

Review Article

Synthesis and Application of Iron Oxide Nanoparticles in Bone Tissue Repair

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Nanoparticles play a vital role in bone tissue repair engineering, especially iron oxide nanoparticles (IONPs), which have magnetic properties, semiconductor properties, and nontoxicity at the same time, and their applications in biomedicine have received widespread attention. This review summarizes the excellent performance of IONPs in enhancing scaffold functions, promoting stem cell differentiation, and labeling positioning, in order to understand the research progress and future development trends of IONPs in bone tissue repair engineering, as well as the security issues. Firstly, IONPs can affect the expression of genes and proteins to accelerate the process of biomineralization under a magnetic field. Then, the composite of IONPs and polymers can synthesize a scaffold which can promote the attachment, proliferation, and bone differentiation of stem cells. Furthermore, IONPs can also mark the location of drugs in the body to follow up the process of bone repair. Therefore, extensive research on the manufacturing and application range of IONPs is of great significance to bone tissue repair engineering.

1. Introduction

As we all know, physical stimulation enhances the bone rebuilding capacity significantly, including stretching, compression, fluid shear stress, and heat [1, 2]. Additionally, the magnetic stimulation of static magnetic fields (SMFs) and electromagnetic fields (EMFs) also improve the bone rebuilding capacity greatly [3, 4]. So, the application of MNPs as an intermediate medium has received extensive attention in medical research, such as targeted drug delivery, magnetic resonance imaging (MRI), local tissue hyperthermia, tumor treatment, bioseparation, and biosensing [5], and compared to other materials, MNPs have lower production cost, more stable physical and chemical properties, and better biocompatibility [6].

Magnetic particles are slowly deposited on the surface of the cell membrane under the action of a magnetic field and are endocytosed by the cell. After the magnetic particles enter the cell, it is easier to affect the physiological function of the cell [7, 8]. If a magnetic field is applied, each magnetic particle will become a magnetic source, so that the magnetic scaffold material can play the role of bone tissue repair treatment. Once magnetic particles are exposed to an external magnetic field, they will be rapidly magnetized. Magnetic particles and magnetic fields work together to improve the effectiveness of bone tissue repair [9, 10]. MNPs can be synthesized through different techniques including coprecipitation [11], microemulsion [12], hydrothermal synthesis [13], sol-gel process [14], polyol synthesis [15], flow injection [16], sonolysis/sonochemical method [17], microwave

irradiation [18], electrochemical synthesis [19], solvothermal method [20], chemical vapor deposition [21], laser pyrolysis [22], green synthesis [23], and using biomass or biological templates.

Scaffolds used to reconstruct an injured bone must have sufficient mechanical strength to carry the load. Therefore, other types of scaffold materials, such as ceramics and biodegradable polymers, are usually not suitable for bone tissue engineering. Porous metals and alloy materials may be used as alternative scaffolds to promote new bone formation. However, other metal particles may release toxic substances and cause tissue pollution [24], and the issues can be overcome by using biodegradable metal materials such as irons and their alloys. In view of the advantages of IONPs, some researchers have incorporated Fe_3O_4 nanoparticles into tissue engineering biomaterials [25–27]. For example, Pan et al. [28] prepared Fe_3O_4 /polylactide composites with extrusion process, and they found that the composites containing IONPs had no cytotoxic effect on fibroblasts and enhanced osteogenesis in vitro experiments. Ge et al. [29] prepared a magnetic scaffold containing Fe_3O_4 /chitosan, which has high biocompatibility to C_2C_{12} cells. Similarly, De Santis et al. designed a magnetic scaffold for bone tissue combining PCL and Fe_3O_4 with different ratios. The results showed that the nanoparticles mechanically enhanced the PCL matrix; the elastic modulus and maximum stress increased by about 10% and 30%, respectively [30]. Thus, IONPs are expected to provide a strategy for bone repair. IONPs improve the three key factors of bone regeneration including stem cells, scaffolds, and growth factors via magnetic fields. Among the factors, magnetic cells' strategies contain cell labeling, targeting, and genetic modification. Magnetic scaffolds can enhance cell differentiation through magnetic-mechanical simulation [31]. And IONPs can also be used as delivery media for growth factors, drugs, and gene [32, 33].

2. Synthesis and Application of IONPs in Bone Tissue Engineering

2.1. Synthesis Method of IONPs. The preparation method has developed well due to its application value, and various element compositions have been used in magnetic nanomaterials, including Fe_3O_4 , Fe, Co, Ni, MgFe_2O_4 , and CoFe_2O_4 [34–37]. The most common component of magnetic nanomaterials is Fe_3O_4 , and the preparation methods of magnetic Fe_3O_4 mainly include dry and wet methods [38, 39]. Among them, the wet method is more commonly used and mainly includes the following technologies: hydrothermal method, solution thermal method, chemical coprecipitation method, ball milling method, sol-gel method, and atomic layer deposition method. The advantages and disadvantages of each preparation method are summarized (see Table 1).

2.2. Application of IONPs in the Function of Scaffold

2.2.1. Scaffold Material Type. Guiding and controlling the delivery of bioactive drugs to bone injuries has always been a hot research area. The design of the delivery platform plays a vital role in the treatment of bone diseases and the activa-

tion of bone regeneration, because they can provide a suitable environment for the cell adhesion and growth and, at the same time, provide a valuable platform for delivery strategies [64]. The nanostructured materials used in the platform have the characteristics of biocompatibility, nontoxicity, and noncarcinogenicity. Several common nanomaterials for the attachment platform of IONPs are exhibited (see Table 2). Nanomaterials can be divided into organic materials and inorganic materials. Organic materials are a combination of the few lightest elements, especially hydrogen, nitrogen, and oxygen, as well as carbon-containing compounds located in organisms. Organic materials include lipids, liposomes, dendrimers, and polymers, including chitosan, gelatin, and collagen [65]. And inorganic materials refer to materials lacking carbon, which are widely used in in vivo and in vitro biomedical research [66].

IONPs with superparamagnetic properties have a high specific surface energy and are prone to agglomeration. Polymers, which can improve the stability of nanoparticle dispersion in water, are often used as wrapping materials on the surface of particles formatting shell-core structure. In addition, polymer-encapsulated magnetic nanoparticles have the characteristics of strong surface modification and easy modification or functionalization, which greatly broaden the application fields of magnetic materials. Polymers frequently used to encapsulate magnetic nanoparticles including proteins, dendrimers, liposomes, chitosan, glucose, starch, polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyvinylpyrrolidone, and polyglyceryl acrylate (PGA). Among them, PEG is used for drug slow-release system because of its electrical neutrality, water solubility, low toxicity, and nonimmunogenic properties. Glycopolymers are often used in medical imaging studies to improve the diagnosis of diseased tissues such as tumors. PGA/PGMA is also a good class of polymers that has a stronger ability to bind to IONPs, greatly improving the stability of the particles. In addition, human-made biodegradable polymers are widely used as tissue engineering scaffold materials because they can be degraded to small molecules in vivo and excreted through the body metabolism. And they have good histocompatibility, mechanical properties, and controllable degradation rate.

There are many ways to synthesize IONP composite scaffolds, and the manufacturing process and the shortcomings shown are also different (see Table 3).

Among these methods, electrostatic spinning technology has become an important process for producing scaffolds due to its modulable properties and simplicity. The basic method of high-voltage electrostatic spinning technology is to apply a voltage of tens of thousands of volts between the jetting device and the receiving device, forming an electrostatic field. As the spinning fluid drips out of the jet, a jet is formed at the cone end of the droplet and is stretched in the electric field. The final result is the formation of long, irregular fibers on the receiving device, with fiber diameters typically in the tens to hundreds of the nanometer range. From the above description of the electrospinning principle, we find that the shape of the electrospun filament can be regulated by several parameters. These parameters include the viscosity and concentration of the spinning solution, the

TABLE 1: Synthesis method of IONPs.

Method	Advantage	Disadvantage	Reference
Organic based	High particle dispersion	Unruly IONPs' appearance	[40–42]
Hydrothermal/solvothermal	Simple operation High reaction efficiency Suitable for wider temperature range	Hard to replicate metastable phase and nanomorphology in other ways	[43–47]
Coprecipitation	Simple operation High-yield	Poor controllability of its particle size and distribution	[48–51]
Ball milling	Granularity reproducibility	IONP aggregation Expensive Long cycle Hard to realize industrial production	[52–55]
Sol-gel	Small particle size Good dispersion	Expensive Long duration Easy to shrink during drying	[56–60]
Atomic layer deposition	Accurate thickness control Good uniformity	Slow deposition rate	[61–63]

conductivity, the charge concentration of the solution, the electric field strength/voltage surface tension, the distance between the needle and the collecting screen, the design and placement of the needle tip, and the composition and geometry of the collecting screen. Of course, there are other factors like ambient constant, dipole moment, dielectric constant, and surface tension that also affect high-voltage electrostatic spinning.

2.2.2. IONPs Enhance Scaffold Function. Artificial bone transplantation has been extensively studied for bone repair due to less immune rejection and low disease transmission ability [102, 103]. Bone morphogenetic proteins and transformation factors are usually incorporated into artificial scaffolds to improve cell viability. However, there is still a phenomenon of slow cell binding. If magnetic field stimulation activates more receptors on the cell surface and further activates related signal pathway, the cell activity will be enhanced [104, 105]. Magnetic scaffolds can attract growth factors and stem cells to migrate in the body through magnetic drive and promote bone repair and regeneration [106]. In addition, magnetic field stimulation can promote the integration of the scaffold with the host bone and increase calcium content and new bone density, thereby accelerating bone healing [107–109]. At present, the role of magnetic scaffold in promoting cell proliferation and new bone tissue growth has been confirmed [110]. The magnetic scaffold has a wide range of components including biological macromolecules, synthetic polymers, polyethylene glycol, and inorganic materials [111–115].

Liu et al. [116] fabricated a magnetic coating composed of Fe_3O_4 nanoparticles and polyamine on the surface of the scaffold, thereby enhancing the cell attachment, proliferation, and bone differentiation of mesenchymal stem cell (MSC) in vitro, and forming new bones at the defect of the rabbit femur. It is found that the magnetic Fe_3O_4 /PDA coating is related to strengthening the regulation signal pathway by protein analysis. Shuai et al. [117] constructed a magnetic microenvironment by selectively sintering Fe_3O_4 magnetic nanoparticles on the PLLA/PGA scaffold. Each nanoparticle

in the environment provides a nanometer-scale magnetic field to activate the cell response. The in vitro results show that the magnetic scaffold not only stimulates cell adhesion and activity but also improves the rate of growth and alkaline phosphatase activity (see Figure 1). Yang et al. [118] used calcium phosphate cement (CPC) and IONPs to prepare a new type of scaffold and explored the effect of the new composite material on the formation and bone formation of human dental pulp stem cells (hDPSC). They found that the addition of IONPs greatly promote the bone formation of hDPSC, enhanced mechanical strength and cell activity, and increased the expression of bone marker genes by 1.5–2 times.

Quite a few studies have shown that IONPs have a significant effect on the function of the scaffold. In fact, the 3D platform with IONPs shows tighter cell-to-cell connections, and highly developed filamentous protrusions (increased number and extension length) enhance the interaction between cells and the platform. The distribution of nanoparticles on the surface of the 3D scaffold increased the surface area of the scaffold, enhanced the mechanical signal transduction of cells, and stabilized the cell's anchoring to the matrix, thus promoting the cell adhesion process. Adding IONPs to the 3D scaffold significantly increases the expression of the osteogenic transcription factor RUNX2 and its two downstream factors. IONPs may promote bone formation through different mechanisms, but they rely more on magnetic genetic responses that activate intracellular magnetic receptors and generate endogenous magnetic fields to promote bone formation even in the absence of external magnetic fields [119].

When nanoparticles are absorbed on the surface of the tissue scaffold, they are in direct contact with the cells and may have unknown effects on the cells through ingestion. Hsin-Yi Lin et al. discussed how to use a three-dimensional printing device to make a chitosan hydrogel scaffold and embed nanoparticles in the hydrogel, so that the surrounding cells do not directly contact the nanoparticles. Some studies have shown that the coupling force induced by the magnetic chitosan scaffold promotes the growth and mineralization of bone cells.

TABLE 2: Types of materials for the attachment platform of IONPs.

Types of nanomaterials	Description	Size (nm)	Applications	Reference
Lipid	Small hydrophobic or amphiphilic molecules	<100	Nanocarriers for anticancer Drug doxorubicin Osteoblastic bone formation Osteoporosis treatment High encapsulation of hydrophilic	[67–70]
Liposome	Same bilayer structure as the skin cell membrane structure and excellent moisturizing effect on the skin	>25	Drug delivery Growth factor delivery Therapeutic gene delivery Used as a template	[71–73]
Dendrimers	Organic molecules with dendritic structure	<10	Multidrug delivery system	[71, 74, 75]
Chitosan	A natural nontoxic linear biopolymer	20-200	Scaffolds Drug delivery Support chondrocyte adhesion Implant coating Osteogenic differentiation	[76–80]
Collagen	The main structural protein of soft and hard tissues in living organisms	—	Drug delivery Scaffolds	[81]
Gelatin	Derivatives of collagen	<200	Scaffolds Drug-loaded gelatin nanoparticles Promote cell growth Drug delivery Scaffolds	[82, 83]
PLGA	A degradable functional polymer organic compound	100-250	Nanostructured film Enhanced cell attachment and growth Promote gene expression	[84–87]
Carbon nanotubes	With cylindrical or tapered structure of different diameters and lengths	20-100	Drug delivery Biosensing Scaffolds	[88–91]

TABLE 3: Synthesis method of IONP composite scaffolds.

Synthesis method	Advantages	Disadvantages	Reference
Traditional method (physical adsorption)	Simple, cheap, stable performance	Lack of control measures for magnetic field gradients	[92, 93]
IONPs mixed with other ingredients	Stable performance, excellent mechanical properties	Difficult to control the surface characteristics of the bracket	[94–97]
Electrospinning	Easy to operate, easy to control the surface characteristics of the bracket	Low output and low strength	[98–100]
3D printing	Good stability, good three-dimensional structure, high efficiency	Expensive, material limitations	[101]

Under the action of inductively coupled magnetic force, the signal transduction in bone cells is achieved through the release of intracellular Ca^{2+} . This leads to an increase in cytoplasmic Ca^{2+} ions and an increase in cytoskeletal calmodulin. The increase in cytoplasmic calcium in bone cells will cause cellular bones to shrink. Calcium-dependent contraction promotes the release of extracellular vesicles. These extracellular vesicles contain key bone regulatory proteins, including cellu-

lar skeletal calmodulin. Calmodulin is a calcium-sensing protein that is involved in signal pathways that regulate many key processes in bone cells, such as growth, cell division, and movement (see Figure 2).

In addition to its outstanding performance in promoting cell proliferation and osteogenic differentiation, the excellent mechanical strength of the magnetic scaffold also deserves our attention. For example, Ghorbani et al. investigated the

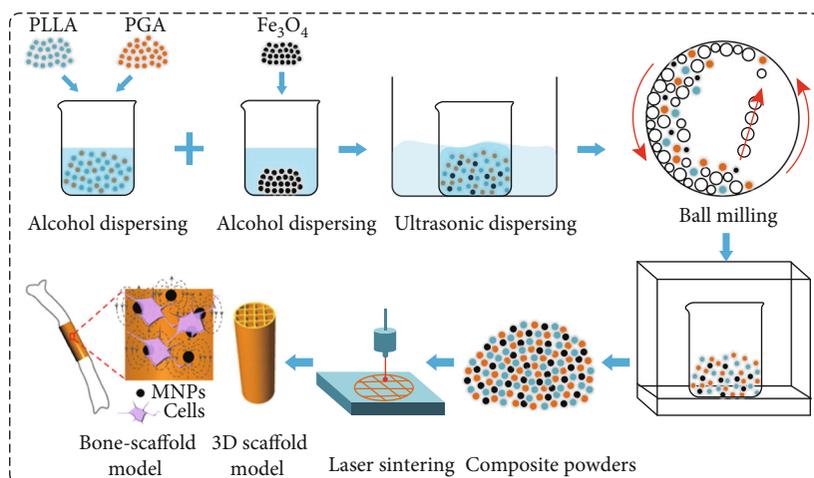


FIGURE 1: Schematic of the magnetic scaffold preparation process. Reproduced with permission from [117].

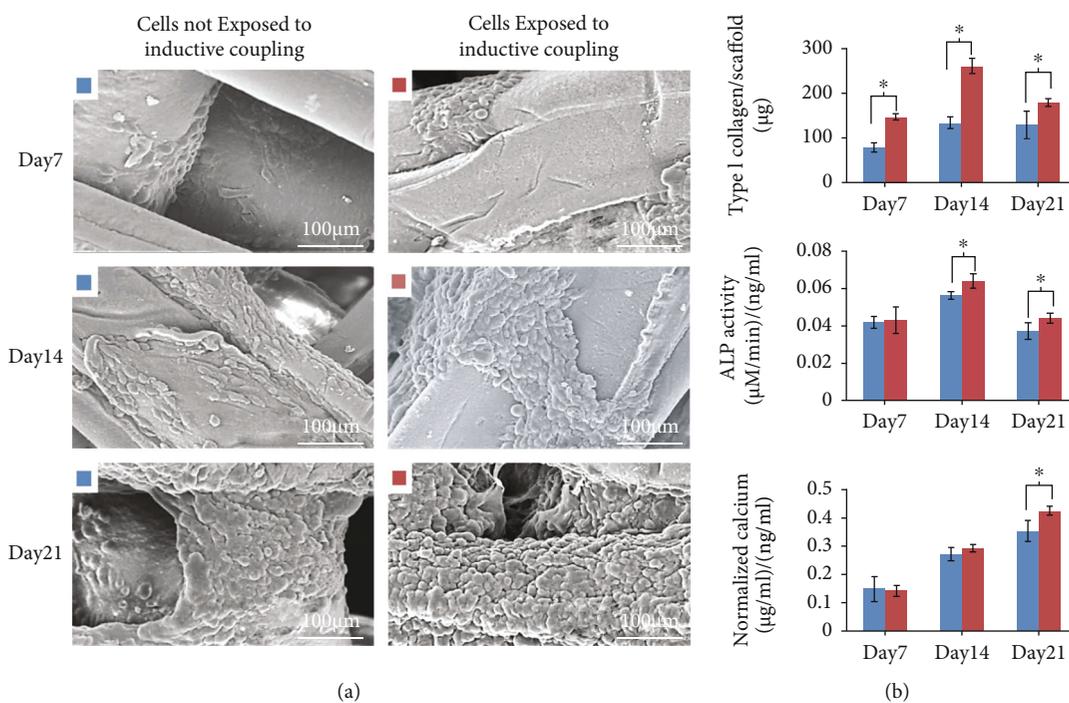


FIGURE 2: (a, b) Cell surface morphology and osteogenic differentiation with and without the inductive coupling magnetic force for 7, 14, and 21 days. (a) The morphology of osteoblast cells on the chitosan hydrogel scaffolds. (b) Osteoblast cell proliferation and differentiation. Reproduced with permission from [120].

effect of IONPs on the scaffold structure. Compared with scaffolds without IONPs, scaffolds with IONPs reduced the pore size, thus improving the mechanical strength of the scaffold, but the absorption capacity and biodegradation ratio were reduced [121]. Similar to Ghorbani et al.'s study, Kim et al. also found that the mechanical stiffness of the PCL scaffold increased significantly with the addition of IONPs. The initial adhesion of cells to the magnetic scaffold was substantially increased by 1.4-fold compared to the pure PCL scaffold [122]. Wang et al. fabricated borosilicate bioactive glass scaffolds loaded with different amounts (5-15 wt%)

of Fe₃O₄ nanoparticles and evaluated their performance in vitro and in vivo. They found that the Fe₃O₄ content was proportional to the compressive strength of the scaffold, and the compressive strength of the scaffolds increased with increasing content of IONPs, from 2.6 ± 0.6 MPa for the BG scaffolds to 3.6 ± 0.6 MPa for the scaffolds loaded with 15 wt% Fe₃O₄ [123]. In the meantime, that scaffold has the largest magnetic saturation and highest temperature of aqueous suspension.

These findings suggest that the scaffold with IONPs has excellent physicochemical, magnetic, mechanical, and biological

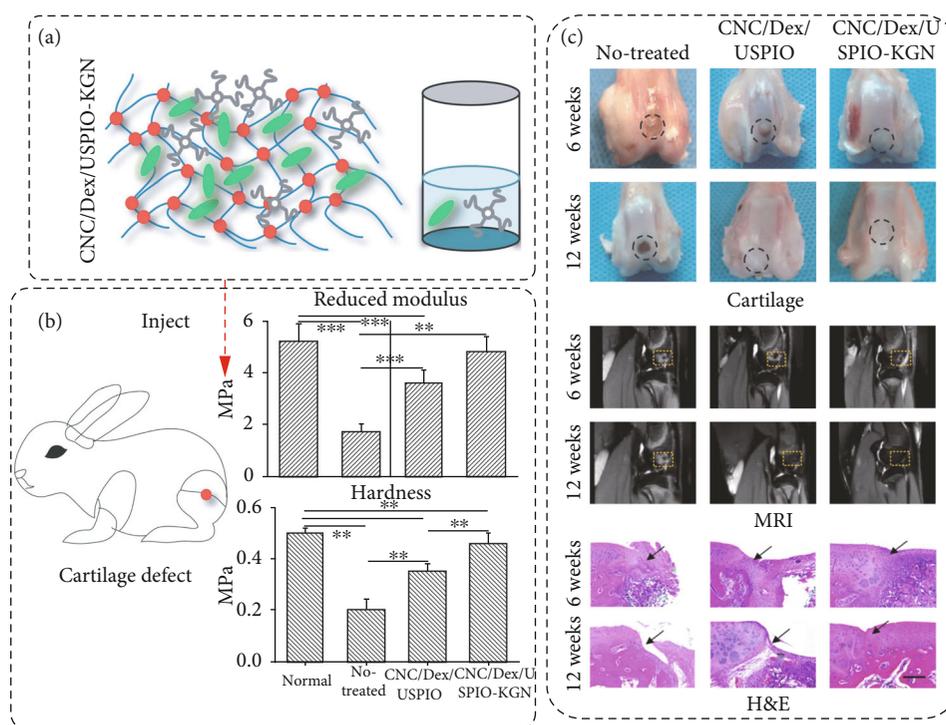


FIGURE 3: Synthesis in vitro and physicochemical properties in rabbit cartilage of CNC/Dex/USPIO-KGN. (a) Schematic illustration of preparation and utilization of USPIO-labeled Dex/CNC/USPIO-KGN hydrogels for engineering artificial cartilage repair. (b) Reduced modulus and hardness (reduced modulus is a “combined” modulus of the tip and the sample and can be directly evaluated from analysis of stress-strain data according to specific equations). (c) Observations of cartilage harvested; MRI and H&E during 6 weeks and 12 weeks. H&E staining shows that the neocartilage integrated with the adjacent normal cartilage. Reproduced with permission from [127].

properties, supporting the potential application of magnetic scaffolds for bone repair and regeneration.

2.2.3. IONPs Promote Stem Cell Differentiation. In addition to scaffolds, stem cells are another vital factor in tissue regeneration. In recent years, although stem cell therapy has provided a strategy for large-scale bone repair and has been successful in the treatment of animal bone defect models, these traditional stem cell transplants have not achieved the desired effect [124]. With the development of materials science and chemical biology, people have been trying to use IONPs as a tool for research and control of stem cells for many years [125]. By combining IONPs with an external magnetic field, it will affect the cell adhesion, proliferation, movement and distribution, and osteogenic differentiation of stem cells. In addition, IONPs can be used to label cells for in vivo tracking and monitoring.

MSCs are a key participant in bone regeneration, and promoting the differentiation of MSCs is an important basis for evaluating the performance of nanoparticles. The combination of different polymers and IONPs greatly promote the differentiation of MSCs. Jia et al. [126] synthesized medium-porous silicon-coated magnetic Fe_3O_4 nanoparticles and evaluated their potential to accelerate bone regeneration in a rat osteoporosis model. After X-ray imaging, micro-CT, mechanical testing, histological examination, and immunohistochemical analysis, local injection of MNPs significantly accelerated bone regeneration. Yang et al. [127] transplanted kartogenin (KGN), which can promote the differentiation of

bone marrow-derived mesenchymal stem cells into chondrocytes, onto a modified magnetic oxide surface, and then integrated into the cellulose nanocrystalline hydrogel. Release and recruit endogenous host cells and differentiate BMSCs into chondrocytes, thereby achieving original cartilage regeneration. The regenerated cartilage tissue is very similar to a natural cartilage. This innovative diagnosis and treatment system improve the convenience and effectiveness of cartilage regeneration (see Figure 3).

Xu et al. [128] prepared hollow IONPs, HMFN, which is of spherical shape with a diameter of about 320 nanometers. It has a negative charge on the surface and has huge super-negative magnetic properties. The -OH bond in it improves the affinity of nanoparticles. For water and biocompatibility, as a result, it was found that electromagnetic fields can use intracellular supersuspended magnetic nanoparticles to manipulate the bone differentiation of BMSCs.

In addition, human dental pulp stem cells (hDPSCs) also provide great potential for research of bone tissue engineering. Their advantages include easy isolation, nonimmunogenicity, strong proliferation, and differentiation ability, similar to bone marrow stem cells (BMSCs) [129]. The osteogenic differentiation ability of hDPSCs has been fully confirmed by previous studies, which is manifested by increased ALP activity [130] and increased expression of bone-specific markers [131, 132]. The WNT/ β -catenin signaling pathway plays an important role in osteogenesis. Previous studies have shown that by activating the SMAD pathway or interacting with the WNT pathway to upregulate

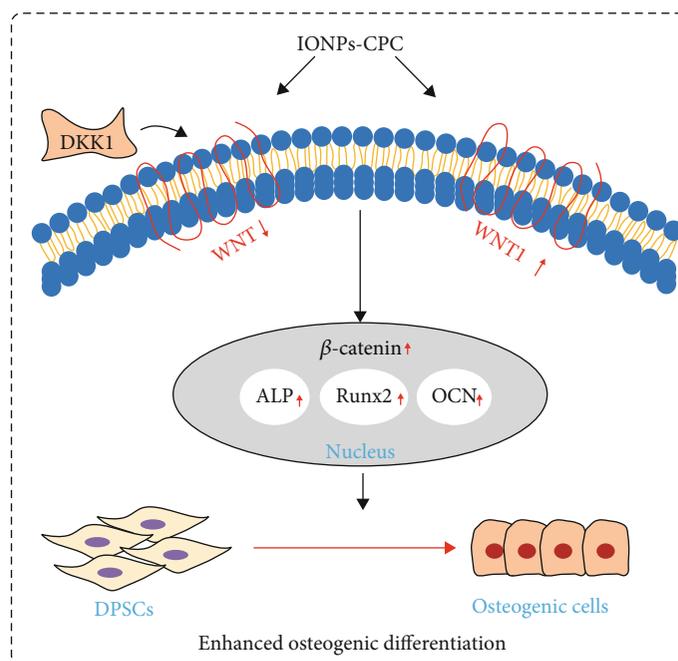


FIGURE 4: WNT signaling pathway promotes osteoblast differentiation.

osteogenic genes, IONPs can promote stem cells by upregulating BMP2, which plays a key role in bone morphogenesis. They added hydrophilic IONP solution to CPC powder to prepare IONP-CPC scaffolds and explored the effect of new composite materials on the formation of bone matrix and osteogenic differentiation of hDPSCs. As the ionosphere content increases, the color of the scaffold turns darker, and the surface of the scaffold forms agglomerates, which significantly enhances the adsorption of proteins. Through the analysis of the WNT pathway, it was found that CPC+IONPs can significantly enhance the expression of β -catenin protein, proving its important role in the osteogenic differentiation of stem cells (see Figure 4) [118]. In fact, another study also confirmed that IONPs can activate the BMP/SMAD pathway [133].

2.2.4. Marking and Positioning of IONPs. Cell migration, distribution, survival, and differentiation play a crucial role in therapeutic efficacy [134]. We understand that these parameters can optimize cell selection, administration route, and therapeutic dose and provide cell-based therapy for specific clinical applications. To solve this problem, researchers have been looking for tools that allow real-time, quantitative, and long-term monitoring of cell behavior in the body. This phenomenon is called cell tracking. IONPs are used as an NMR contrast agent for cell tracking [135].

Because the healing potential of the articular cartilage is limited, the treatment of osteoporotic defects continues to pose a major challenge to patients and orthopedic surgeons. MSCs have a therapeutic potential for the treatment of osteoporotic pain and pathology. However, it is necessary to use appropriate stem cell labels and imaging agents to decipher its role after transplantation. Silva et al. [136] incubated MSCs with magnetic nanoparticles and used external magnetic fields to guide magnetized MSCs in vitro and enhanced

their retention in the lungs in vivo. The results showed that MT improved MSC translocation and expression of chemical hormone receptor. Shelat et al. [137] evaluated the efficacy of bone marrow-derived mesenchymal stem cells (BMSCs) for the treatment of osteoporosis defects in rats and used L-lysine functionalized IONPs (lys-IONPs) to treat stem cells. In vivo monitoring showed that the particles can be used as long-term stem cell markers and imaging agents. Yao et al. [138] used amine-modified silicon-coated nanoparticles to label bone marrow-derived mesenchymal stem cells and then evaluated the stem cell potential. The study found that after labeling, the viability of BM-MSCs remained good and the migration ability was enhanced and had no effect on bone production and adipogenesis, which means that this kind of nanoparticles can not only serve as an ideal tracking marker but also become an accelerator for stem cell positioning during tissue repair (see Figure 5). In addition, scholars have also discovered the advantages of magnetic IONPs as markers in the treatment of diseases such as ischemic heart disease and pulmonary fibrosis [139, 140].

2.2.5. Security Issues of IONPs. IONPs are among the most versatile and safe nanoparticles for a variety of biomedical applications. The dramatic increase in the use of nanoparticles in research, industry, and medicine raises many questions about potential toxicity [141], since the nanoscale properties can potentially induce cytotoxicity by impairing the functions of mitochondria, nucleus, and DNA [141–143]. It is well known that excess reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, and nonradical hydrogen peroxide, can be toxic intracellularly and in vivo [141, 144, 145]. The ROS can be generated from the leaching of iron ions from the surface degradation by enzymatic degradation. Furthermore, ROS can react with macromolecules and damage cells by peroxidizing lipids,

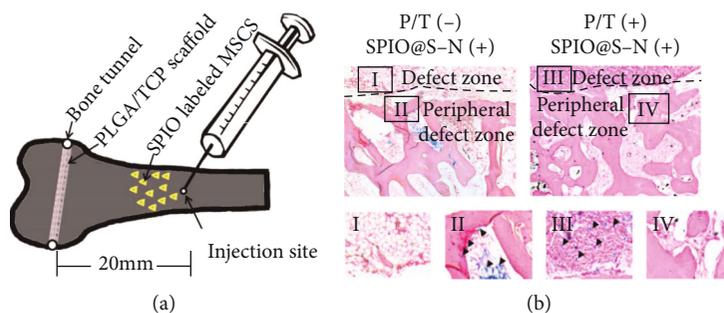


FIGURE 5: (a, b) Marking and positioning of IONPs. (a) Rabbit SAON bone defect model was established, and PLGA/TCP was implanted into the rabbit at the distal femur to track BM-MSC migration in vivo. (b) Dynamic MRI was performed at day 0 and day 7, respectively, after implantation. Reproduced with permission from [140].

changing proteins, disrupting DNA, interfering with signaling functions, modulating gene transcription, and finally causing cell death either by apoptosis or necrosis. Another mechanism by which IONPs can induce toxicity is via iron overload. Since IONPs require magnetic targeting to specific tissues [146], high concentrations of free iron ions may lead to abnormal cellular responses including cytotoxicity, oxidative stress, epigenetic events, inflammation, and DNA damage that may trigger carcinogenesis or have significant effects on offspring [142, 147–150].

The cytotoxicity of IONPs is highly dependent on a number of factors related to their physical properties, such as size, shape, and surface coating. The type of surface coating materials of IONPs and their decomposition products are important in determining their toxicity [151]. As mentioned previously, there are many polymers that can be used to coat IONPs; however, some studies have shown that PEG-coated IONPs produce negligible aggregation in cell culture media and reduce nonspecific uptake by macrophages [152], while dextran-coated IONPs can lead to cell death and reduced proliferation with observable visible membrane disruption [153, 154]. The oxidation state of iron (Fe^{2+} or Fe^{3+}) in IONPs is an additional key factor that determines the cytotoxicity of IONPs [150]. It has been demonstrated that maghemite (Fe_2O_3) with an $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio of 0.118 has a more significant genotoxicity than magnetite (Fe_3O_4) with an $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio of 0.435. More efforts are needed to design and prepare IONPs with good chemical stability.

In vivo, the toxicity of IONPs is dose dependent and is also related to the type of tissue cells on which they act. For example, Hanini et al. [155] reported that IONPs in vivo can induce toxicity in the liver, kidneys, and lungs while the brain and heart organs remained unaffected. Additionally, hemocompatibility is also an aspect that should be taken into account for the in vivo application of IONPs. If IONPs are incompatible with biological fluids such as blood, this can trigger coagulation and clot formation through adsorption of plasma proteins, platelet adhesion, and activation of the complement cascade. IONPs will exist in different proportions in different organs of the body; how to remove the excess particles is also related to the toxic side effects. Generally, clearance and opsonization of IONPs depend on their sizes and surface characteristics [156, 157]. For example, 55% oleic acid/pluronic-coated IONPs of injected dose

were accumulated in the liver of a rat. However, in the same animal model, 25% of injected dextran-coated IONPs was eliminated via urine and feces [158]. Therefore, the toxic effects of IONPs on humans can be effectively reduced by controlling the physical properties of IONPs and selecting the appropriate type of surface coating material and the dose of IONPs. Also, more attention should be paid to the kinetic and metabolic mechanisms of IONPs with different surface coatings, which would allow the development of predictive models of nanotoxicity.

3. Summary and Prospect

We briefly describe the synthesis method of IONPs and the merit and demerit of each method. And the applications of the IONPs in the function of scaffold are also elaborated. Among them, we firstly mentioned the material used to make the scaffold and statistics on their size. Each material has unique properties including nanocarriers for anticancer, drug doxorubicin, osteoblastic bone formation, and osteoporosis treatment. And then, we have mentioned the merit and demerit of several scaffold synthesis methods. Furthermore, the mechanism by which IONPs enhance the function of the scaffold is described. IONPs can impart a magnetic effect to the scaffold, which can attract growth factors and stem cells to migrate in the body, and promote bone repair and regeneration. IONPs can also be used as accelerators to accelerate the binding of the scaffold to the host bone and enhance related signal pathways to promote the attachment, proliferation, and bone differentiation of mesenchymal stem cells. At the same time, as a marker and imaging agent, it is an important tool for drug delivery and stem cell localization.

Through the above literature research, we have summarized the development status and development direction of IONPs. (a) How to precisely control the arrangement of the IONPs to obtain a better magnetocaloric effect has broad prospects. (b) There are many ways to prepare composite scaffolds of iron oxide particles, all of which have excellent performance and significant shortcomings in specific scenarios. How to obtain a scaffold that can control the surface characteristics of the scaffold and has high yield and low cost will become the focus of research. (c) How to maintain long-lasting and effective stem cell characteristics is the key to successful treatment. Therefore, for cells expanded in vitro,

maintaining a stable cell phenotype and reducing the necrosis and loss of the cells at the defect site after transplantation in vitro are necessary for bone defect repair. (d) At present, most researches on IONPs focus on labeling methodologies and monitoring curative effects, but there are not many studies on their effects on labeled cells. IONPs have different effects on different types of cells, even the same type of cell, in different aspects. Functional activities also react differently to IONPs. Therefore, it is necessary to extensively study the short-term and long-term potential cytotoxicities of IONPs to different labeled cells, which is an important reference for avoiding the adverse reactions of IONPs when cell magnetic labeling really enters the clinical trial stage in the future.

With the in-depth research of human cells and virus particles, the continuous improvement of the feasibility and practicability of nanoparticles as drug carriers will inevitably provide a breakthrough in the research of drug carrier systems. It is reasonable to believe that the multifunctional magnetic nanodrug carrier with fluorescence detection, multiple targeting, efficient drug loading, quantitative time-released drug, and nontoxic side effects is of great significance for the diagnosis and treatment of major diseases such as cancer.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

There are no conflicts of interest in this study.

Authors' Contributions

All authors have read and approved this version of the article, and due care has been taken to ensure the integrity of the work.

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