Joints

The prospective opportunities offered by magnetic scaffolds for bone tissue engineering: a review

ALESSANDRO ORTOLANI[1], MICHELE BIANCHI[1], MASSIMILIANO MOSCA[2], SILVIO CARAVELLI[2], MARIO FUIANO[2], MAURILIO MARCACCI[1,2], ALESSANDRO RUSSO[1,2]

[1] Laboratory of Nano Biotechnology (NaBi), Istituto Ortopedico Rizzoli, Bologna, Italy
[2] I Orthopaedic and Traumatological Clinic, Istituto Ortopedico Rizzoli, Bologna, Italy

Abstract

Magnetic scaffolds are becoming increasingly attractive in tissue engineering, due to their ability to enhance bone tissue formation by attracting soluble factors, such as growth factors, hormones and polypeptides, directly to the implantation site, as well as their potential to improve the fixation and stability of the implant. Moreover, there is increasing evidence that the synergistic effects of magnetic scaffolds and magnetic fields can promote bone repair and regeneration. In this manuscript we review the recent innovations in bone tissue engineering that exploit magnetic biomaterials combined with static magnetic fields to enhance bone cell adhesion and proliferation, and thus bone tissue growth.

Keywords: bone, magnetic, regeneration, scaffold, tissue engineering.

Introduction

One of the major challenges in the field of orthopaedics is how to treat patients with critical bone defects in order to restore limb length and function; such defects may be due to degenerative diseases, revisions of prosthetic implants, tumor excisions or traumas (1-3). Bone can spontaneously heal through the recruitment of undifferentiated mesenchymal stem cells (MSCs), together with growth factors and regulatory cytokines, to the site of the defect; these MSCs may eventually differentiate into bone cells, such as osteoblasts and osteoclasts, leading to new bone tissue formation (4). Healing occurs spontaneously when the bone loss is smaller than a critical size, which depends on a variety of factors, such as the bone and segment involved and the animal species (5); otherwise intervention is required.

Nowadays, orthopaedic practice for repairing of critical-sized defects consists of implantation of autologous bone grafts from the iliac crest or autologous vascularized fibular grafts, or alternatively implantation of allografts; another solution is that of bone transport using fixators (Rings or Uniplanner) (6, 7), but this method is associated with prolonged healing times and pain, in addition to psychological and socioeconomic consequences (8). Autografts still represent the gold standard, but their use is often prevented by donor site morbidity and limited availability (9); the availability of allografts, too, is limited and, moreover, their use could give rise to immunogenic responses and/or result in disease transmission (10). One of the most common causes of graft failure is lack of vascularization (11).

To address the above-mentioned issues, several tissue engineering approaches have been developed in recent decades, such as the use of synthetic graft material in addition to the use of novel scaffolds mainly based on calcium phosphates and hydroxyapatite (12, 13), cell seeding (14), and the use of soluble factors like vascular endothelial growth factor (VEGF) and bone morphogenetic protein (BMP) (15). On the basis of the fact that osteocytes themselves function as mechanosensors in bone, mechanical force stimuli have also

Corresponding Author:
Alessandro Russo, MD
II Orthopaedic and Traumatological Clinic, Istituto Ortopedico Rizzoli
Via Cesare Pupilli 1, 40136 Bologna, Italy
E-mail: a.russo@biomec.ior.it

been used successfully to improve bone regeneration (16, 17). In addition, static magnetic field (SMF) has been exploited for many years as a means of inducing magnetic stimulation (18). Inspired by the effects of magnetic stimulation, magnetic biomaterials have become a focus of particular interest for research into bone tissue engineering applications; in particular, superparamagnetic nanoparticles (MNPs), with size below 20 nm, thanks to the possibility of not retaining any residual magnetization upon removal of the magnetic field and thus of avoiding aggregation of MNPs after withdrawal of the magnetic field, according to an on/off mechanism, have shown high potential for *in vivo* applications (11, 19). An attractive property of MNPs is that, through the use of external magnetic fields, they can be guided and used as drug carriers. They may therefore potentially be exploited in a wide range of medical applications, from the antitumoral hyperthermia effect to magnetic drug delivery or as a contrast agent in MRI (20, 21). The possibility of incorporating MNPs into scaffolds for tissue regeneration applications has only recently begun to be investigated. Through the application of an external magnetic field, the superparamagnetic scaffolds thus created could be "activated" in the following way: a high magnetic gradient field is induced by the SMF causing a displacement of the particles along the gradient vector that, in turn, produces compression and tensile forces on the cell membrane with consequent cytoskeleton deformation and cell dragging (22). These mechanical forces, transmitted to the cytoskeleton by membrane receptors such as integrins, determine the activation of intracellular signaling pathways such as changes in intracellular calcium levels and in mitogen-activated protein kinase (MAPK) activity that reproduce the effects of mechanical loading and regulate osteocyte and osteoblast function, eventually leading to the development of new bone tissue (22) (Fig. 1).

Bio-resorbable magnetic scaffolds as "biological stations"

The fact that conventional scaffolds used for bone tissue engineering, once they are implanted, cannot be reloaded with the bio-agents necessary to promote, support and induce tissue regeneration is one of their biggest drawbacks and an issue that needs to be overcome. Bioactive magnetic scaffolds, on the other hand, can be envisaged as "fixed stations" able to offer a prolonged assistance to the tissues in their vicinity: indeed, these "stations" could be reloaded after implantation, in such a way as to mimic the production and consequent delivery of growth factors necessary to direct angiogenesis and tissue regeneration. On application of an external magnetic field, magnetic scaffolds can attract functionalized MNPs bound to growth factors, stem cells and many bio-agents towards the area needing to be regenerated; these bio-agents are thus released in a controlled way by carriers near the scaffolds that can be reloaded (21, 23). Bock et al. (24) proposed an innovative, easily reproducible and non-damaging technique for magnetizing porous scaffolds that consisted of infiltration with different ferrofluid solutions (watery dispersions of magnetite nanoparticles); they infiltrated hydroxyapatite (HA)/collagen (70:30 w/w) and pure collagen scaffolds. As shown by inductively coupled plasma atomic emission spectroscopy, all the scaffolds retained the MNPs, while magnetic characterization showed a higher loading potential of the composite HA/collagen than of the collagen scaffold. *In vitro* testing of adhesion and proliferation of human bone marrow stem cells (hBMSCs) was performed to assess the biocompatibility of these novel magnetic scaffolds: viable cells were increased in number from day 5 to day 15 for both scaffolds, indicating adequate biocompatibility.
Tampieri et al. synthesized magnetic bio-hybrid porous scaffolds through a biologically-inspired mine-

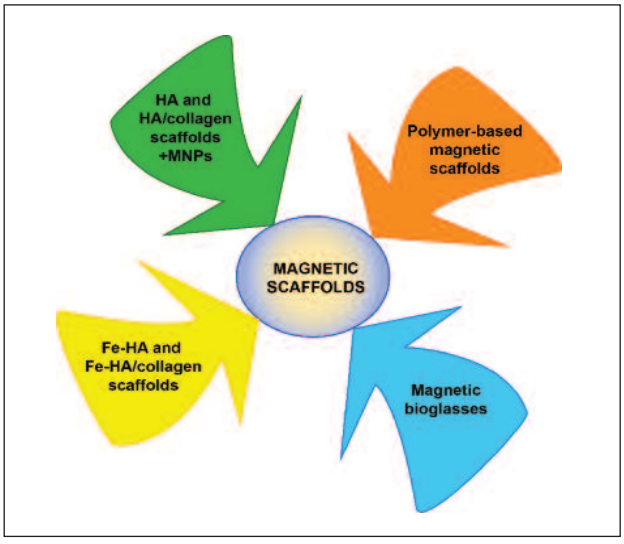

Figure 1. Magnetic scaffolds for bone tissue engineering.

ralization procedure, in which apatite nanocrystals *in situ* nucleated on self-assembling collagen, in presence of magnetite nanoparticles (NPs) (25). The distribution of the magnetite phase within the scaffold framework was found to be uniform on energy-dispersive X-ray spectroscopy (EDS) analysis, while the main peak of magnetite was besides the low-crystalline HA ones on X-ray diffraction (XRD), that is typical of biologically-inspired HA/collagen materials. High-resolution transmission electron microscopy micrographs showed that most of the surface of collagen fibers was covered by calcium phosphate NPs and exhibited a hollow spherical/ellipsoidal morphology (average diameter from 10 to 50 nm), whereas MNPs were made of magnetite nanocrystals (sized between 20 nm to 40 nm), aggregated on collagen fibers. MTT cell proliferation assay and live/dead tests revealed that the magnetic HA/collagen scaffolds were not cytotoxic, and moreover promoted hBMSC adhesion, growth and proliferation; these tests showed a 10-fold increment in cell growth after 10 days of cell culturing. Both these scaffold types, ferrofluid-infiltrated and *in situ* bio-hybrid, were tested *in vivo* by Panseri et al. (26). With the purpose of evaluating biocompatibility, osseointegration and bone healing progression in trabecular and cortical bone, magnetic and non-magnetic scaffolds were implanted in rabbit distal femoral epiphysis and tibial mid-diaphysis. New bone formation, significant scaffold resorption and low residual iron were observed 2 weeks after surgery, independently of the magnetic scaffold type. At 4 weeks, more resorption and more woven trabecular bone were detected in the bio-hybrid scaffold group as compared to the infiltrated one.

The Authors attributed the different *in vivo* behavior to the different magnetic phase distribution within the two groups: the magnetic phase within the bio-hybrid scaffold was completely integrated and finely distributed within the fibrous matrix, while in the infiltrated scaffolds, MNPs were just entrapped on the surface of collagen fibrils.

Static magnetic field and magnetic scaffolds: a synergistic effect to improve implant fixation

If magnetic scaffolds are thought of as "fixed stations" used to bring magnetized soluble factors to the implant site, then they need an external or internal SMF. In particular, an internal magnet close to the magnetic scaffold may improve not only the settling but also the fixation of the magnetic scaffold itself by decreasing micro-motions at the bone/scaffold interface, as shown by the finite element analysis conducted by Shelyakova et al. (27). Rare-earth magnets such as neodymium-iron-boron (NdFeB) magnets can be employed to generate the abovementioned SMF in the bone microenvironment; this type of magnet has attracted considerable attention in recent years thanks to its potential to promote bone formation and inhibit the bone density decrease-associated with surgical procedures. Bio-hybrid and ferrofluid-infiltrated scaffolds were implanted in the lateral femoral condyle of rabbit, with titanium-coated NdFeB magnets (1.2T of permanent magnetization) placed in close proximity to them; non-magnetic HA/collagen scaffolds were employed as controls (28). At 4 weeks from surgery, histological analysis revealed no inflammatory reactions due to the presence of the permanent magnet, and both magnetic scaffolds appeared well integrated within the surrounding bone; on the other hand, the control scaffolds were completely reabsorbed. The newly-formed bone tissue was characterized by thin immature trabeculae within the scaffold and by more mature trabeculae in its vicinity. Interestingly, it was noticed that collagen fibers were aligned in the same direction as the magnetic field lines generated by the permanent magnet.

At 12 weeks post-implantation, it was possible to observe the combined effect of the magnetic forces generated by the permanent magnet and the magnetized collagen fibers of the scaffold: acceleration of bone formation and promotion of a dense and ordered pattern of trabeculae (orthogonally oriented with respect to the magnetic field lines) (**Fig. 2**) (29). By contrast, only partial defect healing was achieved when a non-magnetic scaffold or only the permanent magnet was implanted. The Authors concluded that implantation of magnetic biomaterials together with a permanent magnet generated a force gradient that resulted in more efficient bone structure regeneration than the physiological repair process.

Not only highly-resorbable scaffolds, but also slowly-resorbable magnetic scaffolds have been developed, in particular for application as bone substitutes in large defects, where higher stress resistance is necessary. Microporous HA ceramic composite constructs/

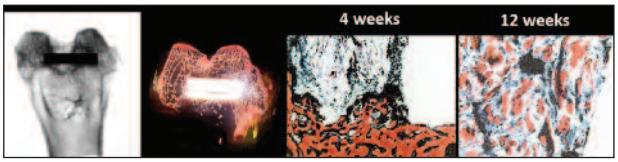

Figure 2. Trabeculae oriented orthogonally to the magnetic field lines.

inserts embedding magnetite NPs in different percentages (wt. % 95/5, 90/10, 50/50 and 100/0, w/w) were fabricated using a foaming technique and sintered at high temperature in a controlled atmosphere (30). As expected, only HA and magnetite phase were revealed at the XRD results; hematite was detected as secondary phase when magnetite cnet was higher than 10%. *In vitro* tests using human osteoblast-like cells were carried out with or without the application of an external magnetic field (320 mT). The whole external surface of the scaffold was completely covered by cells 7 days after seeding, while at 14 days, they filled a good part of the inner micro-pores. Cell proliferation was higher for the 90/10 compound both at 7 and 14 days from seeding, irrespectively of the presence of the external magnetic field.

Thus, the 90/10 scaffold was selected to be tested *in vivo* in a critical-sized lesion in a rabbit femoral condyle. Novel mineralized bone tissue was found within the interconnected porous structure of the scaffold after 4 weeks, both in the magnetic and control scaffold, suggesting good histocompatibility of the newly-developed magnetic scaffold.

The behavior of rat osteoblasts and mouse pre-osteoblasts was investigated by Zeng et al. (31) on various HA scaffolds with different content of MNP (from 0 to 2 wt%). Their results showed that magnetic scaffolds enhanced cell adhesion, proliferation and differentiation when compared to non-magnetic scaffolds. Interestingly, cell proliferation was significantly improved when the magnetic scaffold was combined with an external SMF, suggesting a synergistic effect between them. Finally, the Authors observed a positive correlation between MNP content and cell proliferation. Wu et al. fabricated magnetic HA and HA/tricalcium phosphate (TCP) composite scaffolds that contained MNPs (32); the ability of these two scaffolds to sustain cell proliferation and differentiation was evaluated culturing them with rat Ros17/2.8 and human MG63 osteosarcoma cells. The results revealed that the composite scaffolds had good biocompatibility with bone cells; moreover, it was demonstrated that MNPs do not affect the function of the BMP bonding 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.

Recently, Yun et al. (33) included MNPs in poly(ε-caprolactone) (PCL) scaffolds (0, 5 and 10%w/w). SEM images showed a highly porous structure; the XRD patterns revealed the peaks related to PCL and MNPs (iron oxide magnetite). Mouse calvarian osteoblasts were cultured on these scaffolds with or without application of an external SMF (15 mT): typical osteoblast differentiation gene expression, such as alkaline phosphatase (ALP) and bone morphogenetic protein (BMP-2) expression, was increased at 10 days in the magnetic scaffolds, particularly when SMF was applied. *In vivo* tests were performed creating critical defects in mouse calvarian bone: microCT analysis revealed that newly formed bone volume was significantly increased by the magnetic scaffolds or by the SMF alone, and even more so if they were used together, suggesting that prolonged SMF and magnetic scaffold use created synergistc microenvironments favorable for bone tissue formation.

Intrinsically superparamagnetic Fe^{2+}/Fe^{3+} scaffolds

Iron oxide nanoparticles, such as magnetite and maghemite, have been found to exhibit remarkable properties, however their long-term toxicity effects in the human body were not fully estimated (34, 35). Therefore, a novel synthesis procedure to obtain a superparamagnetic (Fe^{2+}/Fe^{3+}) lattice substituted hydroxyapatite (Fe-HA) was proposed (36): both ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions were introduced into the HA lattice at different Ca^{2+} sites, giving rise to the superparamagnetic behavior and at the same time reducing magnetite formation as secondary phase in the scaffolds.

The biocompatibility of Fe-HA MNPs was assessed *in vitro* by evaluating the proliferation of Saos-2 human osteoblastic-like cells in the absence or presence of an external SMF; these results were compared to the behavior of commercial HA nanoparticles (3).

Different concentrations of MNPs were investigated: 2000, 1000, 500 and 200 μg/ml. Compared with the control group, cell proliferation was found to be

enhanced by Fe-HA MNPs at live/dead assay tests. Moreover, the exposure of cell cultures to a SMF (320mT) induced a significant increase in cell proliferation from day 1 to day 7 compared with groups without exposure to SMF; in particular, the 200 μg/ml concentration promoted the highest cell proliferation. To assess biocompatibility *in vivo* a rabbit critical defect model was used: bone tissue was visible around biomaterials in both magnetic and control groups, indicating a good integration into the surrounding bone tissue.
The same group of researchers also succeeded in directly nucleating crystals of superparamagnetic Fe-HA on self-assembling collagen fibers (telopeptide-free type I, from horse tendon) using a biologically-inspired mineralization process (37). The process was evaluated at different synthesis temperatures: 25°C (Fe-HA/coll25), 40°C (Fe-HA/coll40) and 50°C (Fe-HA/coll50). As reference materials, pure collagen and Ha/collagen (60/40 w/w) non-magnetic scaffolds were prepared. The hybrid composites synthesized at 25° C showed an XRD pattern with the typical shape ascribable to a CaP phase without a long-range periodic regularity close to an amorphous one, while those of the scaffold prepared at higher temperatures exhibited the characteristic broad diffraction peaks of nanocrystalline apatite with very low coherent length.
Interestingly, it was shown that Fe-HA nuclei grew in close contact with the fibers and that their c-axis showed a preferential orientation parallel to the direction of collagen fibers. Analysis of FT-IR spectra of hybrid scaffold confirmed, as expected, the presence of the CaP phase nucleated on collagen fibers; moreover it revealed distinctive bands increased as a function of temperature due to different CaP phase crystallize at different temperatures: amorphous CaP at 25°C and apatite at 40 and 50 °C respectively. The Scanning electron microscope (SEM) analysis showed that the small apatite nanocrystals in Fe-HA/coll25 were closely bound to the collagen fiber and gave rise to a microporous framework, whereas higher synthesis temperature conferred an external layer of large apatite crystals exhibiting a plate-like morphology that caused a collagen fiber thickness increase and total porosity reduction. Higher magnetization values were found for the scaffold synthesized at higher temperatures, suggesting that the substitution of Fe ions in the lattice was promoted by higher temperatures, as confirmed by quantitative chemical analyses. Lastly, viability of osteoblast-like human cells cultured on magnetic hybrid composites was evaluated, in the absence or in the presence of an external magnetic field (320 mT). Independently of the presence of the external magnetic field, Fe-HA/coll25 exhibited the best results in terms of cell adhesion, viability and proliferation.

Polymer-based magnetic scaffolds

In 2011, De Santis et al. prepared by rapid prototyping PCL magnetic scaffolds embedding magnetite (Fe_3O_4) MNPs (38). The 3D fiber-deposition technique made it possible to obtain a highly controlled, well-defined and customized scaffold with a fully-interconnected porous structure: these 3D nanocomposite fibers were manufactured by extruding and alternately depositing fibers along the 0° direction and the 90° direction between two successive layers (0°/90° pattern). PCL/Fe_3O_4 nanocomposite scaffolds were produced starting from PCL/Fe_3O_4 pellets (90:10wt%), obtained starting from PCL pellets and polyvinylpyrrolidone (PVP)-coated Fe_3O_4 NPs (same weight ratio). Tensile tests on PCL and PCL/Fe_3O_4 nanocomposite fibers revealed a ductile behavior for both groups. When the magnetic phase was present the PCL matrix was found to be mechanically reinforced: the elastic modulus and maximum stress increased by about 10 and 30% respectively; however, the maximum strain decreased by about 50%, also suggesting increased brittleness. Magnetic measurement indicated a superparamagnetic behavior. Finally, biological *in vitro* tests revealed a marked increase of adhesion and spreading of hBMSCs on magnetic PCL scaffolds compared to non-magnetic ones, indicating improved biocompatibility.
Recently, Daňková et al. (39) created a similar scaffold by electrospinning a mixture of 24 wt% PCL, using a chloroform:ethanol dissolving system in a ratio of 9:1. SEM analysis revealed that MNPs appeared as spherical objects measuring between 50 and 100 nm, forming irregular agglomerates on the nanofiber surface. These scaffolds were seeded with MSCs obtained from the os ilium bone marrow of miniature pigs: the MSCs' metabolic activity, proliferation and ALP activity were monitored on days 1, 7 and 21 after seeding; the viability of the cells on PCL-MNP scaffolds was significantly higher on days 7 and 21 than that of the cells seeded on a scaffold made from PCL. Also, the ALP activity of cells seeded on PCL-MNP scaffolds was increased on days 7 and 21. Confocal microscopy

analysis showed that the cell layer was fully confluent on the PCL-MNPs scaffolds, while it was not fully confluent on the PCL scaffold. Meng et al. (40) obtained magnetic nanofibrous films based on polylactic acid (PLA). To do this, γ-Fe_2O_3 MNPs with an average diameter of 14 nm, HA nanoparticles with an average diameter of 50 nm, and PLA with a molecular weight of 10 kD were mixed together and processed into fibers by electrospinning. The MNPs were mainly located inside the fibers, while HA nanoparticles distributed near the surface; these films could be folded and fixed to pellets and were thus suitable as bone substitutes. These Authors cultured a pre-osteoblast MC3T3-E1 cell line on the samples, with and without application of an external SMF of 1.0mT. When the SMF was applied, there was a significant improvement in the cell proliferation rate, which indicates that MNPs in the scaffold played a synergistic role in cell proliferation; moreover, cells growing on MNP/HA/PLA films expressed significantly more ALP when exposed to SMF than those growing on non-magnetic films over the experimental period of 17 days.

Cai et al. (41) prepared, also by electrospinning, composite nanofiber scaffolds made of PLA with different content of Fe_3O_4 NPs: 0/100, 2.5/100, 5/100 in w/w [PLA/Fe_3O_4(0), PLA/Fe_3O_4(2.5) and PLA/Fe_3O_4(5), respectively]. SEM images showed that Fe_3O_4 NPs were homogenously distributed within the PLA nanofibers. *In vitro* tests carried out using MC3T3-E1 cells, a mouse calvaria-derived cell line, revealed that cell adhesion and proliferation were enhanced with the PLA/Fe_3O_4(2.5) scaffold, and in particular if an external SMF was applied, while higher NP content [i.e. PLA/Fe_3O_4 (5)] had caused a reduction in cell adhesion and proliferation. Instead, cell osteogenic differentiation, evaluated by analyzing ALP activity and calcium deposition, was enhanced proportionally to Fe_3O_4 NP content; the application of an external SMF further enhanced ALP activity and calcium deposition.

Meng's group, encouraged by previously reported *in vitro* results, validated *in vivo* the osteogenic potential of the magnetic nanofibrous scaffolds with the application of an external SMF (42). They implanted the scaffolds in lumbar transverse defects of New Zealand white rabbits. The animals were housed in cages with fixed permanent magnets in the two opposite sides in order to be exposed to SMF; animals housed in standard cages were used as controls. The evaluation of defect healing was performed 110 days after surgery. Histological analysis 10 days after implantation showed that the scaffold had recruited host-derived cells migrating to the defect area, including macrophages and fibroblasts. At longer time points, the scaffolds started to break into small pieces, osteoblast cells and blood vessels appeared, as well as new bone tissue around the scaffold pieces. Bone apposition rate, osteocalcin (OC) and collagen deposition levels were higher when external magnets were used. Computer tomography images showed that exposure to SMF led to more homogeneous and more natural bone morphology when compared to animal not exposed to SMF. Finally, scaffolds degraded faster under the effect of SMF; Authors speculated that exposed macrophages were more active, contributing to scaffold resorption.

Wei et al. (43) recently fabricated, by electrospinning, magnetic biodegradable Fe_3O_4/chitosan(CS)/ poly(vinyl alcohol) nanofibrous membranes with average fiber diameter ranging from 220 to 380 nm. A homogeneous and smooth nanofibrous composite could be obtained by adding an Fe_3O_4 concentration lower that 5wt%, without altering the crystalline structure and with the MNPs equally distributed in the fibers. The stiffness of the composite on tensile tests was found to improve with increasing MNP concentration. The biocompatibility was assessed by *in vitro* evaluation of human osteoblast-like cells behavior. MTT assay and SEM observations clearly indicated good cell adhesion and proliferation, suggesting that these magnetic nano-fibrous membranes may be suitable for promoting osteogenesis.

In order to reduce the content of magnetite, Gloria et al. developed a magnetic nanocomposite consisting of a PCL matrix embedding bio-resorbable Fe-HA MNPs (44). Fe-HA MNPs were integrated into the PCL matrix with different polymer-to-particle weight ratios: 90/10, 80/20 and 70/30 w/w. As confirmed by XRD analysis, the fabrication process did not alter structure and crystallinity of Fe-HA component; SEM-EDS mapping showed that the distribution of MNPs within the matrix was homogeneous. The PCL/Fe-HA(90/10) scaffolds were the strongest and toughest at small punch tests. Hyperthermia test curves obtained under an alternating field of 27mT with a frequency of 260 kHz revealed a magnetically induced thermal response suitable for *in vivo* applications. The biocompatibility was tested *in vitro* by using hMSCs, which exhibited higher proliferation on PCL/Fe-HA

scaffolds than PCL ones at 7,14 and 21 days. In particular, PCL/Fe-HA 70/30 and 80/20 w/w specimens yielded the highest cell proliferation values.

Magnetic bioactive glasses

Another possibility that has recently emerged is that of fabricating magnetic coatings by incorporation of iron oxide into bioactive glasses. Chen et al. (45) used magnetic bioactive glass coating ($CaO\text{-}SiO_2\text{-}P_2O_5\text{-}Fe_3O_4$), (MBGCs) as sacrificial templates in order to fabricate magnetic HA coatings (MHACs) with oriented nanorod arrays: the conversion of MBGCs to MHACs was done in a simulated body fluid (SBF) via dissolution-precipitation reaction. They obtained HA nanorods with a preferential crystal plane orientation, orthogonal to the coating surfaces. The Authors demonstrated that magnetite nanoparticles present in the coating improved the nucleation rate of HA; instead, when MNPs were not incorporated into the bioglass coatings, the HA nanorods turned into blocky HA particles. In addition, the magnetic coating showed higher hydrophilicity than the non-magnetic coating, ascribed to the magnetic nanoparticles. The biocompatibility of HA nanorods was tested by using hBMSCs that exhibited improved cell adhesion, spreading and proliferation on MHACs when compared with BGCs or MBGCs, thanks to the presence of the HA phase, higher hydrophilicity and oriented nanorod arrays.

A multifunctional mesoporous bioactive glass scaffold system was developed by Wu et al. (46) for hyperthermia and drug delivery applications. Iron scaffolds with a hierarchical macroporous (300-500 μm) and mesoporous structure (4.5 nm) were prepared incorporating 5% or 10% of Fe into bioglass using co-templates of non-ionic polymer P123 (poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide)-based triblock copolymer $(EO)_{20}(PO)_{70}(EO)_{20}$) and sponges of polyurethane, respectively responsible for the mesoporosity and macroporosity. The incorporation of Fe into the mesoporous MBG scaffolds increased mitochondrial activity and expression of bone-related genes, like ALP and OC, in hBMSCs cultured on the scaffolds. The Fe-MBG scaffolds also demonstrated sustained drug delivery.

Wang et al. (47) prepared a bioglass precursor by a route of self-assembly of Ca, P, Si and Fe sources in the sol-gel process; this precursor was stepwise dropped on the surface and the gap of NaCl crystals (200-300 μm), that were then washed out several times with distilled water. Finally, a macro/mesoporous bioglass was obtained. SEM images showed a 3D interconnected macroporous structure (pore size of 200-300 μm); EDS analysis indicated that samples were composed of Si, Ca, P, O and Fe. After immersion in a SBF at 37°C, SEM images showed flower-like crystals covering the surfaces of the samples, which EDS patterns showed to be HA. Finally, the Authors demonstrated that HeLa cells were attached onto each surface after incubation for 3 days.

Conclusions

Magnetic scaffolds have been shown to have a significant influence on bon-derived cells improving adhesion, proliferation and differentiation. In particular, embedded MMPs and *in situ* HA, HA/collagen, polymeric and bioglass scaffolds have been found to have an enhancing effect on cell behavior, likely due to the remote activation of a mechano-trasduction pathway which is converted into the biochemical one. In conclusion, magnetic scaffolds are an excellent resource for bioreactor and scaffold design due to their ability to provide mechanical stimulation that can be enhanced and/or regulated by the application of a remotely controlled external magnetic field.

Acknowledgements

The drafting of the present review was partially supported by the European Project "Magnetic Scaffolds for in vivo Tissue Engineering" (NMP3-LA-2008-214686).

References

1. Oryan A, Bigham-Sadegh A, Abbasi-Teshnizi F. Effects of osteogenic medium on healing of the experimental critical bone defect in a rabbit model. Bone. 2014;63:53-60.
2. Hesse E, Kluge G, Atfi A, et al. Repair of a segmental long bone defect in human by implantation of a novel multiple disc graft. Bone. 2010;46:1457-1463.
3. Panseri S, Cunha C, D'Alessandro T, et al. Intrinsically superparamagnetic Fe-hydroxyapatite nanoparticles positively influence osteoblast-like cell behavior. J Nanobiotechnology. 2012; 10:32.
4. Allori AC, Sailon AM, Warren SM. Biological basis of bone formation, remodeling, and repair-part I: biochemical signaling molecules. Tissue Eng Part B Rev. 2008;14:259-273.

5. Petite H, Viateau V, Bensaïd W, et al. Tissue-engineered bone regeneration. Nat Biotechnol. 2000;18:959-963.
6. Jahangir AA, Nunley RM, Mehta S, et al. Bone-graft substitutes in orthopaedic surgery. AAOS Now. 2008;2:35-37.
7. Sahibzada AS, Khan MA, Khan MS. Management of tibial bone defects due to high energy trauma using the locally manufactured external fixator by segmental bone transport. J Ayub Med Coll Abbottabad. 2005;17:68-72.
8. Lasanianos NG, Kanakaris NK, Giannoudis PV. Current management of long bone large segmental defects. Orthopaedics and Trauma. 2010;24:149-163.
9. Laurencin C, Khan Y, El-Amin SF. Bone graft substitutes. Expert Rev Med Devices. 2006;3:49-57.
10. Calori GM, Mazza E, Colombo M, Ripamonti C. The use of bone-graft substitutes in large bone defects: Any specific needs? Injury. 2011;42 Suppl 2:S56-S63.
11. Willems WF, Kremer T, Friedrich P, et al. Surgical revascularization in structural orthotopic bone allograft increases bone remodeling. Clin Orthop Relat Res. 2014;472:2870-2877.
12. Khan Y, Yaszemski MJ, Mikos AG, et al. Tissue engineering of bone: material and matrix considerations. J Bone Joint Surg Am. 2008;90 Suppl 1:36-42.
13. Bianchi M, Urquia Edreira ER, Wolke JG, et al. Substrate geometry directs the in vitro mineralization of calcium phosphate ceramics. Acta Biomater. 2014;10:661-669.
14. Itani Y, Asamura S, Matsui M, et al. Evaluation of nanofiber-based polyglycolic acid scaffolds for improved chondrocyte retention and in vivo bioengineered cartilage regeneration. Plast Reconstr Surg. 2014;133:805e-813e.
15. Kempen DH, Lu L, Heijink A, et al. Effect of local sequential VEGF and BMP-2 delivery on ectopic and orthotopic bone regeneration. Biomaterials. 2009;30:2816-2825.
16. Sikavitsas VI, Temenoff JS, Mikos AG. Biomaterials and bone mechanotransduction. Biomaterials. 2001;22:2581-2593.
17. Orr AW, Helmke BP, Blackman BR, et al. Mechanisms of mechanotransduction. Dev Cell. 2006;10:11-20.
18. Kotani H, Kawaguchi H, Shimoaka T, et al. Strong static magnetic field stimulates bone formation to a definite orientation in vitro and in vivo. J Bone Miner Res. 2002;17:1814-1821.
19. Dobson J. Remote control of cellular behaviour with magnetic nanoparticles. Nat Nanotechnol. 2008;3:139-143.
20. Barry SE. Challenges in the development of magnetic particles for therapeutic applications. Int J Hyperthermia. 2008;24:451-466.
21. Dobson J. Magnetic nanoparticles for drug delivery. Drug Development Research. 2006;67:55-60.
22. Salter DM, Wallace WH, Robb JE, et al. Human bone cell hyperpolarization response to cyclical mechanical strain is mediated by an interleukin-1beta autocrine/paracrine loop. J Bone Miner Res. 2000;15:1746-1755.
23. Bettini S, Bonfrate V, Syrgiannis Z, et al. Biocompatible collagen paramagnetic scaffold for controlled drug release. Biomacromolecules. 2015;16:2599-2608.
24. Bock N, Riminucci A, Dionigi C, et al. A novel route in bone tissue engineering: magnetic biomimetic scaffolds. Acta Biomater. 2010;6:786-796.
25. Tampieri A, Landi E, Valentini F, et al. A conceptually new type of bio-hybrid scaffold for bone regeneration. Nanotechnology. 2011;22:015104.
26. Panseri S, Russo A, Giavaresi G, et al. Innovative magnetic scaffolds for orthopedic tissue engineering. J Biomed Mater Res A. 2012;100:2278-2286.
27. Shelyakova T, Russo A, Visani A, et al. Application of magnetic rods for fixation in orthopedic treatments. Comput Biol Med. 2015;61:101-106.
28. Panseri S, Russo A, Sartori M, et al. Modifying bone scaffold architecture in vivo with permanent magnets to facilitate fixation of magnetic scaffolds. Bone. 2013;56:432-439.
29. Russo A, Bianchi M, Sartori M, et al. Magnetic forces and magnetized biomaterials provide dynamic flux information during bone regeneration. J Mater Sci Mater Med. 2016;27:51.
30. Panseri S, Cunha C, D'Alessandro T, et al. Magnetic hydroxyapatite bone substitutes to enhance tissue regeneration: evaluation in vitro using osteoblast-like cells and in vivo in a bone defect. PLoS One. 2012;7: e38710.
31. Zeng XB, Hu H, Xie LQ, et al. Magnetic responsive hydroxyapatite composite scaffolds construction for bone defect reparation. Int J Nanomedicine. 2012;7:3365-3378.
32. Wu Y, Jiang W, Wen X, et al. A novel calcium phosphate ceramic-magnetic nanoparticle composite as a potential bone substitute. Biomed Mater. 2010;5:015001.
33. Yun HM, Ahn SJ, Park KR, et al. Magnetic nanocomposite scaffolds combined with static magnetic field in the stimulation of osteoblastic differentiation and bone formation. Biomaterials. 2016;85:88-98.
34. El-Hammadi MM, Arias JL. Iron oxide-based multifunctional nanoparticulate systems for biomedical applications: a patent review (2008 - present). Expert Opin Ther Pat. 2015;25:691-709.
35. Singh N, Jenkins GJ, Asadi R, et al. Potential toxicity of superparamagnetic iron oxide nanoparticles (SPION). Nano Rev. 2010;1 doi: 10.3402/nano.v1i0.5358.
36. Tampieri A, D'Alessandro T, Sandri M, et al. Intrinsic magnetism and hyperthermia in bioactive Fe-doped hydroxyapatite. Acta Biomater. 2012;8:843-851.
37. Tampieri A, Iafisco M, Sandri M, et al. Magnetic bioinspired hybrid nanostructured collagen-hydroxyapatite scaffolds supporting cell proliferation and tuning regenerative process. ACS Appl Mater Interfaces. 2014;6:15697-15707.
38. De Santis R, Gloria A, Russo T, et al. A basic approach toward the development of nanocomposite magnetic scaffolds for advanced bone tissue engineering. Journal of Applied Polymer Science. 2011;122:3599-3605.
39. Daňková J, Buzgo M, Vejpravová J, et al. Highly efficient mesenchymal stem cell proliferation on poly-ε-caprolactone nanofibers with embedded magnetic nanoparticles. Int J Nanomedicine. 2015;10:7307-7317.
40. Meng J, Zhang Y, Qi X, et al. Paramagnetic nanofibrous composite films enhance the osteogenic responses of pre-osteoblast cells. Nanoscale. 2010;2:2565-2569.
41. Cai Q, Shi Y, Shan D, et al. Osteogenic differentiation of MC3T3-E1 cells on poly (L-lactide)/Fe 3 O 4 nanofibers with static magnetic field exposure. Mater Sci Eng C Mater Biol Appl. 2015;55:166-173.
42. Meng J, Xiao B, Zhang Y, et al. Super-paramagnetic responsive nanofibrous scaffolds under static magnetic field enhance osteogenesis for bone repair in vivo. Sci Rep. 2013;3:2655.
43. Wei Y, Zhang X, Song Y, et al. Magnetic biodegradable Fe 3O4/CS/PVA nanofibrous membranes for bone regeneration. Biomed Mater. 2011;6:055008.
44. Gloria A, Russo T, D'Amora U, et al. Magnetic poly(ε-caprolactone)/iron-doped hydroxyapatite nanocomposite substrates for advanced bone tissue engineering. J R Soc Interface. 2013;10:20120833.
45. Chen W, Long T, Guo Y, et al. Magnetic hydroxyapatite coatings with oriented nanorod arrays: hydrothermal synthesis, structure and biocompatibility. Journal of Materials Chemistry B. 2014;2:1653-1660.
46. Wu C, Fan W, Zhu Y, et al. Multifunctional magnetic mesoporous bioactive glass scaffolds with a hierarchical pore structure. Acta Biomater. 2011;7:3563-3572.
47. Wang D, Lin H, Jiang J, et al. One-pot synthesis of magnetic, macro/mesoporous bioactive glasses for bone tissue engineering. Sci Technol Adv Mater. 2013;14:025004.