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REVIEW ARTICLE

Magnetic responsive scaffolds and magnetic fields in bone repair and regeneration

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ABSTRACT: Increasing evidence shows that magnetic fields and magnetic responsive scaffolds can play unique roles in promoting bone repair and regeneration. This article addresses the synergistic effects of magnetic scaffolds in response to external magnetic fields on the bone regeneration *in situ*. Additionally, the exploration of using magnetic scaffolds as tools in the bone implant fixation, local drug delivery and mimicking microenvironment of stem cell differentiation are introduced. We also discussed possible underlying mechanisms and perspectives of magnetic responsive scaffolds in the bone repair and regeneration.

KEYWORDS: bone repair; magnetic scaffold; magnetic field; physical stimulation

Contents

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1 Introduction

Scaffold-guiding regeneration plays crucial roles in the repair of critical size bone defects resulted from trauma, tumor, resection and skeletal abnormalities. Successful regeneration largely depends on interface interactions between bone related cells and scaffolds. In the past decades great efforts have been made to explore varieties of scaffolds as alternatives to autologous or allogeneic bone grafts in the bone regeneration and progresses have been made [1–4]. Nevertheless, there is still a large space for improvement to meet clinical requirements.

As it is well known, bone is a mechanical sensitive

tissue. Mechanical loads generated by gravity and locomotion stimulate local bone remodeling to maintain optimal mechanical performance, while reduced mechanical stimulation associates with sedentary lifestyle, limb paralysis, or space flight results in net bone loss through reduced bone formation and increased bone resorption. Osteoblast cell is one kind of bone related effector cells. It has been accepted that proper physical stimulation can play positive roles in the osteogenesis. Mechanical stimulators such as stress, strain, strain energy, and strain rate have been demonstrated enhancement to bone regeneration and fracture healing [5–10]. Besides mechanical force stimuli, effects of the magnetic stimulation originated from static magnetic field (SMF) or electromagnetic field (EMF) on the wound healing have also been explored for many years. Inspired by the magnetic stimulation effects, magnetic responsive scaffolds have attracted increasing research interests recently. In this article, we will review the investigation progress in the roles of magnetic fields, magnetic scaffolds and the synergy effects of the both in the bone repair and regeneration.

2 Effect of magnetic fields alone on the osteogenesis and bone-graft interface osseointegration

Several groups have reported that the weak or moderate SMF promoted bone formation *in vitro* and *in vivo* [11–15]. Strong SMF of 5–10 T was also reported to have the potency of regulating the orientation of matrix proteins and cells *in vitro* and *in vivo* [16–19]. Yamamoto et al. [20] have established that bone formation from rat calvaria cells was enhanced by SMF to similar extent for the magnetic flux density at 160, 280 or 340 mT. The enhancement mechanism of SMF is involved with the activation of p38 phosphorylation at the cellular level to stimulate osteoblastic differentiation [21]. SMF was also used to prevent the decrease in bone density caused by surgery or implantation [22]. In addition to SMFs, pulse EMFs of Gauss order are one effective stimuli that promotes bone fracture healing [23–25], spinal fusion [26], tibial delayed unions and nonunions [27], and bone ingrowths into ceramics in animal models [28].

In recent years, impacts of the magnetic stimulation on human mesenchymal stem cells (hMSCs) have been explored, as in the past decade, 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. One example is the study of low-frequency EMF on hMSCs during chondrogenic differentiation [29]. In the study, cultures were exposed to homogeneous sinusoidal extremely low-frequency magnetic fields (5 mT) produced by a solenoid or were kept in a control system. Researchers found 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 sinusoidal EMF (5 mT) are able to improve the chondrogenic differentiation of hMSCs under the influence of growth factors (TGF-β3 and FGF-2). Meanwhile, researchers noted that the EMF alone did not induce chondrogenic differentiation. When growth factors were omitted, there was no staining detected for safranin-O and collagen type II. Dental pulp cells (DPCs) can be regarded as stem cells because they are not a homogeneous cell population and contain progenitor/stem cells that can differentiate into dentin-forming odontoblasts and maintain the homeostasis of dental mineralized tissue by polarization and secretion of predentin–dentin components, in response to the appropriate stimuli [30–31]. Effects of the SMF exposure on the cell viability and capacity of osteogenic differentiation in rat DPCs were investigated [32] by the examination of osteogenic marker genes of alkaline phosphatase (ALP), osteopontin (OPN) and osteocalcein (OCN). ALP activity, extracellular calcium concentration, mineralization and ERK-Cbfa1 signaling pathway of rat DPCs in the presence/absence of Dex/b-GP induction were examined *in vitro*. It is interesting to note that exposure to SMF alone (Basal/SMF) did not affect the DPC proliferation either, only exposed to SMF with the Dex/b-GP, the cell differentiation was significantly enhanced at the 7th and 9th day of culture when compared with the control cells (Dex/b-GP/Con). Researchers speculated that the magnetic field may play a cooperative role to accelerate osteogenic differentiation, the maturation of osteoblast-like cells, and mineralization, via the extracellular matrix (ECM)-mediated activation of ERK1/2-Cbfa1 signaling. A further study demonstrated that the magnetic field can stimulate biological functions of MSC; however, the responses of cells depend strongly on the substrate to which they adhere and on the cross-talk between integrin-mediated signals and soluble factors [33].

These results imply that the magnetic stimulation alone on cells is not sufficient for the bone repair and regeneration, the microenvironment where the bone cells live is likely to play crucial synergy roles in the regeneration process.

3 Composites containing magnetic nanoparticles are bone cell-friendly and have potentials as bone substitutes

For the repair *in situ* of bone defects, scaffolds play crucial roles, not only providing the environment for bone cells adhesion, but also guiding cells proliferation and differentiation. In addition, artificial implants are also used as one of important treatments for the bone repair; their osseointegration enhancement is very crucial [34]. Inspired by the enhancement effect of magnetic fields, researchers integrated magnetic nanoparticles (MNPs) in various matrices to fabricate magnetic composites and explore the potential for uses as bone scaffolds or substitutes in recent years. Gu's group fabricated two kinds of ceramic composites containing super-paramagnetic nanoparticles [35]. The two ceramic substrates are hydroxyapatite (HA) and the composite of HA and tricalcium phosphate (TCP). The composite samples were cultured with Ros17/2.8 and MG63 cells *in vitro* to evaluate their effects on the cell proliferation and differentiation. Results indicate that the composites have good biocompatibility with the bone cells. It is also demonstrated that the super-paramagnetic nanoparticles integrated in the composites do not affect the function of the bone morphological protein (BMP) bounding to the composites. In the rat-subcutaneous implantation model, the composite composed of HA–TCP, MNPs and BMP-2 accelerated new bone-like tissue formation. Polymeric materials allow more processing options for the scaffold fabrication when used as substrates. For instance, a magnetic biodegradable Fe_3O_4/chitosan (CS)/poly (vinyl alcohol) (PVA) nanofibrous film was fabricated by electrospinning, with average fiber diameters ranging from 230 to 380 nm and porosity of 83.9%–85.1% [36]. The film exhibits weak ferrimagnetic behaviors and cell-friendly performance to MG63 human osteoblast-like cells, suggesting that this magnetic biodegradable Fe_3O_4/CS/PVA nanofibrous film can be one of promising biomaterials for facilitation of osteogenesis. A recent study reported that the Fe_3O_4 nanoparticles even benefit the improvement for the substitute osseointegration [37]. Researchers fabricated magnetic hydroxyapatite coatings (MHACs) with oriented nanorod arrays using magnetic bioglass coatings (MBGCs; $CaO–SiO_2–P_2O_5–Fe_3O_4$) as sacrificial templates. The MBGCs were converted to the MHACs in a simulated body fluid (SBF) via a dissolution–precipitation reaction. The formed HA nanorods with a preferential (002) orientation were perpendicular to the coating surfaces. The Fe_3O_4 nanoparticles in the coating improved the nucleation rate of HA, so the elongated HA nanocrystals were remained even after hydrothermal reaction for 3 d. It is interesting that the HA nanorods were turned into the blocky HA particles with increasing the reaction time from 12 to 24 h if there was no MNPs in the bioglass coatings (BGCs). Moreover, the presence of Fe_3O_4 nanoparticles improves the MHACs hydrophilicity. Human bone marrow stromal cells (hBMSCs) exhibited the better adhesion, spreading and proliferation on the MHACs than those on the BGCs or MBGCs due to the presence of HA phase, good hydrophilicity and oriented nanorod arrays.

4 Synergistic effects of magnetic scaffolds on guiding bone regeneration in response to external magnetic fields

Paramagnetic materials can response to external applied magnetic fields, showing small and positive susceptibility. The magnetic properties do not persist if the external magnetic field is removed. Regarding to the magnetic responsive property of super-paramagnetic nanoparticles, we particularly address ourselves to the synergy effect of magnetic scaffolds with magnetic fields on osteoblast cells [38]. To fabricate a paramagnetic nanofibrous film, nanoparticles of γ-Fe_2O_3 with an average diameter of 14 nm (MNP) and nano-hydroxyapatite (nHA) with an average diameter of 50 nm were dispersed in N, N-dimethyl-acetamide (DMAc) with the aid of sonication, followed by dissolving polylactic acid (PLA) with a molecular weight of 10 kD in the suspension. The mixture solution was processed into nanofibrous films (MNP/nHA/PLA) by electrospinning. The film has nanofibrous network under scanning electron microscopy (SEM) with the average diameter of (805±113) nm. The pores formed by entangled fibers are mostly in micrometer scale, majority of the pore size under SEM is about 10–20 μm. The MNP mainly located inside the fibers, while nHA distributed near the surface of the fibers. The films can be folded and fixed to pellets with the fixed volume and mass, allowing for uses as bone substitutes in the implantation. In the nanofibrous composite, MNP is used to give super-

paramagnetic response, nHA is for bone conductivity and PLA for nanofibers fabrication. Importantly, the nanofibrous composite films display a paramagnetic property with the saturation magnetization of 0.0492 emu/g and show an almost immeasurable coercive force and remanence (Fig. 1).

In our study, MC3T3-E1 was used, which is a pre-osteoblast cell line that differentiates into osteoblasts when cultivated in inductive culture medium. Four groups were set: group I, nHA/PLA film; group II, nHA/PLA film under a magnetic field; group III, MNP/nHA/PLA film; and group IV, MNP/nHA/PLA film under a magnetic field. The magnetic field of 0.9–1.0 mT was applied to the cultured cells using magnet rods. Results from the viability assay show that groups II and IV obtained significantly increased proliferation rate in reference to groups I and III respectively, which is consistent with the results reported in the literatures that SMFs stimulated bone tissue regeneration. It is noticeable that group IV gained the higher degree of proliferation increase than group II before 15th day of the culture in reference to the corresponding control, which clearly indicates super-paramagnetic nanoparticles integrated in the scaffold play a synergy role in the cell proliferation with the applied magnetic field. Furthermore, osteoblast cells in group IV reached the end of proliferation period and started their differentiation faster than those in the other groups.

In the new bone formation, the ECM production by osteoblast cells is one of the very crucial factors that lead to the ultimate formation of new bone tissue. ALP is one of the key substances in the ECM, which is also indicative whether osteoblasts have entered the period of ECM development and maturation. Under the magnetic field, cells growing on MNP/nHA/PLA films produced a significantly more ALP than those growing on the nHA/PLA films over the experimental period of 17 d (Fig. 2). Moreover, cells in group IV were surrounded by thicker ECM substance. Similar experimental results *in vitro* were reported by other groups. Gu's group fabricated magnetic responsive HA-based scaffold using a simple immersing method [39]. The scaffolds response to the external magnetic field and engender some synergistic effect to intensify the stimulating effect of a magnetic field to the proliferation and differentiation of cells. Tampieri et al. reported similar porous ceramic composite made of HA and magnetite showing biocompatibility *in vivo*. Additionally, enhanced cell proliferation was observed at the early stage *in vitro* under the external magnetic

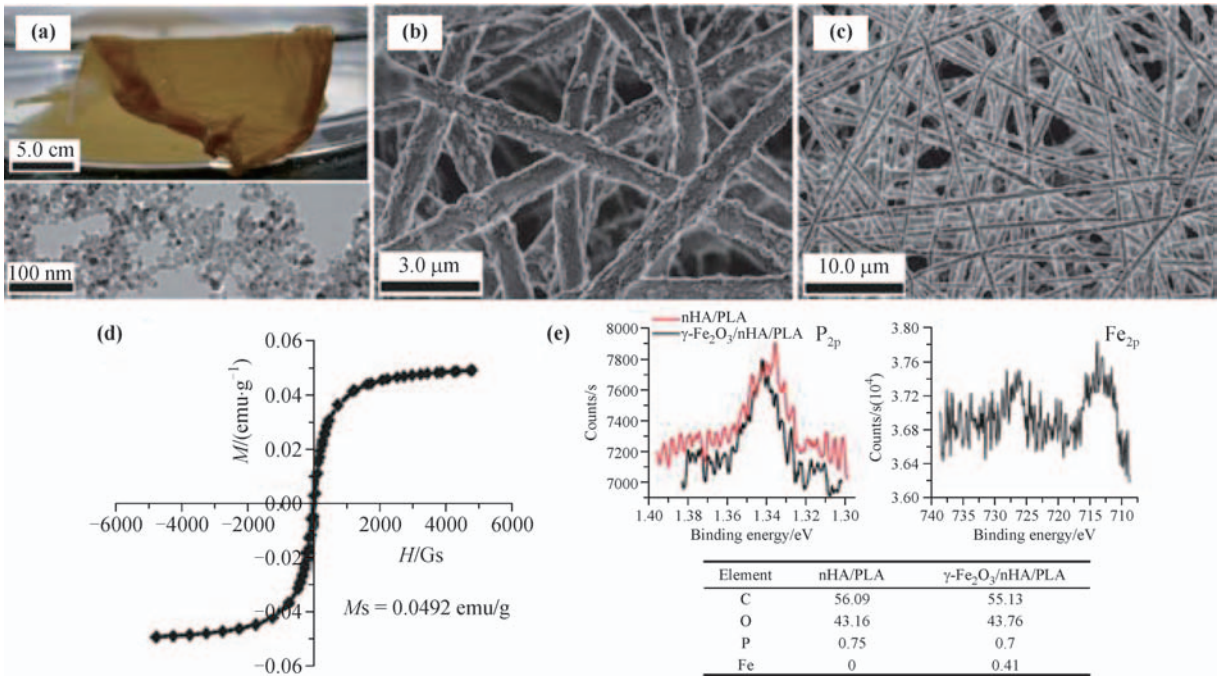

Fig. 1 Characterization of the nanofibrous composite film of γ-Fe_2O_3/nHA/PLA. **(a)** Optical graph of the film combined with a transmission electron microscopy (TEM) image of the γ-Fe_2O_3. **(b)(c)** SEM images showing that the fibers formed nonwoven mesh-like structures. **(d)** Magnetization curve of the film. **(e)** X-ray photoelectron spectroscopy (XPS) analysis for the nanofibrous composite film of γ-Fe_2O_3/nHA/PLA and nHA/PLA. The inserted Table summarizes the content of the element Fe and P on the film surface. (Reproduced with permission from Ref. [38])

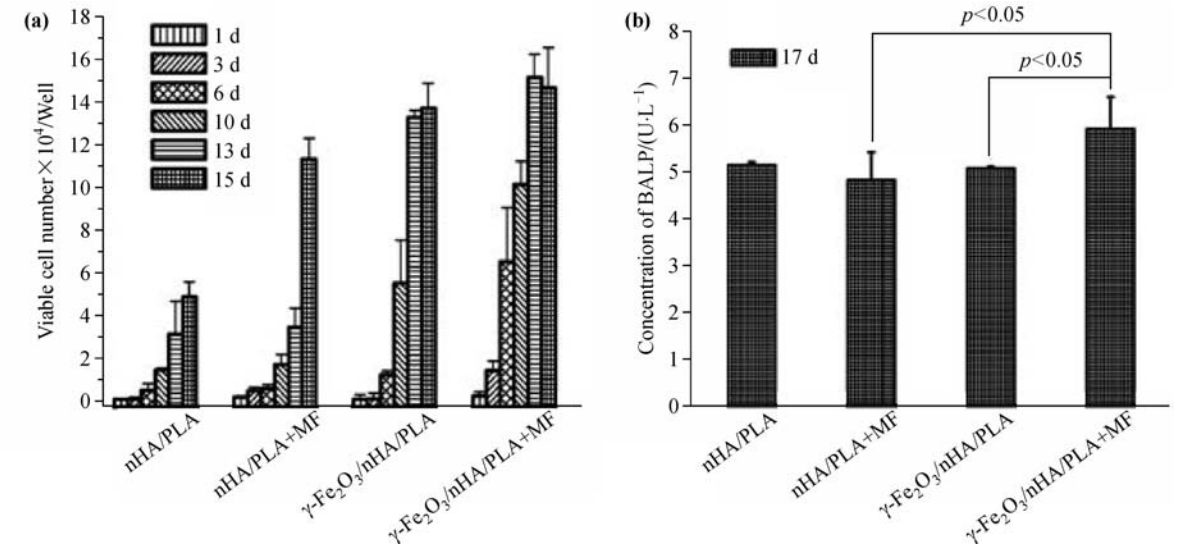

Fig. 2 **(a)** Proliferation and **(b)** ALP secretion of the pre-osteoblast cells seeded on different nanofibrous films. ''+ MF'' means applying a static magnetic field of 0.9–1.0 mT to the cells. (Reproduced with permission from Ref. [38])

field [40]. Zhou's group fabricated a nanofibrous scaffold composed of PLA and iron oxide nanoparticles. The scaffold exhibits biocompatibility as well as guided cells orientation along the fibers under the external magnetic field [41].

Encouraged by above results *in vitro*, we further validated *in vivo* osteogenesis enhancement of superparamagnetic nanofibrous scaffolds synergizing with external SMF [42]. The scaffolds were implanted in the lumbar transverse defect of New Zealand white rabbits. In order to have the rabbits exposed to an external magnetic field, animals were housed in cages with fixed permanent magnets in the two opposite sides (group S + M), while animals housed in standard cages were taken as a control (group S). The observation for the defect repair was lasted 110 d post the implantation.

Observations from hematoxylin–eosin (HE) and Perl's staining indicate that on the day 10 post the implantation surgery, the implanted scaffolds recruited host-derived cells migrating to the defects area, mainly including macrophages and fibroblasts. As time over, the implanted scaffolds were separated into smaller pieces by the cells, while osteoblast cells appeared and new bone tissue formed around the scaffold pieces, vessel structures were seen very near around the scaffold pieces, suggesting that oxygen and nutrients were supplied to support proliferation and differentiation of the osteoblast cells. During the repair process, both degradation rate and new bone formation for group S + M are faster than those for group S at each time point (Fig. 3). Moreover, Group S + M exhibited higher level of osteocalcin (OC) and collagen deposition than group S, indicating that the scaffold induces the higher activity of new ECM secretion in response to the applied magnetic field (Fig. 4).

Consistent with the pathological observation, computed tomography (CT) images show that the applied SMF accelerated the repair process. The shape of newly formed bone tissue in group S + M became homogenous faster than that in group S and very similar to that of natural bone on the day 90 when the shape in group S still showed thick and not continuous (Fig. 5). On the day 110, the bone amount for group S + M was lower than that for group S, suggesting that the remodeling process for group S + M is faster than that for group S. Bone remodeling is one crucial step in the late of defect repair, in which the newly formed bone tissue will be reshaping by osteoclast cells, thus the newly formed bone tissue can match the shape and size of natural bone well.

Besides new bone tissue formation, degradation rate for the scaffold is another important factor that influences the bone repair. We found that under the external magnetic field, the scaffold degraded faster than that without the external magnetic field. It is speculated that the recruited macrophages are more active when exposed with the external magnetic field. Underlying mechanisms and the relationship between macrophages, the magnetic field and the scaffold degradation are worth further exploitations, which will benefit the optimization of magnetic strength and scaffold composition to elicit stronger osteogenesis effects.

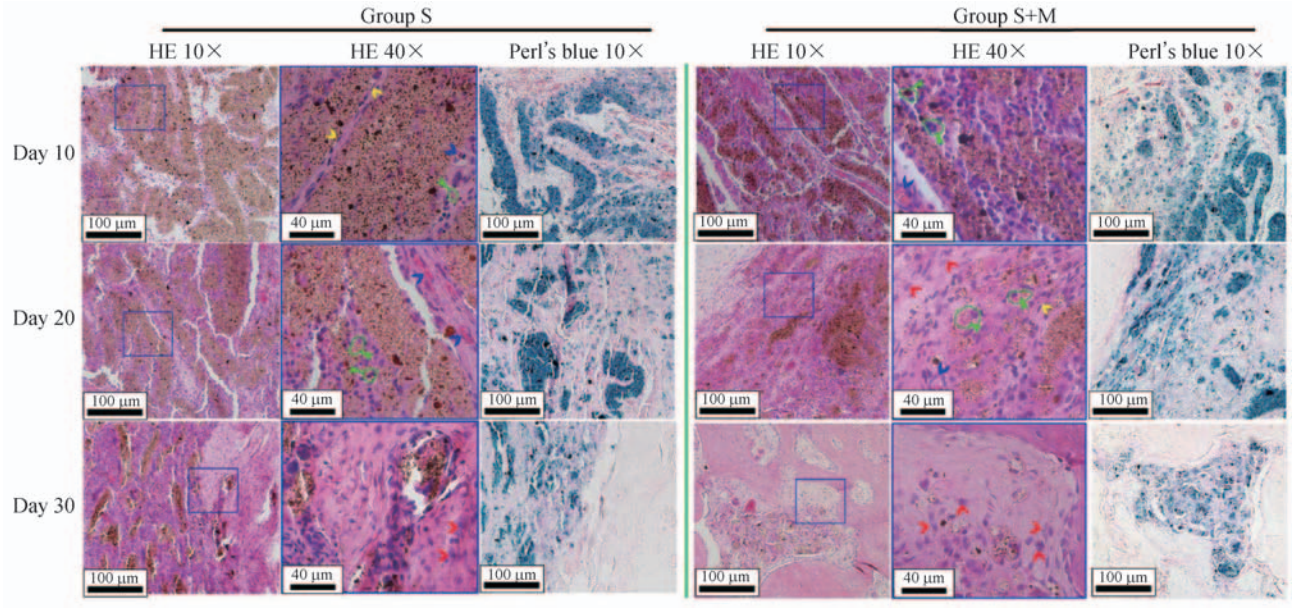

Fig. 3 Representative histological images of the scaffolds implanted in the bone defect on day 10, 20, 30 post implantation. Left column: Groups S; Right column: Group S + M. Macrophages were circled by green line and pointed by green arrow; Fibroblasts were pointed by yellow arrow; Vessels were pointed by blue arrow; Osteoblast cells were pointed by red arrow. (Reproduced with permission from Ref. [42])

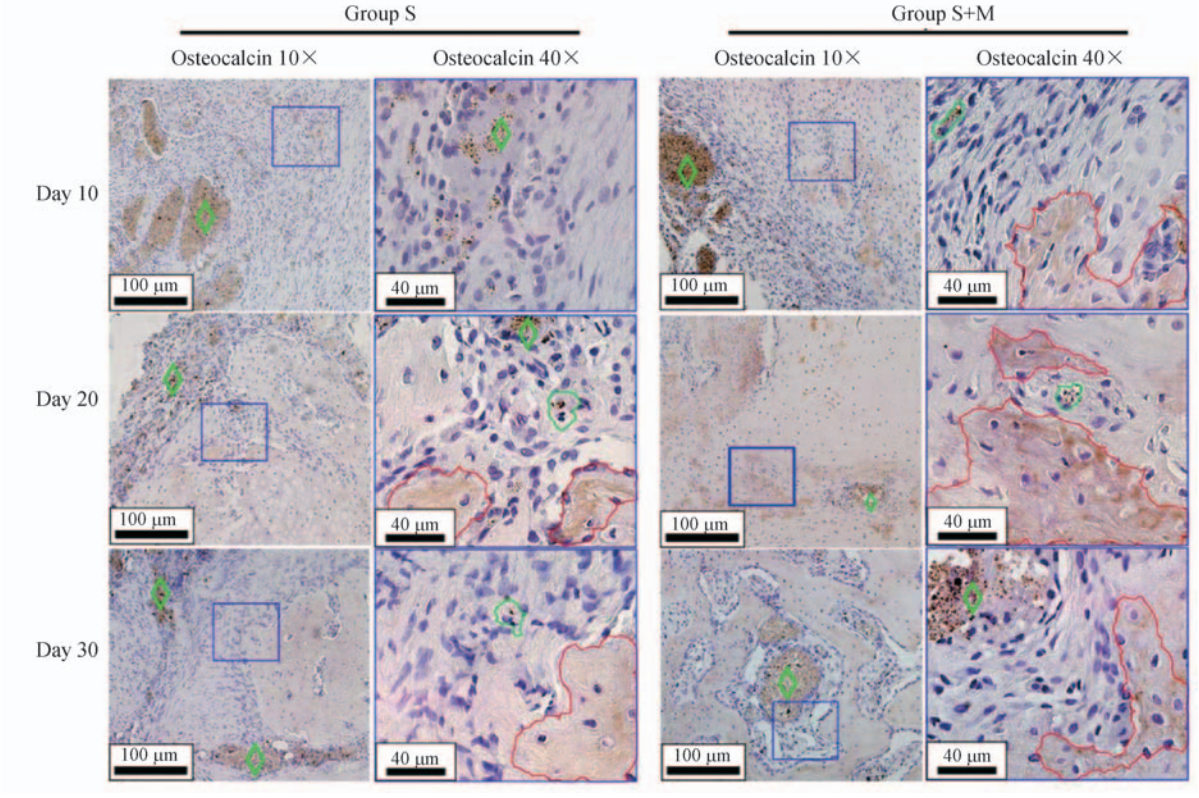

Fig. 4 OC expression induced by the scaffolds implanted in the bone defect on day 10, 20, and 30 post implantation. Left column: Groups S; Right column: Group S + M. OC positive cells were circled by red line; the scaffolds were labeled by green rhombus. (Reproduced with permission from Ref. [42])

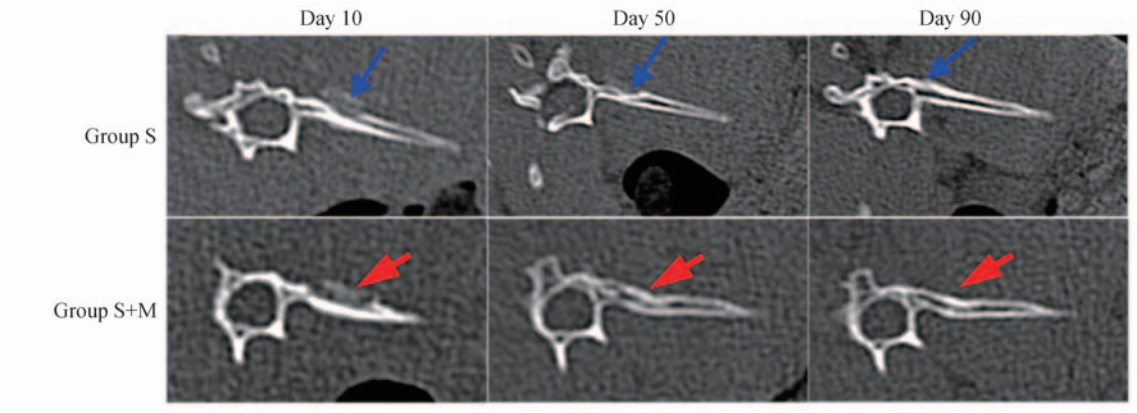

Fig. 5 CT images of the bone defects for group S and Group S + M post 10, 50 and 90 days implantation. The arrows pointed to the defects. (Reproduced with permission from Ref. [42])

5 Magnetic scaffolds as tools in bone tissue regeneration

The content of iron in the composite is expected to be useful in the local drug delivery for bone defects resulting from malignant bone tumors. For instance, Xiao et al. fabricated an iron (Fe)-containing multifunctional mesoporous bioactive glass (MBG), so called (Fe-MBG) scaffold to meet this target [43]. The incorporation of Fe into MBG scaffolds enhances the mitochondrial activity and the expression of bone-related genes (ALP and OCN) in human bone marrow MSCs. It is interesting that the content of Fe leads to morphological changes in the scaffolds; the mesopores varied from straight channels to curved fingerprint-like channels as well as possesses high specific surface areas, which allows sustained drug delivery. Furthermore, the magnetism of the composite scaffolds can be tailored by controlling the Fe content, which is advantageous for hyperthermia treatment. Regarding to the magnetic property of iron oxide nanoparticles, Dediu et al. proposed that magnetic scaffolds could act as a conceptually "station" for *in vivo* attracting growth factors or stem cells that are bound with MNPs by the magnetic guiding process [44]. Researchers fabricated magnetic scaffolds by two ways: dip-coating conventional HA scaffolds in aqueous ferrofluids containing iron oxide nanoparticles coated with various biopolymers or direct nucleation of biomimetic phase and superparamagnetic nanoparticles on self-assembling collagen fibers [45]. The nanoparticles integrated in the scaffolds provide the substrate with magnetization values suitable for generating magnetic gradients, enabling magnetic guiding in the vicinity and inside the scaffold. Besides, the magnetic phase in the collagen scaffold also acted as a sort of cross-linking agent for the collagen, inducing a chemico–physical–mechanical stabilization of the material and allowing controlling the porosity network of the scaffold [46].

The iron content in scaffolds is also designed for the fixation of implanted substitutes to avoid the micromotion *in situ* [47]. In the experiment, two kinds of magnetic scaffold were fabricated: (i) MAG-A, composed of superparamagnetic nanoparticles and collagen, and (ii) MAG-B, composed of HA, super-paramagnetic nanoparticles and collagen. The scaffolds were implanted within the lateral condyles of the distal femoral epiphyses of rabbits; meanwhile, one cylindrical NdFeB magnet was implanted in close proximity to the magnetic scaffolds (Fig. 6). The NdFeB magnets not only provide SMF *in situ*, but also resulted in a well-ordered bone tissue and scaffold fixation at the tissue/biomaterial interface (Fig. 7) [48].

Magnetic scaffolds can even be an ideal model for investigating stem cells differentiation and cartilage and ligament tissue. As it is known, chondrocyte growth has been associated with demanding physical input such as cell deformation, hydrostatic pressure gradients, fluid flow, streaming currents and physicochemical changes [49]. For very porous, soft and elastic materials, direct mechanically connected systems are unfavorable and lead to scaffold distortions. Researchers mixed nano-sized metal magnets to the polymer strands of soft and flexible hydrogels, applying a force at each polymer strand to pull them moving using external magnetic field [49]. The vertical motion of the magnetic and soft hydrogel scaffold was controlled by a magnetic field (0.8 T) induced by an external electromagnet. The pressureless, soft mechanical stimulation precipitated by the cyclic deformation of soft, magnetic hydrogel scaffolds with an external magnetic

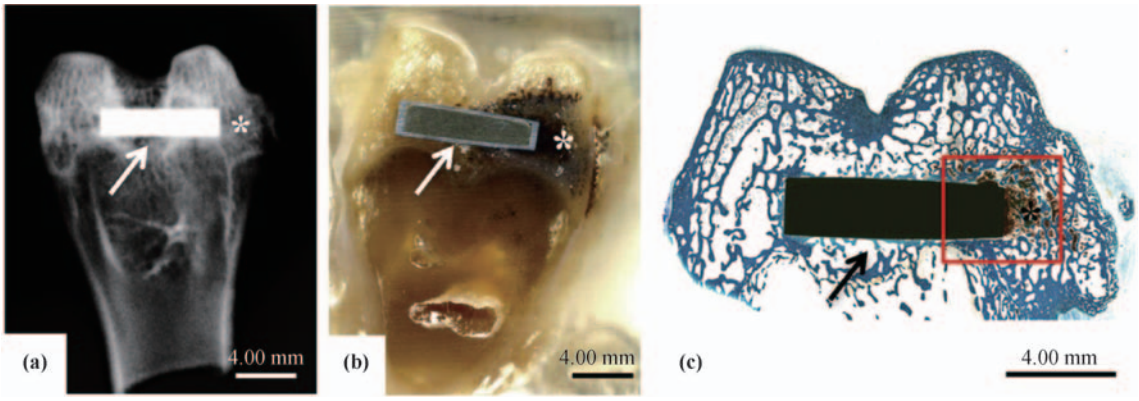

Fig. 6 *In vivo* positioning of magnets and scaffolds. **(a)** Radio graphic image of NdFeB magnet and scaffold placement. **(b)** Example of the bone tissue methyl-methacrylate embedded after the longitudinal cut. **(c)** Sample images digitalized with Aperio (Aperio Scanscope CS System, Aperio Technologies, Vista, CA — USA) at the highest resolution (1781×1467 pixels) for the histological evaluations and histomorphometric measurements performed in the selected ROI located at the interface between NdFeB magnets and magnetic or control scaffold. Toluidine Blue, Acid Fucsin and Fast Green staining were used. The arrows indicate NdFeB magnets while the asterisks highlight the magnetic scaffolds. (Reproduced with permission from Ref. [48])

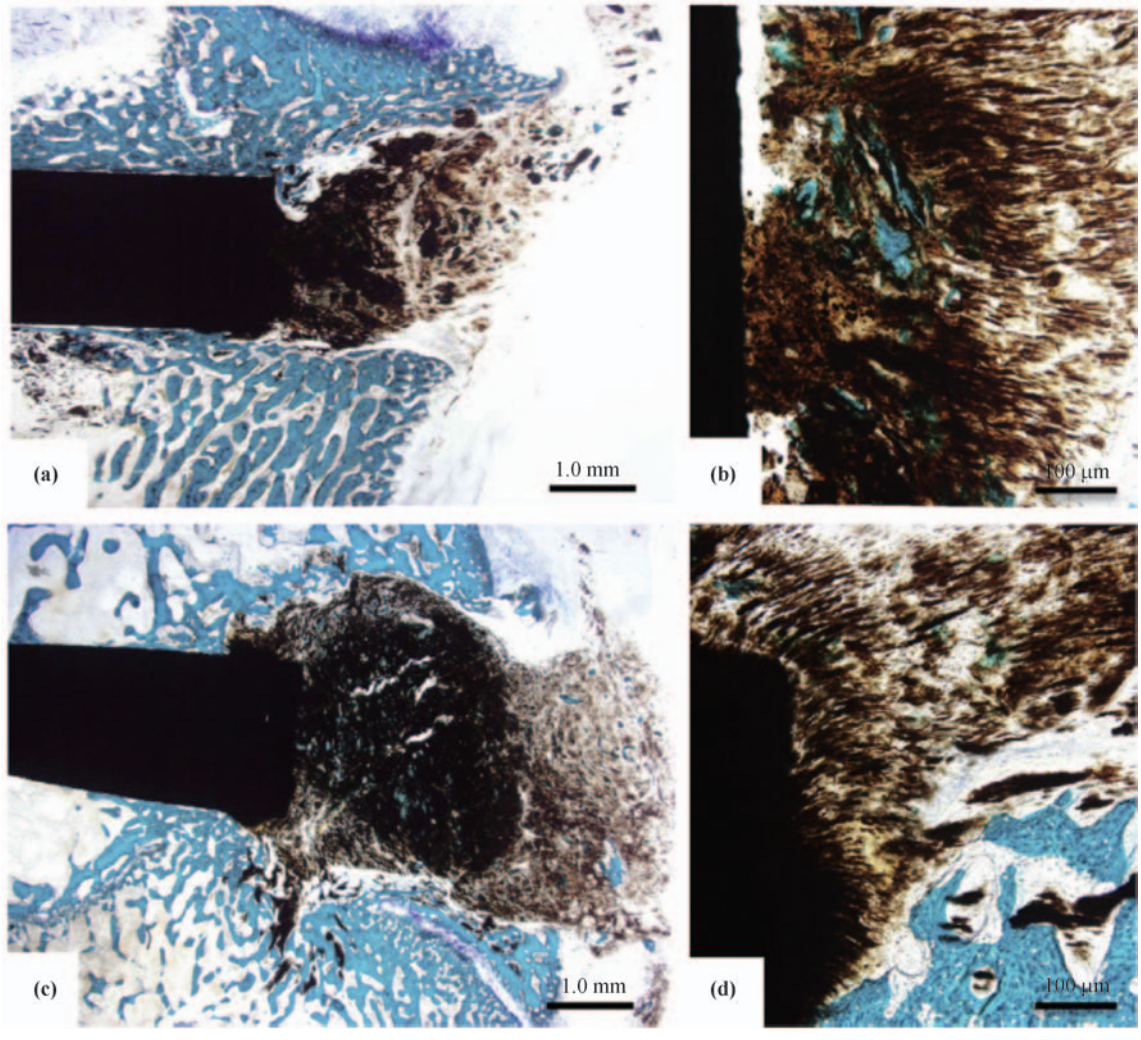

Fig. 7 Representative overview of the undecalcified histological sections obtained from **(a)(b)** MAG-A and **(c)(d)** MAG-B at 4 weeks. Magnetic scaffolds were attracted to NdFeB magnet. Thin bone trabeculae inside the scaffolds and mature trabecular tissue at the periphery of the magnetic scaffolds characterize the histological pattern of both magnetic scaffolds (b)(d). Images were acquired with opticmicroscope (BX51, Olympus Optical Co. EuropaGmbH, Germany) connected to Olympus XC50 camera. Toluidine Blue, Acid Fucsin and Fast green staining were used. (Reproduced with permission from Ref. [48])

field, can induce chondrogenesis in MSCs without any additional chondrogenesis transcription factors (TGF-β1 and dexamethasone). A systematic study on the role of movement frequency revealed a classical dose-response relationship for hMSCs differentiation towards cartilage using mere mechanical stimulation. The effect was synergistically amplified when exogenous chondrogenic factors and movement were combined. The elastic, soft, mechanical stimulation to the cell culturing device uses the highly magnetic, active part of hydrogel scaffolds to convey force through external application of EMFs (Fig. 8).

6 Perspectives for magnetic responsive scaffolds in bone regeneration

Increasing experimental data point out the clear role of magnetic stimulation on the bone cell viability and

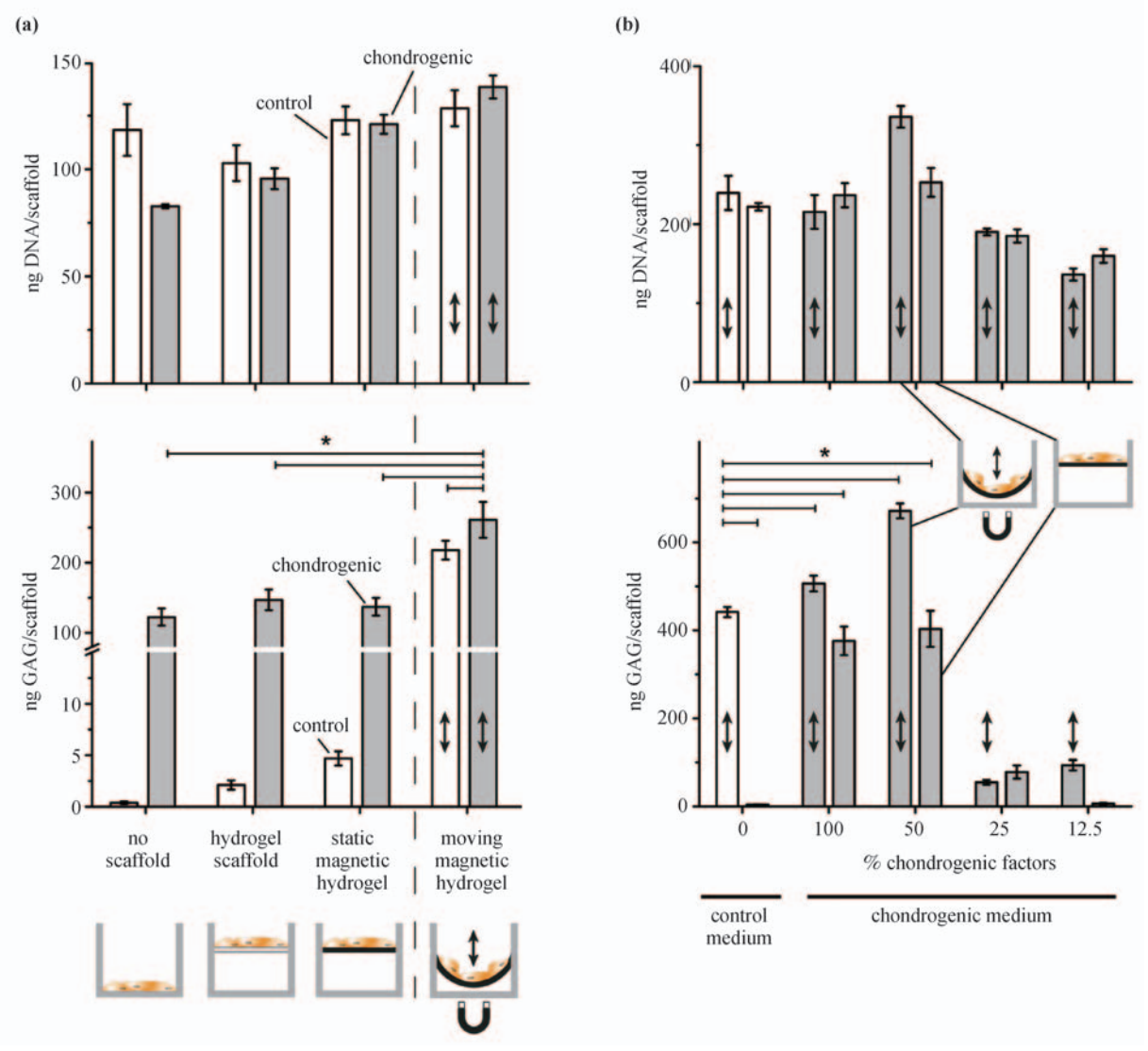

Fig. 8 Mechanical stimulation induced chondrogenesis. **(a)** Cell numbers (DNA amount per scaffold) confirmed good cell expansion and growth. Below is the glycosaminoglycan (GAG) deposition per scaffold over a period of 5 weeks. Control medium (white bars) and chondrogenic medium (grey bars) were applied on cells seeded into either tissue culture plate (no scaffold), hydrogel scaffold (no nanomagnets, i.e. no movement is possible) or magnetic hydrogel. Mechanical stimulation (arrow) triggered higher GAG deposition. **(b)** Comparable DNA amount indicated good cell growth for cells seeded into magnetic hydrogels with both medium types and no negative effects from mechanical stimulation. GAG deposition using diluted chondrogenic (grey) versus control medium (white bars). Mechanically stimulated hMSC in control medium showed comparable GAG deposition as in standard chondrogenic medium under magnetic actuation (indicated by ↕). * $p < 0.01$ cells cultured with control medium under mechanical stimulation versus non stimulated and mechanically stimulated hydrogel using both cell culture media. (Reproduced with permission from Ref. [49])

organization, in particular, the magnetic scaffold exposed to an external magnetic field enables synergic enhanced stimulation of osteoblasts and bone formation *in vitro* and *in vivo*. However, the mechanism of the bone cell stimulation in magnetically responsive scaffolds is not clear yet and urgently necessary to be investigated. We hypothesize that MNP in scaffolds would generate the microdeformation of scaffolds under the magnetic field, which give strain stimulation to cells growing on the scaffolds. The strain stimulation would activate the cells to proliferate and differentiate and form new bone tissue. The strategy of utilizing magnetically responsive scaffolds in response to the external applied magnetic field to accelerate the new bone formation is probably applicable to other tissues in the regenerative medicine. In addition, the synergy effect of this strategy may be amplified by combining with chemical signals (i.e. growth factors). Understandings of the mechanisms will help to develop optimal magnetic scaffolds to response proper magnetic fields for bone repair and regeneration.

Abbreviations

ALP	alkaline phosphatase
BGC	bioglass coating
BMP	bone morphological protein
CS	chitosan
CT	computed tomography
DMAc	N, N-dimethyl-acetamide
DPC	dental pulp cell
ECM	extracellular matrix
EMF	electromagnetic field
FGF	fibroblast growth factor
GAG	glycosaminoglycan
HA	hydroxyapatite
HE	hematoxylin–eosin
hBMSC	human bone marrow stromal cell
hMSC	human mesenchymal stem cell
MBG	mesoporous bioactive glass
MBGC	magnetic bioglass coating
MHAC	magnetic hydroxyapatite coating
MNP	magnetic nanoparticle
MSC	mesenchymal stem cell
nHA	nano-hydroxyapatite
OC	osteocalcin
OCN	osteocalcein
OPN	osteopontin
PLA	polylactic acid
PVA	poly (vinyl alcohol)
SBF	simulated body fluid
SEM	scanning electron microscopy
SMF	static magnetic field
TCP	tricalcium phosphate
TEM	transmission electron microscopy
TGF	transforming growth factor
XPS	X-ray photoelectron spectroscopy

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