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Nanotechnology in Regenerative Medicine and Drug Delivery Therapy

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ISBN 978-981-15-5385-1 ISBN 978-981-15-5386-8 (eBook)
https://doi.org/10.1007/978-981-15-5386-8

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Acknowledgments

The authors would like to thank for supports from the National Key Research and Development Program of China (2017YFA0205500, 2017YFA0205001), the National Natural Science Foundation of China (31571013, 21673055, 21773042, 31971295, 21790394, 31901007, 81471793, 81801771), the CAMS Innovation Fund for Medical Sciences (CIFMS 2016-I2M-3-004, CIFMS 2018-I2M-3-006, CIFMS 2019-I2M-1-004), the State Key Laboratory Special Fund (2060204), the Fundamental Research Funds for the Central Universities (3332019064, 2018PT31028), the Key Research Program of Frontier Sciences, Chinese Academy of Science (QYZDJ-SSW-SLH048), the Youth Innovation Promotion Association of Chinese Academy of Science (2018048), the Open Project Fund provided by Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, CAS (NSKF201812), the Zhongyuan Thousand Talents Project (204200510016), the Program for Innovative Research Team (in Science and Technology) in the University of Henan Province (19IRTSTHN026), the Guangdong Natural Science Foundation of Research Team (2016A030312006), and the Shenzhen Science and Technology Program (JCYJ20160429191503002).

Contents

Abbreviation 1

124 polymer	Poly(octamethylene maleate (anhydride) 1,2,4-butanetricarboxylate)
AFM	Atomic force microscopy
AML	Acute myeloid leukemia
APDCs	Amphiphilic peptide-drug conjugates
APL	Acute promyelocytic leukemia
ATRA	All-trans retinoic acid
BP	Bispyrene
BSA	Bovine serum albumin
CA	Cellulose acetate
CD	Circular dichroism
CEC	*N*-carboxyethyl chitosan
CMC	Carboxymethyl cellulose
CNCs	Cellulose nanocrystals
Col	Collagen
CR	Complete remission
cryo-EM	Cryo-electron microscope
CS	Chitosan
CTCs	Circulating tumor cells
DM1	Maytansinoid
DTT	Dithiothreitol
EGCG	(−)-epigallocatechin-3-gallate
EPR	Enhanced permeability and retention
ESEM	Environmental scanning electron microscopy
FDA	Food and drug administration
Gel	Gelatin
GelMA	Methyl acrylic anhydride-gelatin
GGMA	Methacrylated gellan gum
GS	Gelatin sponge
HA	Hyaluronic acid
HA-SH	Thiol-modified hyaluronic acid

HCD	Hydrophobic central domain
HDAC	Histone deacetylase
hIAPP	Human islet amyloid polypeptide
HLB	Hydrophilic-lipophilic balance
HME	Hot melt extrusion
HPLC	High-performance liquid chromatography
IC_{50}	50% inhibiting concentration
IHC	Immunohistochemical
LbL	Layer-by-layer
MAAG	Methacrylated aminated gelatin
MDS	Myelodysplastic syndrome
MEL	Melamine
MeTro	Methacryloyl-substituted recombinant human tropoelastin
MIDAS	Metal ion-dependent adhesion site
MNPs	Magnetic nanoparticles
NFs	Nanofibers
NMR	Nuclear magnetic resonance
Nrp-1	Neuropilin-1
OHA	Oxidized hyaluronic acid
OPF	Oligo(poly(ethylene glycol) fumarate)
P(VDF-TrFE)	Poly(vinylidene fluoride-co-trifluoroethylene)
PAA	Poly(acrylic acid)
PAAm	Polyacrylamide
PAH	Poly(allylamine hydrochloride)
PANi	Polyaniline
PCL	Poly-ε-caprolactone or polycaprolactone
PDA	Polydopamine
PDCs	Peptide-drug conjugates
PDLLA	Poly(D, L-lactic acid)
PEDOT	Poly(3,4-ethylenedioxythiophene)
PEG	Poly(ethylene glycol)
PEGDA	Polyethyleneglycol diacrylate
PEGS	Poly(ethylene glycol)-co-poly(glycerol sebacate)
PG	Polyglycolide
PGS	Poly(glycerol sebacate)
PHEMA	Poly(2-hydroxyethyl methacrylate)
pI	Isoelectric point
PLA	Poly-DL-lactide or poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
PLL	Poly-L-lysine
PLLA	Poly(L-lactide)
PNIPAAm	Poly(*N*-isopropylacrylamide)
PPY	Polypyrrole
PSS	Poly(4-styrene sulfonate)
PTAA	Poly(thiophene-3-acetic acid)

PU	Polyurethane
PVA	Polyvinyl alcohol
PVDF	Polyvinylidene fluoride
PVP	Polyvinylpyrrolidone
QCSG	Glycidyl methacrylate functionalized quaternized chitosan gel
RIF	Realgar-indigo naturalis formula
ROS	Reactive oxygen species
RTG	Reverse thermal gel
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SF	Silk fibroin
SPR	Surface plasma resonance
SSM	Sterically stabilized micelle
STM	Scanning tunneling microscopy
TCP	Tissue-culture-treated polystyrene
TEAR	Transformation-enhanced accumulation and retention
ThT	Thioflavin T
TKIs	Tyrosine kinase inhibitors
TNBC	Triple-negative breast cancer
TPU	Thermal plastic polyurethane
TTR	Transthyretin
VEGFR2	Vascular endothelial growth factor receptor 2
VNTR	Variable number of tandem repeats
κC	κ-carrageenan

Abbreviation 2

Cell types	Cell name	Abbreviation
Muscle cells	Rat smooth muscle cells	SMCs
	Cardiomyocyte/cardiac myocytes	CMs
	Neonatal rat ventricular myocytes	NRVM
	Neonatal rat cardiomyocytes	/
	hiPSC-derived cardiomyocytes	hiPSC-CMs
	Embryonic-stem-cell-derived cardiomyocytes	/
	Mouse embryonic stem cell derived cardiomyocytes	mES-CM
	Primary cardiomyocytes	/
	Murine neonatal cardiomyocytes	/
Myoblast cell	H9c2 rat cardiac myoblast cells	H9c2
	C2C12 myoblast cells	C2C12
Stem cell	Human pluripotent stem cells	hPS
	Human pluripotent stem cells-derived cardiomyocytes	hPSC
	Brown adipose-derived stem cells	/
	Human induced pluripotent stem cells	hiPSCs
	Mouse embryoid bodies	Ebs
	Mesenchymal stem cells	MSCs
	Human embryonic stem cells	hESC
	Umbilical cord blood (UCB)-derived multipotent mesenchymal stem cells	CB-hMSCs
	Stem cells	/
	Human mesenchymal stem cells	hMSCs
	Bone marrow derived stem cells	BMSCs
	Rat bone-marrow-derived mesenchymal stem cells	MSCs
Osteoblasts	Osteoblasts	/
	Osteosarcoma	MG-63
	Mouse osteoblast cells	/

Cell types	Cell name	Abbreviation
Nerve-related cells	Nerve stem cells	/
	Rat pheochromocytoma 12	PC12
	Neural stem cells	/
	Schwann cells	SCs
	Rat Schwann cell	RSC
	Dorsal root ganglion neurons	DRG neurons
	Neuron	/
	Human neural stem/progenitor cells (hNSPCs)	/
	Central nervous system neurons	CNS neurons
Stromal cell	Adipose tissue-derived stromal cells	ADSCs
	Fibroblasts	/
	Human cardiac fibroblast	/
	Mouse osteoblast cells	/
Epithelial cell	Epithelial cells derived from breast adenocarcinoma	MCF-7
Blood-related cells	Neutrophil	/
	Macrophage	/
	RAW 264.7 cells	/
	Platelet	/
	Endothelial cell	/

Chapter 1
Magnetically Actuated Scaffolds to Enhance Tissue Regeneration

Haiyan Xu, Suisui Hao, and Jiawei Zhou

Abstract Tissue defects beyond critical sizes and organ failure are great challenges in human health care. Biomaterial scaffold-guided tissue regeneration is one important and promising way to develop more effective therapeutic strategies to meet the clinical demands by creating microenvironments mimicking natural physiological conditions from the aspects of biochemistry and biophysics. The combination of nanotechnology in the biomaterial scaffolds have opened a new field in regenerative medicine and provided more powerful tools for developing strategies to modulate biochemical and mechanical microenvironments for tissue regeneration. In this chapter, we focused on the evolution and research progress of magnetic nanoparticles-based tissue engineering scaffolds. This chapter emphasized the synergistic effects of magnetic scaffolds in combination with external magnetic fields on cells differentiation and tissue regeneration through comprehensively reviewing the applications in multiple cells and tissues including bone, cartilage, cardiac, endothelial, nerve, Schwann cells, fibroblasts and macrophages, following the introduction of biological effects exerted by magnetic fields and magnetic scaffolds separately. The combination strategy allows for providing mechanical stimulations directly and remotely controls cells behaviors and fates in vivo without invasion, and provides promising platforms for drug screening in vitro and ex vivo.

Keywords Regenerative medicine · Tissue engineering · Magnetic scaffold · Mechanical stimulation · Remotely control

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H. Xu, N. Gu (eds.), *Nanotechnology in Regenerative Medicine and Drug Delivery Therapy*, https://doi.org/10.1007/978-981-15-5386-8_1

1.1 Introduction

Magnetism is a long historical and intriguing physical cue that can alter the behaviors of a broad range of cells; therefore, the implementation of magnetic features in biomaterial scaffolds may offer innovative opportunities to control cell populations within engineered 3D microenvironments, with the potential to enhance their use in tissue regeneration or in cell-based therapies. For decades, magnetic nanoparticles, especially iron oxide nanoparticles, have attracted intense research interests in biomedical fields because the manufacture technologies are maturing, and they have shown promising perspectives in various applications, such as bio-separation, detection and diagnosis, medical imaging, thermal treatment against tumor, and targeting drug delivery [1–3]. In the past decade, a new application potential of magnetic nanoparticles in tissue engineering and regenerative medicine has been explored with increasing interests from the aspects of magnetically actuated mechanical stimulation and remote control, by integrating magnetic nanoparticles into substrates or implantable scaffolds to provide mechanical forces directly to cells grown on and inside. The application modality included magnetic scaffolds alone or in combination with external applied magnetic fields.

Motivations of integrating magnetic nanoparticles into various matrices for uses in tissue engineering can be summarized into following aspects: First, magnetic nanoparticles would form magnetic phases in microscale that distribute in matrices. These aggregated nanoparticles would provide local magnetic moments or magnetic fields, which is assumed to be beneficial to the tissue regeneration according to the literatures of biological effects of magnetic fields. Second, the induction of magnetic nanoparticles brings magnetic property to scaffolds, which endows the scaffolds capacity of reloading with bioagents in situ after implantation. For example, magnetic scaffolds are expected to attract and take up growth factors, stem cells or other bioagents bound to magnetic nanoparticles in vivo by the guidance of an external magnetic field. The magnetic property of scaffolds can also be used to control microstructures of scaffolds (for example, to form aligned morphology) or to improve implant fixation and stability in vivo, by the remote control of external magnetic fields. And third, implantable scaffolds containing superparamagnetic nanoparticles can be magnetized by responding to external applied magnetic fields, which allow for generating local strain-induced deformation to apply mechanical stimulations directly to cells grown on the scaffolds.

This chapter will emphasize the synergistic effects of magnetic scaffolds in combination with external magnetic fields on cells differentiation and tissue regeneration, through comprehensive reviewing the applications in multiple cells and tissues including bone, cartilage, cardiac, endothelial, nerve, Schwann cells, fibroblasts, and macrophages, following the introduction of biological effects exerted by magnetic fields and magnetic scaffolds separately. The combination strategy allows for providing mechanical stimulations directly and remotely controls cells behaviors and fates in vivo without invasion and provides promising platforms for drug screening in vitro and ex vivo.

1.2 Biological Effects of Magnetic Fields on Tissue Regeneration

Magnetic fields include static (SMF) and electromagnetic fields (EMF). The therapeutic functions of magnetic fields have attracted clinical and research attentions for many years [4]. The SMF is usually generated by permanent magnetic materials, and there are various methods for obtaining the electromagnetic field generated by an arbitrarily moving point charge in free space. The EMF can be subdivided into pulse electromagnetic fields (PEMFs), alternating magnetic field (AMFs), or rotating magnetic fields (RMFs). Among them, SMFs and PEMFs are used most widely. According to the field intensity, SMF can be classified into weak field (less than 1 mT), moderate field (1 mT–1 T), strong field (1–5 T), and ultra-strong field (>5 T). It has been reported that the magnetic field with moderate intensity could enhance pre-osteoblasts proliferation and ECM production, and templated mineral deposition more rapidly than the control culture [5]. As an example, exposure to SMF of 160–340 mT enhanced the bone formation of rat calvaria cells [6] through the activation of p38 phosphorylation at the cellular level to stimulate osteoblastic differentiation [7]. There are also literature reports about the effects of magnetic fields on nerve system. For example, low-frequency magnetic fields could activate *N*-methyl-D-aspartate receptors in glutamatergic Ca^{2+} channels to promote neuronal differentiation [8] and might improve depressant behavior and cognitive dysfunction mainly by modulating synaptic function [9].

Impacts of the magnetic stimulation on human mesenchymal stem cells (hMSCs) have also been explored, as hMSCs are widely accepted as seeding cells, and the control of mesenchymal stem cells (MSC) by physical cues is of great interest in regenerative medicine. A group reported that equine adipose-derived mesenchymal stem cells (ASCs) exhibited higher mRNA levels of bone morphogenetic protein-2/4 (BMP-2/4), Sox9, and type I collagen when cultured under the magnetic field of 0.2 T. In addition, the cells cultured in the magnetic field reached the population doubling time earlier, because the colony-forming potential of equine ASCs was higher when cells were cultured under magnetic field conditions. Interestingly, exposure to the magnetic field increased the number of secreted microvesicles in comparison to the control culture as well as increased the production of growth factors [10]. Another study reported the effect of low-frequency EMF on hMSCs during chondrogenic differentiation, the cultures were exposed to the low-frequency magnetic fields (5 mT) [11]. It was observed that the effect of EMF on collagen type II expression was cell passage-dependent and exclusively detected at higher cell passages. Under the EMF and the addition of human fibroblast growth factor 2 (FGF-2) and human transforming growth factor-β3 (TGF-β3), hMSCs showed a significant increase in collagen type II expression at passage 6, indicating that the very low-frequency EMF was able to improve the growth factor-induced chondrogenic differentiation of hMSCs, however, when growth factors were absent, there was no staining detected for safranin-O and collagen type II, indicating that the EMF could not induce chondrogenic differentiation alone. SMF exposure could

also affect the cell viability and capacity of osteogenic differentiation in rat dental pulp cells (DPCs) [12]. It is interesting to note that exposure to SMF alone did not affect the dental pulp cell proliferation, only when exposed to SMF with the supplementary Dex/b-GP, the cell differentiation was significantly enhanced at the seventh and ninth day of culture when compared with the control culture, which was speculated that the magnetic field played a cooperative role via the ECM-mediated activation of ERK1/2-Cbfa1 signaling, while the new ECM deposition was time consuming. Another study showed that the magnetic field could stimulate biological functions of MSC; however, the responses of cells depended strongly on the substrate to which they adhere and on the crosstalk between integrin-mediated signals and soluble factors [13]. The results again implied that the magnetic stimulation alone on cells is not enough for the bone repair and regeneration, the microenvironment where the bone cells live is likely to play crucial synergy roles in the regeneration process. Another group had MSCs seeded in a composite scaffold composed of hydroxyapatite and collagen I, the construct was placed in a dynamic perfusion bioreactor and exposed to EMF of 15 Hz/1 mT. The applied EMF could promote osteogenic differentiation with the inducing medium. When implanted in a defect hole of 4 mm in width and 8 mm in depth that was created in each lateral femur condyle of rabbits, micro-CT results revealed that EMF could promote integration at bone–implant interface and bone regeneration after 12 weeks [14]. These results implied that scaffolds might be necessary as media for magnetic fields to elicit promoting effects on MSCs differentiation.

1.3 Biological Effects of Magnetic Materials on Tissue Regeneration

Inspired by the biological effects elicited by the low or moderate intensity of magnetic fields, it has been considered that magnetic materials may have positive effects on scaffold-guided tissue regeneration as well, because magnetic materials usually consist of iron (Fe), cobalt (Co), or nickel (Ni), and these materials can produce magnetic fields directly or indirectly, as it is well known that permanent magnets produce static magnetic fields (SMFs). As examples of the efforts, the roles of magnetic scaffolds in the guidance of bone tissue regeneration have been investigated largely. In case of degenerative disease or lesion, bone tissue replacement and regeneration are an important clinical goal. In long period the repair for critical size defects rely on implantable grafts that are not biodegradable. The emerging of tissue engineering and regenerative medicine provide the concept of developing biodegradable scaffolds that are 3D structural supports, allowing cellular infiltration and subsequent integration with the native tissue. Several ceramic hydroxyapatite (HA) scaffolds with high porosity and good osteointegration have been developed in the past few decades but they have not solved completely the problems related to bone defects, a major challenge is the speed of scaffold-induced new bone formation is

too low to meet the clinical demand [15, 16]. Researchers have been making great efforts to overcome the problem; the utilization of magnetic nanoparticles is one of the directions.

Early investigations mainly addressed the biocompatibility of magnetic nanoparticles incorporated in matrices. For example, researchers developed a simple and inexpensive technique able to transform standard commercial scaffolds made of hydroxyapatite and collagen into magnetic scaffolds by dip-coating the scaffolds in aqueous ferrofluids containing iron oxide nanoparticles coated with various biopolymers. By this way, the nanoparticles were integrated into the structure of the scaffolds, providing the latter with magnetization values as high as 15 emu/g at 10 kOe [17]. These values were considered suitable for generating magnetic gradients, enabling magnetic guidance in the vicinity and inside the scaffold. It was reported that the magnetic scaffolds did not suffer from any structural damage during the process, maintaining their specific porosity and shape. Moreover, they do not release magnetic particles under a constant flow of simulated body fluids over a period of 8 days, indicating the good stability of the scaffold. Computer simulations revealed that the magnetic field generated by magnetic scaffolds was ready within the useful range for in vivo applications. Cellular experimental results showed that the composite scaffold supported the proliferation of human bone marrow-derived stem cells (HBMSCs), the cells covered the whole surface and filled most of the macropores of scaffolds, and no statistically significant differences in cellular viability and osteoblastic differentiation among all types of scaffolds at each time point were observed. Furthermore, the composite of hydroxyapatite (HA) dipping magnetic fluids was tested in a magnetic field of 320 mT, no negative effects arising from the presence of magnetite [18]. A comprehensive comparison study for the bone tissue formation was conducted by the same group in a preclinical rabbit model of critical femoral defect treated either with a HA/magnetite (90/10 wt%) or pure HA porous scaffolds at 4 and 12 weeks after implantation, indicating that both scaffolds were able to allow bone regeneration, and that the HA scaffold dipping magnetic fluids did not have short-term adverse effects on biocompatibility and bone formation ability [19]. Another pioneer work is to fabricate magnetic composites that were composed of CaP ceramic and magnetic nanoparticles (CaP-MNP). In the fabrication, superparamagnetic nanoparticles were integrated to two kinds of CaP ceramics, one was hydroxyapatite (HA), the other one was a composite of HA and tricalcium phosphate in a ratio of 65:35, named HT. When bone-related cell line Ros17/2.8 and MG63 cells were seeded in the composites and incubated for 4 or 7 days, the cell proliferation was significantly promoted compared with that of non-magnetic scaffolds. The possible influence of magnetic materials on bone morphological protein (BMP) was concerned in this investigation. BMP-2 was loaded on the magnetic scaffolds that were implanted subcutaneously in the fasciae of rat back muscles for 30 days. The in vivo test showed that the expression of BMP-2 was accelerated by HT containing superparamagnetic nanoparticles, and new bone-like tissue formation was observed, indicating the function of loaded BMP-2 was not affected by the incorporation of superparamagnetic nanoparticles. Importantly, it was observed that ALP secretion of Ros17/2.8 cells was increased on the magnetic

composites more than those on nonmagnetic composites after 14 days incubation, but the magnetic composites did not affect the ALP secretion of MG63 cells. Those results showed an important cue that the CaP-MNP composites could promote osteogenetic differentiation as well as the proliferation [20].

In addition to inorganic scaffolds, iron oxide nanoparticles are widely employed in polymer-based scaffolds, aiming to improve the biocompatibility and mechanical strength. One typical example is that a nanofibrous scaffold made by electrospinning technique from a mixture of poly-ε-caprolactone (PCL) and γ-Fe_2O_3 nanoparticles of 23 nm in diameter. The scaffolds obtained the magnetic responsive property, which contributed to the enhancement of adhesion and proliferation of porcine mesenchymal stem cells. Importantly, the PCL-based scaffold-induced osteogenic differentiation of mesenchymal stem cells, which was evidenced by the increased ALP activity [21]. Similarly, magnetic nanofibrous scaffolds of PCL incorporating citrated magnetic nanoparticles (MNP) of 12 nm in diameter were fabricated by using electrospinning technique, the content of MNPs was up to 20% in the solution. The incorporation of MNPs greatly improved the hydrophilicity of the nanofibers as well as enhanced the tensile mechanical properties of the nanofibers. In particular, the tensile strength increase was as high as 25 MPa at 15% MNPs, much higher than 10 MPa for pure PCL. The PCL-MNP nanofibers exhibited magnetic behaviors, with a high saturation point and hysteresis loop area, which increased gradually with MNP content. The incorporation of MNPs substantially increased the degradation rate of the PCL nanofibers in a content dependent manner. When subcutaneously implanted in rats, the composited nanofibers significantly improved initial cell adhesion and subsequent penetration through the nanofibers for rat bone marrow MSCs, compared to the pure PCL control, and increased the alkaline phosphatase activity and the gene expression of collagen I, osteopontin, and bone sialoprotein. Noted that they induced substantial neoblood vessel formation which greatly limited in the pure PCL, while exhibited minimal adverse tissue reactions [22]. The same formulation of PCL and MNPs was processed into porous scaffolds by using salt-leaching approach, which also showed the superparamagnetic behavior. When applied on bone repair, similar results were obtained, confirming the effects observed in the nanofibrous scaffolds. As shown that the incorporation of MNPs greatly improved the hydrophilicity and water swelling of scaffolds. More mineral nanocrystallites covered on the surfaces of the composite scaffolds than on the PCL scaffolds in the in vitro acellular mineralization experiment. The mechanical stiffness increased significantly with the addition of MNPs when tested under both static and dynamic compressed wet conditions. The subcutaneous implantation in rats for 2 weeks revealed favorable tissue compatibility, with substantial fibroblastic cell invasion and neoblood vessel formation while exerting minimal inflammatory reactions in the testing groups [23]. From these investigations we can notice the important roles of iron oxide nanoparticles in the scaffolds for enhancing bone tissue regeneration. When make further comparison with data from above references, it seemed the smaller the nanoparticles (23 nm vs. 12 nm), the stronger the enhancing effects. Another example is magnetic PLGA/PCL scaffolds that were fabricated using a combination of the electrospinning technique and layer-by-layer

assembly of superparamagnetic iron oxide nanoparticles (IONPs) greatly enhanced the hydrophilicity and increased the elastic modulus of the scaffolds. At the early stage of cell culture, the adhesion and spreading of ADSCs of IONPs group were more stretched and spindle-like compared to those on the control groups, which indicated that IONPs-containing scaffolds affected cellular behavior at an early stage. It could be noticed that IONPs group induced ADSCs osteogenic differentiation, evidenced by the highest ALP activity. Higher mineralization rate also confirmed the importance of magnetic nanomaterials as a bioactive interface between cells and scaffolds [24].

Chitosan and collagen are two kinds of biological materials widely investigated for scaffolds fabrication. A group prepared composite scaffolds containing hydroxyapatite nanoparticles (nHAP) and Fe_3O_4 nanoparticles (Fe_3O_4 NPs), and the substrate of the scaffolds was the mixture of chitosan and collagen (CS/Col). It is interesting that during the cell culture, a mass of hydroxyapatite microparticles could be observed forming on the surfaces of scaffolds containing Fe_3O_4, whereas did not on the surface of CS/Col/nHAP, suggesting that Fe_3O_4 NPs enhanced mineralization. The composite scaffolds (CS/Col/Fe_3O_4/nHAP) not only showed superior structural and mechanical performance for pre-osteoblasts cell line MC3T3-E1 cells adhesion and proliferation, but also had a higher bone regeneration ability when implanted into the SD rats' skull defects comparing to control group [25]. In another kind magnetic scaffold composed of HA, collagen, and magnetic nanoparticles, the magnetic phase could act as a sort of cross-linking agent for the collagen, inducing a chemical-physical-mechanical stabilization of the material and allowing for the control of the porosity network of the scaffold, while the hydroxyapatite (HA) was directly nucleated onto collagen fibers during their self-assembly, representing the biomineral calcium phosphate phase [26]. Gelatin is also one widely applied biological material in tissue engineering. When integrated with superparamagnetic nanoparticles (SPIONs), the magnetic gelatin sponge (GS) was implanted in the incisor sockets of Sprague-Dawley rats sacrificed at 2 and 4 weeks. In addition to the enhancement of the bone regeneration, the scaffold degradation and interaction with host tissues could be visually monitored over time by MRI, and a significant decrease in the signal intensity of T2-weighted magnetic resonance imaging (MRI) was also observed. This is another advantage of superparamagnetic iron oxide nanoparticles, the residual SPIONs, together with newly formed bone [27]. Magnetic composites also showed application potentials in the treatment of tumor related-bone defects. For example, magnetic $SrFe_{12}O_{19}$ nanoparticles modified-mesoporous bio-glass (BG)/chitosan (CS) porous scaffold (MBCS) not only triggered tumor apoptosis and ablation by elevating the temperatures of tumors under the irradiation of near-infrared (NIR) laser, because the $SrFe_{12}O_{19}$ nanoparticles in MBCS improved the photothermal conversion property, but also promoted the expression levels of osteogenic-related genes (OCN, COL1, Runx2, and ALP) and the new bone regeneration by activated BMP-2/Smad/Runx2 pathway, indicating the osteogenic differentiation was effectively induced by the magnetic composite nanoparticles. In this process, the residual SPIONs and newly formed bone could be monitored in the treatment of tumor related-bone defects [28]. Another example

is the fabrication of 3D porous Mg_2SiO_4-$CoFe_2O_4$ nanocomposite scaffolds with interconnected porosity and desirable mechanical properties close to cancellous bone, loading chemotherapeutics for bone cancer treatment. When exposed with electromagnetic fields of 100, 125, 150, and 200 Oe under 200 kHz, the scaffolds could produce heat for hyperthermia-based chemotherapies and displayed good biocompatibility with MG63 cells [29]. Nevertheless, it should be noted that there were literatures reporting no osteogenestic differentiation was induced by polymeric magnetic scaffolds. For instances, the induction of osteogenestic differentiation was not detected upon the electrospun membrane of integrating magnetic nanoparticles of Fe_3O_4 into biodegradable chitosan (CS) and poly vinyl alcohol (PVA), though human osteoblast-like cells MG63 grew well on the composite scaffold [30]. The electrospun membrane composed of poly(lactic-co-glycolic acid) (PLGA) and hydrophobic superparamagnetic nanoparticles (MNPs) showed good biocompatibility and significantly promoted cell proliferation compared with PLGA nanofibrous scaffold. Again, the composite scaffolds had no effects on the differentiation of MC3T3-E1 cells [31].

As for the dental tissue regeneration, magnetic scaffolds are being investigated in recent years, which is still an unmet clinical need. So far, no therapies have been completely successful in regenerating dental tissue complexes such as periodontium. One of the major challenges is the lack of scaffolds that can guide and direct cell fate toward the reconstruction of different mineralized and nonmineralized dental tissues. The osteogentic effect was reported by using magnetic scaffolds in some investigations. For example, composite foam-like scaffolds of PCL and Fe_3O_4 nanoparticles were fabricated by a salting out procedure, there were pores ranging from 250 to 500 μm inside of the scaffold, the size distribution was considered suitable for cellular population and odontogenic differentiation. When human dental pulp cells (HDPCs) were cultured on the magnetic composite scaffolds containing Fe_3O_4 nanoparticles up to 10 wt%, they exhibit good viability. It is interesting that the migration rate of the cells was enhanced after incubated with the composite scaffolds, determined by the in vitro scratch assay. At the same time, the adhesion of HDPCs was significantly promoted. Furthermore, the magnetic scaffolds significantly influenced the odontogenic differentiation of HDPCs, supported by the increased ALP activity, mRNA expression of odontogenic markers, and mineralized nodule formation. Mechanisms may include the upregulation of integrin subunits of α1, α2, β1 and β3, and the activation of downstream pathways including FAK, paxillin, p38, ERK MAPK, and NF-κB [32]. The magnetic scaffolds with nanofibrous structures were also showed similar positive effects on the human dental pulp cell (HDPCs) behaviors. The growth of HDPCs was significantly enhanced on the magnetic scaffolds compared with that on the nonmagnetic counterpart. The odontogenic differentiation of cells was significantly upregulated by the culture with magnetic scaffolds. Furthermore, the magnetic scaffolds promoted the HDPCs-induced angiogenesis of endothelial cells. Mechanistic studies indicated that the expression of signaling molecules, Wnt3a, phosphorylated GSK-3β and nuclear β-catenin, was substantially stimulated by the magnetic scaffolds; in parallel, the MAPK and NF-κB were highly activated when cultured on the magnetic nanofiber scaffolds [33]. Considering that tooth is a complex organ made of highly

mineralized tissues where the nonmineralized periodontal ligament (PDL) connects the alveolar bone to the cementum and ensures tooth functionality and stability, a very compact scaffold was made by integrating three layers, one was the bone-like layer, consisting iron-doped hydroxyapatite (FeHA), acting as mineral phase nucleated on collagen fibers; the second one was a ligament-like layer consisting of collagen, the third one was the cementum-like layer consisting of FeHA and cellulose acetate (CA), preserving its dense morphology. The scaffold was endowed with a superparamagnetic ability and showed that the chemical composition and morphology of the scaffolds favor substantial cell viability and proliferation [34].

Magnetic nanoparticles are also utilized as carriers of loading growth factors in the treatment of spinal cord injuries and might act together as imaging contrast to monitor the healing process by MRI. For example, free bFGF has short half-life of about 3–10 min due to rapid enzymatic degradation, this disadvantage leads to loss of biological activity and functions. A magnetic fibrin hydrogel scaffolds were prepared by blending thrombin-conjugated γ-Fe_2O_3 nanoparticles with fibrinogen to form magnetic fibrin hydrogel, with the addition of magnetic nanoparticles conjugated with basal fibroblast growth factor (bFGF). The composite hydrogel was applied to human nasal olfactory mucosa (NOM) cells that are primary cells of interest for implantation into spinal cord injuries. The conjugated bFGF enhanced the growth and differentiation of the NOM cells in the fibrin scaffolds significantly, compared to the same or even five times higher concentration of the free bFGF. In the presence of the bFGF-conjugated magnetic nanoparticles, the cultured NOM cells proliferated and formed a three-dimensional interconnected network composed mainly of tapered bipolar cells [35].

Data collected from some of above investigations were summarized in Table 1.1. It is seen that all the magnetic scaffolds showed biocompatibility, in some cases magnetic scaffolds alone did not induce osteogenesis though some did. It seems whether magnetic scaffolds could induce osteogenic differentiation or maturation is largely involved with the cell type and the chemistry and microstructures of scaffolds. And should be noted that even the osteogenesis was induced by magnetic scaffolds, there was still a large space for the degree to increase.

1.4 Combination of Magnetic Scaffolds and Magnetic Fields to Accelerate Tissue Regeneration

The combination of magnetic scaffolds and magnetic fields has emerged since 2010. This strategy's aim is to provide stronger and remotely controllable stimulations to bone-related cells to enhance osteogenesis and new bone formation. In recent years, this combination strategy has gained increasing research interests and has been demonstrated effective to multiple cell type including bone, cartilage, endothelial, nerve, fibroblasts, macrophages, and Schwan cells. Data from some investigations are summarized in Table 1.2, the magnetic component, magnetic field strength, scaffolds composition, and biological effects were emphasized.

Table 1.1 Magnetic scaffolds and biological effects on cells

Magnetic component	Other components	Preparation methods	Structure	Cell and animal model	Biological effects	Refs.
Fe_3O_4 NPs (5 nm)	Hydroxyapatite and/or $Ca_3(PO_4)_2$	Foamed and sintered, followed by immersed into Fe_3O_4 NPs colloid	Porous	Ros17/2.8; MG63; implanted in the skin incision at the midline of the SD rat back	Enhanced proliferation and ALP activity increased; new bone-like tissue formation observed	[1]
Fe_3O_4 NPs	Hydroxyapatite nanoparticles, Chitosan and Collagen	in situ crystallization and freeze-drying	Porous	MC3T3-E1; Full-thickness defects with diameter of 5 mm on the middle ridge of skull in SD rats	Enhanced adhesion, proliferation, and differentiation; better tissue compatibility and higher bone regeneration ability	[17]
Fe_3O_4 NPs (10.7 nm)	PCL	Salt-leaching	Porous	MC3T3-E1; subcutaneous implantation of SD rats	Enhanced proliferation, differentiation, and mineralization Favorable tissue compatibility, neoblood vessel formation, minimal inflammatory reactions	[8]
Iron-doped hydroxyapatite (FeHA)	PCL/FeHA	Rapid Prototyping	3D porous	Bone marrow mesenchymal stem cells (hBMSCs) Implantation in critical bone defect in lateral femoral condyle of male rabbits	Promote adhesion, proliferation, and differentiation; new bone formation after 4 weeks	[10]
Magnetic nanoparticle (Mgn)	Hydroxyapatite	Casting	Porous	Saos-2; critical bone defect in lateral femoral condyle of male rabbits	Enhanced proliferation Good compatibility and bone regeneration in vivo	[5]
Fe_3O_4 NPs (12 nm)	PCL	Electrospinning	Nanofibrous	Rat bone marrow mesenchymal stem cells (MSCs) Small subcutaneous pouches in the back area laterally from the spine of SD rats	Promote adhesion, penetration and differentiation Induce neoblood vessel formation and bone regeneration	[7]

Iron oxide nanoparticles (<50 nm)	Hydroxyapatite	Foamed and sintered	Porous	Critical femoral defect of rabbit	No short-term adverse effects on biocompatibility and bone formation ability	[19]
SPOINs (9 nm)	Gelatin sponge	Sponge immersed into SPIOs colloid	Sponge	A rat model of mandible incisor sockets	Enhanced bone repair and preserved the alveolar ridge after tooth extraction	[18]
Fe_3O_4 NPs (12–15 nm)	Chitosan and PVA	Electrospinning	Nanofibrous	MG63	Enhanced adhesion and proliferation	[2]
Fe_3O_4 (8.47 nm)	PLGA	Electrospinning	Nanofibrous	Ros17/2.8; MC3T3-E1	Enhance proliferation and induce differentiation	[4]
Commercial magnetic NPs (50–100 nm)	PCL	Electrospinning	Nanofibrous	Porcine mesenchymal stem cells (MSCs)	Enhanced adhesion, proliferation, and ALP activity increased	[12]
Fe_3O_4 NPs (20–40 nm)	Hydroxyapatite and collagen	Assembly and drop-wise	Porous	Human bone marrow mesenchymal stem cells (hBMSCs)	Support adhesion, proliferation, and differentiation	[3]
$SrFe_{12}O_{19}$ (30 nm in thicknesses, 50–150 nm in widths	Bio-glass and Chitosan	Sol-gel	Mesoporous	Human bone marrow mesenchymal stem cells (hBMSCs)	Promote proliferation and induce differentiation	[20]
Mg_2SiO_4-$CoFe_2O_4$		Sol-gel	3D porous	MG63	Good biocombability	[29]
γ-Fe_2O_3 (10 nm)	PLGA/PCL	Electrospinning and layer-by-layer assembly	3D macroporous	Adipose-derived stem cell (ADSCs)	Enhanced adhesion and differentiation	[23]
Fe_3O_4 NPs	Chitosan	Covalent conjugation and co-precipitation	3D sphere	mesenchymal stem cells (MSCs)	Promoted viability and mineralization	[21]
FeCaP (<2 μm)	Calcium phosphate	One-pot hydrothermal and post-reduction annealing	Microsphere	Adipose-derived stem cell (ADSCs)	Good biocompatibility	[16]

(continued)

Table 1.1 (continued)

Magnetic component	Other components	Preparation methods	Structure	Cell and animal model	Biological effects	Refs.
Fe_3O_4 NPs	Hydroxyapatite	Dissolution–precipitation reaction, coating	Orientated nanorods	Human bone marrow mesenchymal stem cells (hBMSCs)	Better adhesion, spreading and proliferation	[9]
Fe_3O_4 NPs (5–10 nm)	PCL	Salt-leaching	Porous	Human dental pulp cells (hDPCs)	Enhance adhesion, migration, and induce differentiation	[13]
Fe_3O_4 and Fe_2O_3 NPs	Hydroxyapatite/collagen	Hybrid assembly	Tri-layer porous	Mesenchymal stem cells (MSCs)	Favor viability and proliferation	[22]
Fe_3O_4 NPs (10.8 nm)	PCL	Electrospinning	Nanofibrous	Human dental pulp cells (hDPCs) and human umbilical vein endothelial cells (HUVECs)	Enhance proliferation and induce differentiation, pro-angiogenesis	[15]
γ-Fe_2O_3 (19.8 nm)	Thrombin/bFGF	Physical/covalent conjugation	3D porous	Nasal olfactory mucosa (NOM) cells	Show migration, growth, and differentiation	[6]
Fe_3O_4 NPs (9 nm)	Collagen	Electrospinning	Aligned fibers	Neurons PC12	Leading neurons to be elongated and directed	[11]

Table 1.2 Effects of magnetic scaffolds in combination with magnetic fields on tissue regeneration

Magnetic component	Other components	Preparation	Structure	Magnetic field	Cell and animal model	Biological effects	Refs
γ-Fe_2O_3 NPs (14 nm)	PLA/hydroxyapatite nanoparticles	Electrospinning	Nanofibrous	1 mT to cells, 25 mT to rabbit	MC3T3-E1 Rabbit model of lumbar transverse defects	Enhanced proliferation and differentiation in cell Accelerated repair for bone defect	[1, 5]
Fe_2O_3 NPs (10 nm)	PCL	Salt-leaching	Porous	15–25 mT	Mouse calvarial osteoblasts and Human umbilical vein endothelial cells (HUVECs) 5-mm-diameter calvarial critical-sized defect of mice	Differentiation, endothelial cells angiogenesis In vivo results showed improved bone formation	[9]
Commercial magnetic NPs (50 nm)	Composite of hydroxyapatite and collagen	Concurrent nucleation and immersed in MNPs colloid	Porous	1.2 T	Cylindrical defects in the lateral condyle of the distal femoral epiphysis on rabbits	Well-ordered regenerated tissue and higher level of bone maturation	[6, 10]
Fe_2O_3 NPs (8 nm)	Hydroxyapatite	Microwave-assisted foaming and immersing	Porous	10 Oe, 50 Hz	ROS 17/2.8 MC3T3-E1	Enhanced proliferation and differentiation	[4]
FeHA	Collagen	pH-driven self-assembly	Porous	320 mT	MG63	Promote proliferation and differentiation	[7]
Fe_2O_3 NPs	nHA/PLLA	Low-temperature rapid prototyping	3D porous	100 mT	Bone marrow mesenchymal stem cells (bMSCs)	Induce differentiation	[12]
Fe_3O_4 NPs	PLLA	Electrospinning	Nanofibrous	100 mT	MC3T3-E1	Promote adhesion, proliferation, and differentiation	[8]

(continued)

Table 1.2 (continued)

Magnetic component	Other components	Preparation	Structure	Magnetic field	Cell and animal model	Biological effects	Refs
Fe_3O_4 NPs (10 nm)	PLA/hydroxyapatite nanoparticles	Electrospinning	Nanofibrous	10 mT	Bone marrow mesenchymal stem cells (BMSCs)	Induce differentiation	[9]
γ-Fe_2O_3 NPs (10 nm)	PLA/nHA	Electrospinning	Nanofibrous	10 mT	MC3T3-E1, Bone marrow mesenchymal stem cells (bMSCs)	Promote differentiation, angiogenesis and osteogenesis	[36]
$CoFe_2O_4$ nanoparticles	PVDF and methacrylated gellan gum (GGMA) gel	Casting	Film	220 mT	MC3T3-E1	Good viability	[52]
$CoFe_2O_4$ nanoparticles	$CoFe_2O_4$/PVDF nanoparticles	Using a nylon template	3D porous	230 Oe at a frequency of 0.3 Hz	MC3T3-E1	Higher proliferation rate and elongated morphology	[53]
Terfenol-D particles	P(VDF-TrFE) polymer	Casting	Film	Varying magnetic field	MC3T3-E1	Enhanced the proliferation of pre-osteoblast	[54]
Fe_3O_4 NPs	Collagen gel	Plastic compression	3D collagen	72–144 mT	MG63	Promote proliferation, mineralization, and differentiation	[16]
Iron oxide nanoparticles (4–20 nm)	Collagen/ hydroxyapatite	Direct laser writing	3D multilayered	100–250 mT	MG63	Enhance proliferation and induce differentiation	[17]
Fe_2O_3 NPs	Silk fibroin/chitosan	Freeze-casting	Hydrogel with lamellar structure	3 mT	MG63	Good biocompatibility	[42]
Fe_3O_4 (250 nm)	κ-Carrageenan hydrogels	Casting	Membrane	350 mT, 2 Hz	Human adipose-derived stem cells (hADSCs)	Promote chondrogenic differentiation	[18]

Commercial magnetic NPs	RADA16 peptide		Hydrogel	1 mT with a pulse frequency of 15 Hz	Human adipose-derived mesenchymal stem cells (hASCs)	Enhanced osteogenic differentiation	[44]
FeHA nanoparticles	PCL	Pellets assembly	Cylindrical scaffolds	30 mT under the frequency 70 Hz for 6 h with 20 intervals of 18 min	Human mesenchymal stem cells	Better cell adhesion and spreading	[50]
Commercial magnetic NPs (100 nm)	Agarose gel	Partial thermal setting	Trilayered	0.4, 0.5 or 0.75 T	Bovine chondrocytes	Good viability	[19]
Fe_3O_4 MNPs	Poly(lactic-co-glycolic acid) (PLGA)		3D porous	45 mT	D1 mouse MSCs	Increase adhesion and migration and chondrogenic differentiation	[61]
Fe_3O_4	Agarose hydrogels	Covalently bound	Porous	0.8 T	Human mesenchymal stem cells (MSCs)	Induce chondrogenesis	[20]
Superparamagnetic iron oxide NPs (11–12 nm)	PLLA/collagen	Electrospinning	Fibrous	0.03–0.62 T	Primary neurons	Promote neurite outgrowth and directional guidance	[35]
Magnetic NPs (100 nm)	Alginate gels	Magnetic manipulation	3D hydrogel	255 G	Primary neurons PC12	Enhanced growth and elongation	[68]
Fe_3O_4 NPs (30 nm)	Chitosan/ glycerophosphate	Freeze-dry	Porous	2 mT, 50 Hz	Schwann cells; left sciatic nerve of SD rats	Enhance proliferation and higher regeneration-related gene expression In vivo, viability increased, nerve regeneration and functional recovery promoted	[28, 80]

(continued)

Table 1.2 (continued)

Magnetic component	Other components	Preparation	Structure	Magnetic field	Cell and animal model	Biological effects	Refs
γ-Fe_2O_3 (10 nm)	PLA/Hydroxyapatite nanoparticles	Electrospinning	Nanofibrous	5 mT, 10 mT	Macrophage cell line RAW264.7 Subcutaneous implantation of C57 mice	Enhanced proliferation, promote M2 phenotype polarization Conditioned medium-enhanced angiogenesis and osteogenesis Enhanced angiogenesis around the implant	[32]
Fe_3O_4 NPs	Alginate	Freeze-dry	Macroporous	10–15 Oe, 5 Hz	Cardiac cells, fibroblasts	Promote adhesion, spreading, and integration of cardiac patch	[26]
γ-Fe_2O_3 (10 nm)	PLA/nHA	Electrospinning	Nanofibrous	5 mT, 10 mT	NIH3T3	Promote pro-regeneration phenotype polarization, induce angiogenesis, and osteogenesis	[33]
Fe_3O_4 nanoparticles	Mixture of fibrin and agarose	Casting	Hydrogel	0–48 kA/m in intensity	Oral mucosa fibroblasts	The mechanical properties of the substitutes could be reversibly tuned by the magnetic fields	[78]
Fe_3O_4 NPs	Alginate	Freeze-dry	Macroporous	10–15 Oe, 40 Hz	Bovine aortic endothelial cells	Promote proliferation and vasculogenesis	[24, 25]

1.4.1 Bone

To verify whether magnetic scaffolds can be actuated by external applied magnetic field and play roles together in the scaffold-guided bone tissue regeneration, a nanofibrous composite film was fabricated with poly lactide (PLA), hydroxyapatite nanoparticles (nHA), and γ-Fe_2O_3 nanoparticles of 14 nm in diameter by using electrospinning technique, while nanofibrous films composed of PLA and nHA was fabricated as control. The magnetic composite film displayed superparamagnetic property, showing an almost immeasurable coercive force and remanence. For the cell culture, the films were placed on the bottom of cell culture wells, and the static magnetic field (MF) was set up by fixing permanent magnets to the culture system. There was a high intensity area along the plate sides, which decreased sharply and form a homogeneous and low intensity area in some culture wells with the intensity of 1 mT, where pre-osteoblasts (MC3T3-E1) were seeded. It was not surprise that the superparamagnetic nanoparticles of γ-Fe_2O_3 embedded in the fibers and the applied static magnetic field acted in a synergistic way to boost the cells proliferation, but striking and more importantly, the combination strategy enhanced the osteogenic differentiation of cells, evidenced by the significantly increased ALP amount after 17 days of culture than that on the same composite films but without the applied magnetic field. This result for the first time clearly showed a sign that γ-Fe_2O_3 nanoparticles incorporated in the PLA and the magnetic field enhanced the osteogenesis in a synergistic way [36]. Further animal experiments validated this concept in vivo. The magnetic composite film was folded into scaffolds with proper shape and size and implanted in white rabbit model of lumbar transverse defects. The rabbits after surgery were housed in cages, permanent magnets were fixed to the two sides of the cages to provide static magnetic field of 25 mT in the middle area or housed in normal cages as control. The rabbits implanted with magnetic scaffolds and stayed in the magnetic cages showed faster new bone formation and scaffold degradation than those stayed in normal cages (Fig. 1.1). Besides, the former exhibited the higher level of OC and collagen in the tissue of implanted scaffold, indicating more new bone formation. More evidence came from the monitoring by CT scan. The CT images showed incomplete fracture in the right transverse process in group of magnetic scaffold (group S) and the combination of magnetic scaffold and magnetic field (group S+M) on day 10 after implantation, when the filled defect exhibited not homogeneous with blurred bright spots in the two groups. On the day 50, the cortical bone had become connected and homogeneous, and the bone marrow cavity was mostly clear for group S+M, instead, there was still nonhomogeneous bone density hyperplasia for group S (Fig. 1.2). These indicated that the applied static magnetic field promoted the density increase of the defects area. On the day 90, bony connection was further improved in both groups. The newly formed bone in group S+M became more homogenous, and the shape was close to that of natural bone, suggesting the repair process had completed, while the process in group S not yet [37]. These results indicated that the combination of superparamagnetic scaffold with external applied magnetic field provided a novel strategy for

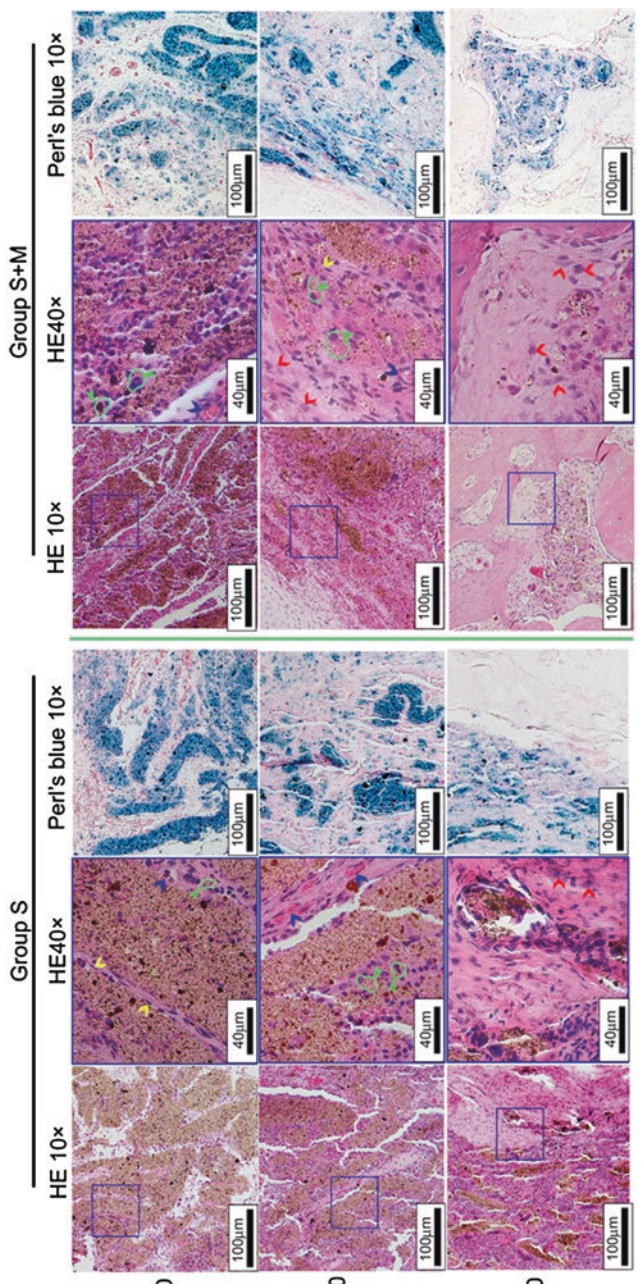

Fig. 1.1 Representative histological images of the scaffolds implanted in the bone defect on day 10, 20, 30 post implantation. Left column: Groups S; Right column: Group S1M. Macrophages were circled by green line and pointed by green arrow; Fibroblasts were pointed by yellow arrow; Vessels were pointed by blue arrow; Osteoblast cells were pointed by red arrow [37]