized iron oxide nanoparticles as a novel three-dimensional nano-matrix for the controlled fabrication of hydroxyapatite due to its well dispersibility and stability in aqueous medium.

Nano iron oxide

Based on oxides and pure metals, iron oxides have better biocompatibility. Thus, they are investigated most extensively in biomedical applications in contrast to other magnetic materials. The degree of atomic order or crystallinity in the iron oxide lattice, as well as the dispersity in terms of size and shape of the nanoparticles, also affect their performance in some biomedical application.[23] Among iron oxides, maghemite (α-Fe_2O_3) and magnetite (Fe_4O_3) can well fulfill several essential demands of biomedical applications, including chemical stability in physiological conditions, sufficiently high magnetic moments, and low toxicity.

Recently, nano iron oxide attract a great deal of attention from researchers. For example, Figuerola reviewed the applications of iron oxide nanoparticles in biomedicine. The superparamagnetic iron oxide nanoparticles have been used for the detection of lesions and tumors in the liver. In

addition, they were also used for *in vivo* tracking iron oxide doped stem cells and *in vivo* monitoring transplanted tissues.[37] In addition, size-controlled synthesis of superparamagnetic iron oxide nanoparticles with proper surface architecture and combined MNPs with targeting ligands/proteins have attracted a great deal of attention for biomedical applications.[38] Ultra-small superparamagnetic iron oxide nanoparticles with a small scale <50 nm are suitable for intravenous administration.[39] PEGylation coating of MNPs by polyethylene glycol (PEG) is one of the most favored ways to enhance biocompatibility. Illés et al. have studied the effect of the synthesis conditions on the colloidal stability, biomedical applicability and biocompatibility of the PEGylated nanomagnets.[40] They have found that the adsorption of the first oleic acid layer proceeded via surface complexation to synthesize MNP, while it could also be accomplished by H-bonding when purified MNPs react with oleate. Their study was beneficial to control the synthesis of PEGylated nanomagnets. Similarly, Anbarasu et al. reformed the preparation of PEG coated Fe_4O_3 nanoparticles by a novel chemical co-precipitation method.[41] They used PEG as a stabilizer and dispersant. The PEGylated Fe_4O_3 nanoparticles exhibited superparamagnetic behavior and high saturation magnetization, which made them be promising for MRI and biosensors applications.

MNPs-based composites

MNPs-based composites have better biocompatibility and more applications than pure MNPs. They have been hotly studied for biomedical applications such as specific cell targeting, bioimaging, drug storage and release.[42,43] For example, MNPs coated with chitosan and linoleic acid can be used for gene delivery to hepatocytes.[44] And, Badruddoza et al. encapsulated Fe_3O_4 in a shell of SiO_2 to improve biocompatibility of their nanocomposite.[45] Their magnetic nanocomposite was demonstrated to be highly dispersible in an aqueous medium and benign toward healthy cells. And the nanocomposite could act as optically trackable, cancer-cell targeting and delivery vehicle for anticancer drugs. In addition, Chen et al. used tissue plasminogen activator (tPA) covalently bound to chitosan-MNP and obtained the chitosan-MNP-tPA.[46] Their chitosan-MNP-tPA was biocompatible and may be used for magnetic targeted delivery of the thrombolytic drug in clinical thrombolytic therapy. In addition, Nanocomposites containing iron and zinc have great potential in biomedical applications, such as $ZnO/ZnFe_2O_4$ nanocomposite. Due to their small size, nontoxic metal constitutes and high surface area, these metal based magnetic resonance imaging (MRI) contrasts targeting magnetic drug delivery vehicles are superior than the more widely used gadolinium (toxic metal) based chelate MRI contrast. Furthermore, different magnetic polymer beads also have been studied widely by researchers. Put MNPs into beads can help beads be sensitive to magnetic field. Due to different magnetic properties and sizes, MNPs encapsulated in polymer would be used for different applications. Small MNPs were suitable for drug delivery, because they did not exist remanence when removed magnetic field. For example, $MgFe_2O_4$ and $MgCr_{0.9}Fe_{11}O_4$ nano-ferrites were synthesized using a citrate-gel technique with a size of 23 nm and 7.6 nm respectively. And they have been applied in targeted drug delivery.[47] MNPs with diameters >200 nm can be applied in the exploratory researches of the gastrointestinal tract.[48]

BIOMEDICAL APPLICATIONS OF MNPS

Increasing attention has been attracted in utilizing MNPs for biomedical applications over the past two decades. Since MNPs were used as contrast agents in MRIs successfully, this excitement has continued to the present. To be applied in biomedical field, MNPs must consider a wide range of chemical, physical, physiological, and biological factors and conditions.[49] However, by adjusting the nature of the core, shell and ligands, these factors can be made use of to provide the expected biocompatibility and biofunctionality for better biomedical applications. Next, we will introduce various aspects of biomedical applications utilizing MNPs.

Magnetic resonance imaging (MRI)

MRIs are one of the most powerful techniques not only for diagnostic radiology but also for therapeutic medicine. Today, the rapid development of different kinds of imaging techniques such as MRI, positron emission tomography, ultrasound and optical imaging do great help for the early detection of diseases, understanding basic molecular aspects of living organisms and the evaluation of medical treatment.[29] Due to the following advantages: the extreme imaging flexibility, high patient acceptance, capability to evaluate anatomic and physiologic parameters, as well as the acquisition of unique clinical information, MRI is regarded to be one of the most powerful diagnostic tools in medical domain.[50,51]

From a historical perspective of view, the clinical MRI studies first appeared in 1979. The first commercial MRI scanner was produced in 1980. As early as 1985 magnets with a field strength of 1.5 T were available even for commercial clinical MRI systems. In the decade between 1985 and 1995, numerous researches were done to obtain faster gradient systems.[52,53] Currently, several iron oxide-based nanoparticles formulations had been approved by Food and Drug Administration for biomedical applications, such as Feridex, Resovist, and Endorem.[54] About the recent developments, Andreas et al. investigated novel experimental anionic citrate coated with superparamagnetic iron oxide nanoparticles and commercial Endorem and Resovist nanoparticles.[55] They confirmed that low citrate superparamagnetic iron oxide nanoparticles concentrations are good for sufficient contrast in the MRI and important to cell viability and function in cell-based therapies. And, Shokrollahi et al. presented a short review. They reviewed the physical principles and some advances in MRI, as well as providing a summary of the synthesis methods and properties of contrast agents (CAs), like different core materials and surfactants.[56]

MNPs have been widely used in MRI applications. For example, iron oxide nanoparticles can be used for cancer imaging, *in vivo* tracking of iron oxide doped stem cells and

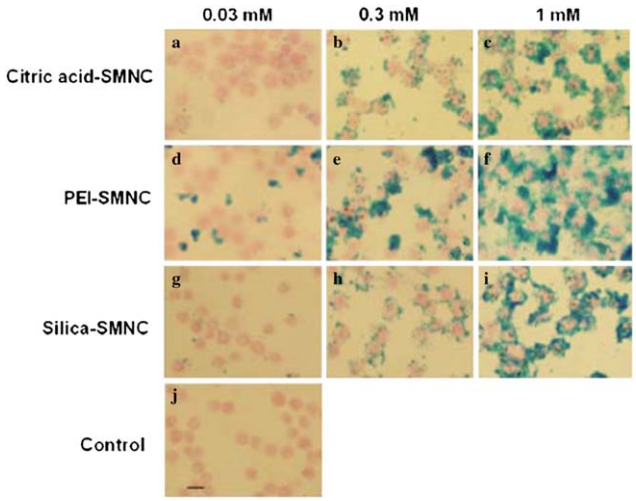

FIGURE 2. Prussian blue staining of cells incubated with citric acid SMNC (a–c), PEI SMNC (d–f) and silica SMNC (g, h) at the different iron concentrations for 1 h. (j) Control cells. Bar: 10 μm (Adapted from Ref. 58, with permission from Elsevier Ltd).

in vivo monitoring of transplanted tissues. In addition, superparamagnetic magnetite nanocrystal clusters (SMNC) can served as a tool for cell imaging. Researchers used a mini emulsion/sol-gel method and a polyol technique to synthesize SMNC, with hybrids polyetherimide and citric acid and silica coated.[57] These SMNC possessed a very high magnetic resonance sensitivity, and had no adverse effect on cell viability, as well as showing a dose and time-dependent feature (Fig. 2).[58] Similarly, superparamagnetic iron oxide MNPs (SPIO) have been extensively used as tracers in treatment of cardiovascular system complications and central nervous in animal models. Zhao et al. investigated survival and proliferation of SPIO-labeled bone marrow mesenchymal stem cells. They concluded that transplanting cells via liver or spleen injection could effectively accelerate liver healing. *In vivo* MRI of SPIO-labeled cells are propitious to trace real-time liver healing during clinical treatment after hepatectomy.[59]

Most CAs used in MRI are made of gadolinium chelates.[60,61] However, increasing attention has been attracted in utilizing MNPs as CAs because MNPs can easily integrate multiple contrast functions and finely tune their structure. For example, the modified Mn-ferrite act as CAs due to their higher saturation magnetization and r_2-relaxivity.[62] Similarly, it has been experimentally proved that the suspensions of multifunctional Au-Fe_3O_4 hybrid nanoparticles can be good candidates for negative CAs because of the special magnetic, optical, and other properties of them.[63] Besides, taking advantage of metal nanoparticle impurities attached to carbon nanotubes can serve as a T_2 CA in MRI.[64] Choi et al. attached iron oxide nanoparticles at the ends of single walled carbon nanotubes to act as T_2 CAs in MRI.[65] Advanced MRI CAs consisting of MNPs and appropriate targeting agents can enable the ultrasensitive detection of various types of cancer in MRI. Wu et al. synthesized multiwalled carbon nanotube (MWNTs)/cobalt ferrite by a solvothermal method. The obtained nanocomposite significantly enhanced the negative contrast in T_2 weighted MRI of cancer cells.[66] In addition, the fabrication processes of CAs have been studied to obtain better CAs. Novel micro electro mechanical system and nanoelectro mechanical system based fabrication processes for biocompatible, hollow cylindrical ferromagnetic structures were found as potential use as CAs for MRI.[67]

Lately, research has been tempted to develop the parallel imaging to increase the speed and to decrease the imaging time, which leads to lower patient throughput and further movement artifacts.

In cancer therapy

In the past decades, MNPs have been applied for the treatment of cancer. The development of nanotechnology improves the ability to combine the unique features and properties of MNPs for cancer.

MNPs have been studied for the detection, diagnosis, and treatment of malignant tumors. Different sizes of MNPs exhibit different characteristics. Ferromagnetism with large size can be used to alternate magnetic hysteresis under heat, and used in cancer targeted therapy.[68] MNPs with small size are suitable to hyperthermia. To improve the applicability and efficacy of those MNPs, some studies incorporate highly specific targeting agents and some functional ligands, such as fluorophores and permeation enhancers.[26] For example, Chen et al. had used graphite coated on highly magnetic FeCo to make one MNP microarray chips by chemical vapor deposition method. The chips could help to easily capture and efficiently detect cancer cells from 1 mL of whole blood.[69] And, MNPs and polymer can synthesize magnet responsive polymeric beads, which possess both features of them. Thus, Shi et al. synthesized polyethyleneoxide modified polystyrene-Fe_3O_4 nanospheres by miniemulsion/emulsion polymerization. The Fe_3O_4 nanoparticles could respond to an external magnetic field by increasing the temperature of the surrounding environment that made the prepared composite nanospheres potential for local therapy of cancer.[70]

Following surgery, radiotherapy, chemotherapy and immunotherapy, hyperthermia treatment has become the fifth largest method in cancer therapy and applied in clinical practice. Magnetic hyperthermia put heat material on the tumor site by direct injection or intravenous injection. By heating the tumor tissue to 41 to 46°C temperature, the injection can cause cancer cells apoptosis. Take advantage of the magnetic properties, the MNPs have been utilized as heat generators in hyperthermia. Owing to relaxation processes and hysteresis losses, they would transform magnetic energy to heat.[71] This phenomenon is the primary and import principle for cancer therapy by hyperthermia.[72,73] Hyperthermia based on MNPs can through controlled heating of the damaged tissue to make the tumor cells selectively apoptosis.[74] The ability to control the generated heat by MNPs is one of the most important parameters in hyperthermia based therapy. Many related studies have been done to get the progress of hyperthermia. Rahimi et al. have

studied the effect of polyvinyl alcohol surface passivation of $Ni_{0.3}Zn_{0.7}Fe_2O_4$ ferrite nanoparticles on the loss power density and sensitivity to magnetic field frequency.[75] Their results indicated that the magnetic interactions and particle size could affect the sensitivity of nanoparticles to magnetic field frequency. This result is important for controlling the generated heat by MNPs in hyperthermia. In addition, magnetic hyperthermia by direct injection, intravenous guide, or intervention methods such as delivery to the tumor site, tend to be inaccurate and is likely to cause damage to normal tissue around the tumor. MNPs exhibit superparamagnetic behavior. Once the magnetic field is removed they do not retain any magnetization. Hence they are suitable for magnetic hyperthermia treatment. By measuring the hyperthermia heating behavior in a high frequency alternating magnetic field, Sadat et al. investigated the effect of nanoparticle confinement on the magnetic relaxation of Fe_3O_4 nanoparticles.[76] They found that specific absorption rate of the unconfined MNPs systems were higher than those of confined, at the same time, they proposed one physical model to explain the effect of dipole interactions on the hyperthermia heating behavior of the Fe_3O_4 nanoparticles. And Tomitaka et al. investigated the characteristics of the surface properties of Fe_3O_4 nanoparticles.[77] They studied the composite with surface-coated Fe_3O_4 nanoparticles, and reported the effect of the magnetic properties and heat dissipation. All the studies help to control the generated heat by MNPs in hyperthermia. What is more, Lyubutin et al. prepared one hollow microcapsules with the core-shell structure by *in situ* synthesis.[78] The MNPs were synthesized from the iron salt and then directly coated on the capsule shells using the "layer by layer" technique. Lyubutin et al. combined biopolymer polylysine and dextran sulfate, then, they heated the reagents to a suitable temperature for biomaterials. The structural and magnetic properties of the nanocomposite capsules was demonstrated suitable for hyperthermia.

In the delivery of drugs

In magnetic drug delivery, MNPs used for treatment are generally injected into the blood stream, then magnets are used to concentrate them to disease locations. An advantage of MNPs is that they can delivery drugs into specific cells by exerting an external field. Many factors can influence the behaviors of MNPs *in vivo,* such as blood convection, diffusion (in blood and in tissue), extravasation, and the applied magnetic fields.[79] Nacev et al. comprehensively studied the behavior of MNPs in blood vessels and surrounding tissue. They found that there were three types of behaviors allow researchers to easily determine the drug behaviors in the experiments: velocity dominated, magnetic dominated, and boundary-layer formation.

Numerous kinds of MNPs have been used for drug delivery. For example, the spinel $Co_{0.5}Ni_{0.5}Fe_2O_4$ system demonstrated its potential for drug delivery by a variety of techniques.[80] And, the nano nickel ferrite also may be applied in drug delivery. Dumitrescu et al. synthesized nano nickel ferrite by sol-gel method and embedded them within the polyacrylamide-based hydrogel matrix by an original polymerization crosslinking simultaneous method.[81] The samples had high purity and good antimicrobial activity. And they could act as a novel magnetic drug carrier by combining with drugs such as antibiotics. Similarly, a novel magnetic-upconversion luminescent bifunctional coaxial nanoribbons may be used as drug target delivery materials due to its special magnetic and upconversion luminescent properties. Besides, based on magnetic cylindrical ordered mesoporous silicon dioxide nanocarrier has been synthesized for targeted drug delivery.[82] The nanocarrier can efficiently enter the HeLa cells, which may be helpful for developing novel controlled-release nanocarriers. In addition, the stimuli-responsive particles are controllable and self-assembling. In different physical conditions, stimuli-responsive particles can change their morphologies or aggregation properties to control drug release behaviors. Some advances for different types of stimuli-responsive particles used for drug delivery have been reviewed by Yang and Liu, including temperature responsive polymers, pH responsive polymers and temperature/pH-dual-responsive polymers.[58] Moreover, the superparamagnetic iron oxide nanoparticles (SPIONs) have better biocompatibility and proper surface architecture. Combining SPIONs with targeted ligands/proteins has attracted a great deal of attention for drug delivery applications.[83]

In hard tissue repair

In the past decade, employing nanotechnology to hard tissue repair has attracted a great deal of attention of researchers, especially applying nanotechnology to bone repair and orthopedic and dental implants.[84–87] In the market, there have been some micro/nanofabricated implantable products, such as micro/nano bio chip, nano-artificial bone. In the laboratory, some experimental products such as coating materials, bone repair materials have been also studied. Therefore, there must be new developments in the future.

A bone injury creates a gap, which causes the accumulation of necrotic bone debris. Blood from broken blood vessels accompanies with inflammatory cells and releases a set of signals ultimately.[88] A bone injury triggers a series of both inflammatory and bone building signals, then the signals mitigate the migration of phagocytic cells. Next, in order to provide nutrients to the injured cells, organism will remove the necrotic tissue and propagate vascularization. This ultimately initiates the healing cascade. Most therapies try to produce a new bone, strengthen synergistically acting processes of osteoinduction and osteoconduction.[89] Besides the traditional studies, some nanotechnology used MNPs for bone repair have been studied recently.

MNPs can be applied as bone repair materials. For example, Kharaziha and Fathi synthesized the forsterite nanopowder by the sol-gel process, and introduced the preparation, characterization, and bioactivity evaluation of them.[90] The forsterite nanopowder presented good *in vitro* bioactivity and biocompatibility, which were potential to be used as bioactive bone repair materials. To further study

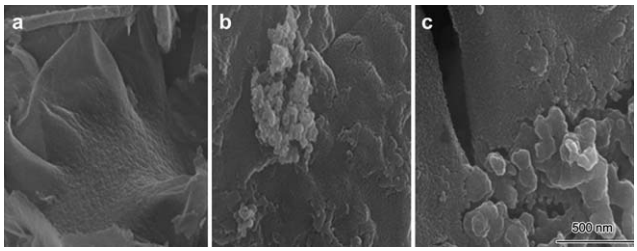

FIGURE 3. SEM images of the forsterite powder fired at: (a) 800°C, (b) 900°C, (c) 1000°C (Adapted from Ref. 91, with permission from Elsevier Ltd).

the structural characterization and biocompatibility of forsterite nanopowder, Naghiu et al. did a series of detailed experiments.[91] The SEM images on the forsterite powder are presented in Figure 3.

The sample fired at 800°C [Fig. 3(a)] did not reveal the presence of forsterite crystals. However, at 900°C [Fig. 3(b)] small crystals were visible. The forsterite powder fired at 1000°C [Fig. 3(c)] was well- crystallized. They immersed forsterite for 7 days and studied the formation of hydroxyapatite on its surface. By numerous experiments, they confirmed that forsterite powders dissolution had no significant cytotoxicity effects and promoted the human- type osteoblasts proliferation.

Bone scaffolds combine with magnetic materials are better choices for bone healing, especially when the therapy process accompanies the external magnetic stimulation. MNPs inside bone scaffolds can promote bone healing. For example, Heidari et al. applied a novel method to synthesize iron oxide nanoparticles inside hydroxyapatite/chitosan bone scaffolds.[92] The prepared MNPs were superparamagnetic, which would help to control the magnetic field. In addition, chitosan/nanohydroxyapatite/Cu-Zn alloy nanoparticles composite scaffolds exhibit better antibacterial activity and show great potential for bone tissue engineering.[93] And, nano-silica in composite scaffolds is also suitable for bone tissue engineering.[94] In addition, the titanium surface characteristics, from sub-nano to sub-micron scales, have been investigated along with the concomitant effects on mesenchymal stem cell response of osteoblast differentiation and osteoblastic phenotype cells. From the study, nano-submicron features had more superiorities in accelerating subsequent osteoblast differentiation,[95] and it developed a great preparation for the nanoscale surface studies of bone implants.[96]

MNPs used in orthopedic and dental implants also attracted much attention of researchers, especially nanocomposite containing nanoTiO_2 and nano titanium.[97] For instance, Sasani et al. developed one simple method to prepare fluorapatite-titania-carbon nanotube decorated with antibacterial agent. Their new dental implant coating with fluorapatite, TiO_2 and copper decorated multi walled carbon nanotubes showed suitable morphological features.[98] Moreover, some other MNPs also have been studied for orthopedic and dental implant applications. Rau et al. used pulsed laser deposition technique to synthesize iron-substituted hydroxyapatite (Fe-HAp).[99] The obtained Fe-HAp crystalline films with 35 nm mean crystallite size and could be used as coating material for orthopedic and dental implant applications.

To summarize, it is promising for using MNPs in hard tissue repair, such as various studies for bone graft solutions, bone regeneration, and dental applications.[100] In the near future, more and more micro/nanoscale bone and dental implants will quickly from laboratory to the market.

In tissue engineering

Tissue engineering is a method of trying to successfully achieve tissue regeneration without damage. This approach generally includes three parts: the *in vitro* culture of autologous cells, combining the cells with three-dimensional (3D) biodegradable scaffolds, and promoting the reformation of their original structure. Cells normally stay on the surface of these structures and do not enter the scaffold, thus, they cannot lead to tissue necrosis. It is worthy to pay attention to find a effective method to seed the cultured cells to the center of the 3D scaffold. Biopolymeric scaffolds associate with MNPs can achieve the requirement.[101] In particular, scaffolds have been applied in combination with multifarious bioagents and become fundamental tools in bone graft substitution.

In recent years, attention on using MNPs to improve tissue engineering has increased significantly.[102–105] Standard commercial scaffolds is made of hydroxyapatite and collagen in magnetic scaffolds. Bock et al. successfully transformed standard commercial scaffolds by a low-cost and simple-preparation technique.[106] They dip-coat the scaffolds in aqueous ferrofluids with various biopolymers and iron oxide nanoparticles. And, the obtained scaffold is a novel magnetic guiding tool for tissue engineering applications. They have through PI/AO tests to do parallel assessment of cell viability (Fig. 4). The cell growth dynamics (according to MTT and PI/AO assays) showed a significant increase in the number of viable cells from 5 to 15 days for all types of scaffolds (from MAG_0 to MAG_3). From the picture, we can ensure that the magnetic scaffolds with non-significant cytotoxic potential (MAG_1, MAG_2, MAG_3). In addition, Mattix et al. prepared biological magnetic cellular spheroids by using magneto ferritin nanoparticles.[107] The magneto ferritin nanoparticles were able to serve as a biological alternative to the magnetic cellular spheroids, which made them using as tissue engineered building blocks. The magneto ferritin nanoparticles exhibited no cacoethic effects on cell viability, and their cell concentration were much higher than using with other MNPs in tissue applications. Almost at the same time, Mattix et al. reported a Janus structure of magnetic cellular spheroids (JMCSs). The JMCS accompanied with spatial control of MNPs in order to form two distinct domains: cells and extracellular MNPs.[108] These structures were divided into two segregated domains, one composed of cells and another of extracellular MNPs to form a Janus magnetic cellular spheroid (JMCS, Fig. 5). Safely incorporated MNPs into cellular spheroids, JMCSs were demonstrated that they were able to magnetic manipulate. JMSCs also were capable of using magnetic forces during magnetic force assembly mediate integration into controlled patterns

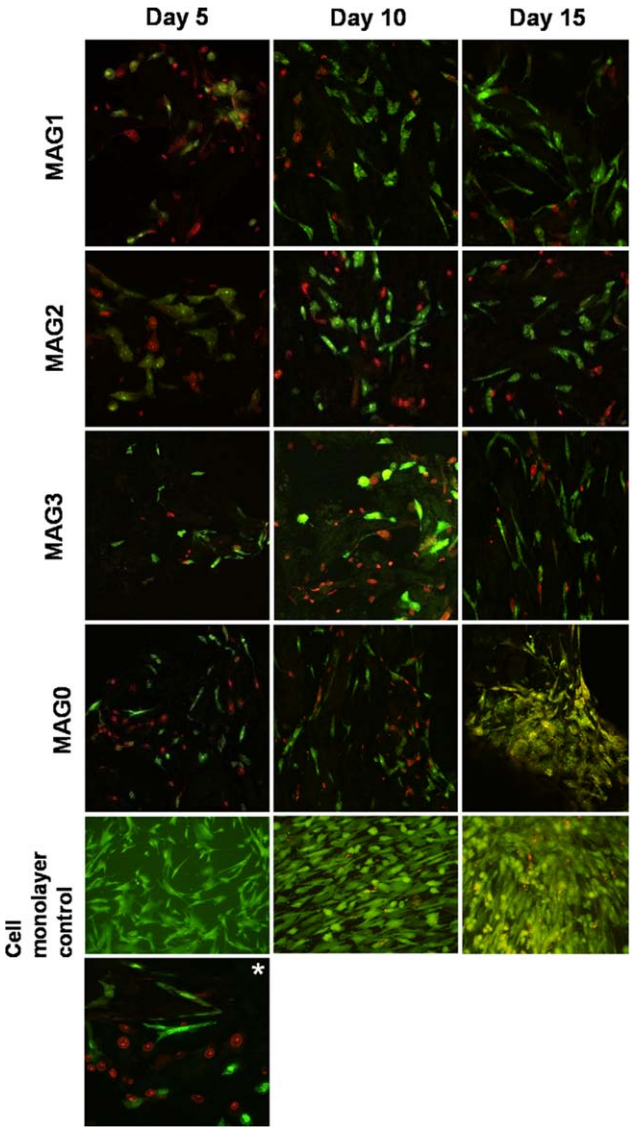

FIGURE 4. Morphology of cells stained with PI/AO after cocultivation with all types of HA/COLL composite scaffolds (MAG0 to MAG3) after 5 days (first column), 10 days (second column) and 15 days (third column) in osteogenic medium (magnification 100×). The cells with red nuclei are scored as unviable cells. The cells with green cytoplasm and nuclei are scored as viable cells. Unviable cells found in all types of scaffolds had a typical necrosis morphology (uncondensed nuclei) as shown for MAG3 at day 5(*) (magnification 200×) (Adapted from Ref. 106, with permission from Elsevier Ltd).

and complex tissues. Moreover, Prabu Periyathambi et al. used goat blood as the starting material for the preparation of novel magnetic fibrin nanoparticles in tissue engineering.[109] They performed Saos-2 cells viability assay and quantified alkaline phosphatase levels to evaluate the magnetic fibrin nanoparticles. The MNPs showed good biocompatibility and could serve as promising biomaterial in bone tissue engineering. In addition, the adipose-derived regenerative cell sheets were produced by using magnetite Fe_3O_4 nanoparticles for the treatment of myocardial infarction. Ishii et al. have investigated their therapeutic potential.[110] Magnetite Fe_3O_4 nanoparticles were combined with liposomes to create magnetic cationic liposomes with a positive surface charge, which was applied subsequently for culturing adipose-derived regenerative cells. The special cell sheets are promising to be a novel and worthy regenerative strategy for ischemic heart disease.

In biosensors

A biosensor is essentially considered as a device capable of transforming a biological event into an easily detected cells. MNPs are suitable for serving as platforms for biosensors because the surface of MNPs can be easily modified with numerous receptors, and MNPs can serve as ferromagnetic labels.[111] They can also disperse in the sample and be used onto the active detection surface of the biosensor. MNPs have been extensively used in sensing applications, such as directly applied in tagging supports to the sensor, associated with the transducer materials.[112]

Different biosensors can mainly detect different biological substance. Chen et al. used MNPs and a two-marker recognition system to develop a sensitive biosensor based on surface plasmon resonance spectroscopy.[113] The cytosensor has great potential on exploring new applications for the detection of multitudinous separation products based on MNPs. In addition, the glucose sensor based on chemical oxidative polymerization of pyrrole on $ZnFe_2O_4$ nanoparticles surface showed good activity. They are sensitive to detect glucose.[114] Similarly, Xiong et al. synthesized a novel electrochemiluminescence glucose biosensor based on Fe_3O_4 nanoparticles.[115] The prepared biosensor has been used to determine the glucose in plasma samples. And, Nouira et al. developed one glucose conductometric biosensor using gold and MNPs. The two kinds of nanoparticles were modified by glucose oxidase coating on poly-allylamine hydrochloride, which finally deposited on a planar interdigitated electrode.[116] Gold and MNPs also have been applied to develop a multilayered polymeric DNA sensor by the radio frequency technology.[117] Moreover, Chauhan et al. described a method of preparing an amperometric biosensor to determined glutathione.[118] They immobilized a glutathione oxidase onto the surface of gold coated MNPs to modify Pt electrode, then, they used chitosan introduce amino groups

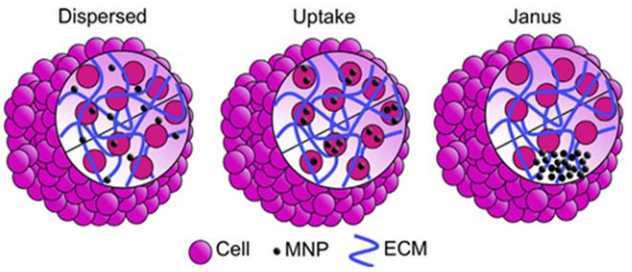

FIGURE 5. Structure of JMCSs. (a) Janus magnetic spheroid structures avoid cytotoxic affects associated with MNP internalization, common to disperse and uptake methods, by separating MNPs and cells into segregated and distinct domains. The combination of MNPs (black), cells (pink), and ECM (collagen, blue) into segregated domains decreases cellular toxicity related to MNPs by decreasing interactions with cells (Adapted from Ref. 108, with permission from Elsevier Ltd).

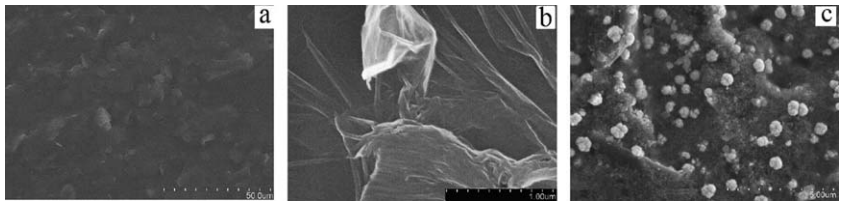

FIGURE 6. SEM images of SPCE (a), SPCE|GS-Nafion (b), and SPCE|GS-Nafion/Fe3O4-Au-HRP (c) electrode (Adapted from Ref. 119, with permission from Elsevier Ltd).

onto the surface of MNPs. The obtained amperometric biosensor showed good analytic performance and could be used for the development of highly sensitive glutathione biosensor. Besides, a disposable biosensor based on Fe_3O_4-Au MNPs for the determination of hydrogen peroxide was presented.[119] The MNPs coated horseradish peroxidase (HRP) and graphene sheets (GS)-Nafion film to modify screen-printed carbon electrode (SPCE), Figure 6 shows the characterization of the surface of different electrodes.

Figure 6(a) shows the flake graphite on the surface of SPCE. The magnified scanning electron microscope (SEM) image shows that there are a few thin wrinkles in the surface of SPCE|GS-Nafion electrode [Fig. 6(b)]. The diameter of Fe_3O_4-Au-HRP biocomposites are about 200 to 500 nm in average, and they form randomly on the GS [Fig. 6(c)]. Obviously, the Fe_3O_4-Au-HRP biocomposites could homogenously distribute onto the GS-Nafion matrix. Compared with other H_2O_2 biosensors, the biosensor were disposable design, low cost, renewable and long stability. Therefore, it may be suitable for drug residues analysis.

What is more, many other kinds of biosensors have been studied. A novel fluorescence biosensor based on aptamer was developed to detect chloramphenicol.[120] The fluorescence biosensor used aptamer-conjugated MNPs for recognition and concentration elements. And the biosensor used upconversion nanoparticles as highly sensitive signal labels. The prepared biosensor was convenient, sensitive, and stable, thus it showed a great advantage on nano label applications for bioassays. In addition, Wang et al. improved the performances of uric acid biosensor by taking advantage of the special properties of Fe_3O_4 nanoparticles, chitosan and polyaniline.[121] They served a simple and novel approach to determine serum uric acid concentration in gout patients. Besides, based on magnetic core/shell Fe_3O_4/SiO_2 and Fe_3O_4/Ag/SiO_2 nanoparticles, surface plasmon resonance biosensors were developed for using in immunoassay. Furthermore, Chandra et al. tried to reduce the interference from ascorbic acid during the detection of dopamine. They used various techniques to prepare and characterize a suitable electrode material based on amperometry and MNPs.[122] Their biosensor showed high sensitivity and selectivity, micromolar detection limit, high reproducibility and easily prepared. It was suitable for the determination of dopamine.

In other aspects

MNPs have developed widely in biomedical applications, in addition to the above aspects, many other aspects should be paid close attention. For example, the research and application of stem cell requires a series of existing cell labeling and targeting strategies. Pullulan coated on MNPs could be applied for the labeling of mesenchymal cells due to their low cytotoxicity and good biocompatibility.[123]

There are several ways for adequate bio-functionalization of MNPs to make biocomposites with appropriate characteristics for biomedical and clinical applications. Bio-functionalized MNPs can be used to enrich proteins and immobilize enzymes because their surface coated on chemical functional groups (hydrophilicity, hydrophobicity, and affinity).[124] Modified MNPs with antibodies are commonly applied in isolation of antigen-expressing cells through special immune reactions. Grafted MNPs combine with appropriate oligonucleotides via specific base pairing to be used for separation and purification of nucleic acids.[23] Based on MNPs modified with antibodies against surface antigens of cells, immunomagnetic separation for the separation of eukaryotic and prokaryotic cells has been exploited. Recently, the same approach appeared in the detection and isolation of bacteria from biological samples. Antigens immobilized on MNPs can isolate antibody-expressing or antigen-specific cells though immune response.[125]

For bioseparation processes, MNPs usually coated with various polymeric materials in order to increase colloidal stability, morphology and functionality. MNPs have great potential to be used in adsorptive separations because their small size and large surface area to unit volume ratio.[126] El-Boubbou et al. fabricated silica-coated magnetic lycol-nanoparticles that could detect *Escherichia coli* strains quickly. Concomitantly, the composites were able to 88% removal from the sample and exploit the bacterial interaction with mammalian cell surface carbohydrates.[127] Moreover, Mejias et al. studied the biocompatibility of dimercaptosuccinic acid and MNPs composites *in vitro*, as well as long-term biodistribution and clearance *in vivo*. Their results showed that the composite was promising for clinical application.[128]

Multimode nanoprobes can pick-up biological information at different object levels. They can also achieved the *in vivo* detection and *in vitro* validation of tumor angiogenesis. As one kind of multimode nanoprobes, fluorescent magnetic nanoprobes (FMNPs) were synthesized and existed. The FMNPs could show excellent magnetic resonance sensitivity and high illumination intensity with strong signal strength. The FMNPs were proved to be non-toxic and prominent multimode imaging probes after numerous imaging studies

of *in vitro/vivo* and *in vivo* using mice models.[129] Similarly, Ravichandran et al. reported a novel approach to synthesize $CoFe_2O_4$ nano-needles. They applied a co-precipitation method by using ferric chloride and cobalt nitrate as precursors at 80°C. The $CoFe_2O_4$ nano-needles may be used for cancer therapeutics.[130]

CONCLUSION AND PERSPECTIVES

MNPs are being widely employed in biological applications. In this review, we introduced the latest research progress on MNPs for biomedical applications, such as in MRI, cancer therapy, the delivery of drugs, hard tissue repair, tissue engineering, biosensors and other aspects, which indicated that MNPs possess great potential for many kinds of biomedical applications due to their unique properties.

Although many researches on MNPs for biomedical applications have been done and many achievements have been obtained, there is still a lot of work to do. Firstly, the new synthesis techniques and methods still need to be developed to prepare novel MNPs with satisfactory biocompatibility. On the other hand, the toxicity of MNPs cannot be neglected. When deliver MNPs to target tissue, the majority of MNPs often distribute in tissues of the reticuloendothelial system, particularly in the liver and spleen. Therefore, finding new methods to reduce the cytotoxicity and improve the biocompatibility of the current available MNPs is also important. Secondly, more effective methods need to be exploited to prepare MNPs-based composites with fine microstructures and high performances for biomedical applications. Thirdly, more appropriate techniques of experiments both *in vitro* and *in vivo* are necessary to detect and analyze the biocompatibility and cytotoxicity of MNPs and understand the possible influencing mechanism of the two properties. Fourthly, because MNPs-based composites with "core-shell" structure, stated in this review, have unique biological and physicochemical properties that make them have great potential for biomedical applications, further investigations into the diagnostic and therapeutic applications of them are necessary. Finally, deeper understanding and further study of the properties of MNPs are still necessary in order to reveal their new biomedical applications.

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