Review Article

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Magnetic nanoparticles in 3D-printed scaffolds for biomedical applications

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Abstract: Magnetic nanoparticles (MNPs) have recently attracted considerable attention, mainly due to their unique magnetic properties and biocompatibility. Although MNPs have been extensively studied for biomedical applications, there are still very few studies on them as part of three-dimensional (3D)-printed scaffolds. Thus, this review aims to show the potential of MNPs to modulate various properties of 3D-printed scaffolds. 3D Printing is for itself a contemporary method in biomedicine, owing to its ability to produce versatile scaffolds with complex shapes enabling a homogeneous distribution of cells or other entrapped compounds, as well as possible precise control of pore size and shape, porosity, and interconnectivity of pores that contribute to structural stability. All mentioned properties can be upgraded or complemented with the specific properties of MNPs (e.g., biocompatibility and positive effect on cell proliferation). Considering the latest related literature and a steadily increasing number of related publications, the fabrication of magnetically responsive scaffolds is among the most interesting strategies in tissue engineering. According to the literature, incorporating MNPs into scaffolds can improve their mechanical properties and significantly affect biological properties, such as cellular responses. Moreover, under the influence of an external magnetic field, MNPs significantly promoted cell adhesion, proliferation, and differentiation.

Keywords: magnetic nanoparticles, 3D-printed scaffolds, iron oxide nanoparticles, mechanical properties, cellular responses

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Abbreviations

AMF	alternating magnetic field
β-TCP	beta tri-calcium phosphate
BMSCs	bone marrow mesenchymal stem cells
FeHA	iron-doped hydroxyapatite
GC	glycol chitosan
GelMA	gelatin methacryloyl
GO	graphene oxide
HA	hydroxyapatite
hASCs	human adipose stem cells
IONPs	iron oxide nanoparticles
MBG	mesoporous bioactive glass
MGO	magnetic graphene oxide
MH	magnetic hyperthermia
MNPs	magnetic nanoparticles
NPs	nanoparticles
OHA	oxidized hyaluronate
PCL	polycaprolactone
PGA	polyglycolic acid
PLGA	poly(lactic-*co*-glycolic acid)
PLLA	poly-L-lactic acid
SPIONs	super-paramagnetic iron oxide nanoparticles
TE	tissue engineering

1 Introduction

Magnetic nanoparticles (MNPs), especially iron oxide nanoparticles (IONPs), have attracted increasing attention in recent years due to their unique magnetic properties such as appropriate Curie temperature, superparamagnetism, and magnetic hyperthermia (MH), as well as their

biocompatibility, which makes them suitable for biomedical applications [1]. In addition, MNPs exhibit many important properties, such as high specific surface area, chemical stability, low intraparticle diffusion rate, and high loading capacity [2,3]. MNPs are usually composed of magnetic elements such as iron (Fe), nickel (Ni), and cobalt (Co), and their oxides such as magnetite (Fe_3O_4) and its oxidized form maghemite (γ-Fe_2O_3). Their ability to be remotely controlled with an external magnetic field is one of the most important properties of MNPs [4]. In the last decade, MNPs have been extensively investigated for biomedical applications such as MH, magnetic resonance imaging, and targeted drug delivery [5,6]. Meanwhile, very few studies have been conducted on using MNPs as part of three-dimensional (3D)-printed scaffolds for tissue engineering (TE), although the interest in such applications is steadily rising. Figure 1 shows the increasing number of scientific publications on MNPs use in 3D-printed scaffolds in recent years. This statistic shows that incorporating MNPs into 3D-printed scaffolds is a promising research area that will continue to grow in the coming years. Considering the overall still a low number of related articles and especially the lack of any review articles, related to the use of MNPs to manipulate the properties of 3D-printed scaffolds to improve their properties for biomedical applications, we present a summarized review of the opportunities arising from this combination.

2 Methods

A literature review was conducted *via* the biggest medical literature databases (Medline, PubMed, and ScienceDirect) to obtain studies related to MNPs and 3D printing. The employed search terms in the form of keywords were "magnetic nanoparticles" and "3D-printing." With the help of specific filters (5-year review), we were able to find relevant new impactful studies on MNPs in 3D-printed scaffolds, which were included in this review.

3 General properties of MNPs and 3D printing

Although a wide variety of MNPs can be used for this purpose, most research has focused only on IONPs. This is at least partially related to the approval of their clinical use by the US Food and Drug Administration (FDA) [7]. In addition to the general advantages of MNPs, IONPs offer many other benefits like relatively simple synthesis, high saturation magnetization, high magnetic susceptibility, and low cytotoxicity [8]. Moreover, when the size of ferrimagnetic Fe_3O_4 nanoparticles (NPs) is reduced below 20 nm, they exhibit super-paramagnetic properties, as each particle becomes a single magnetic domain [9].

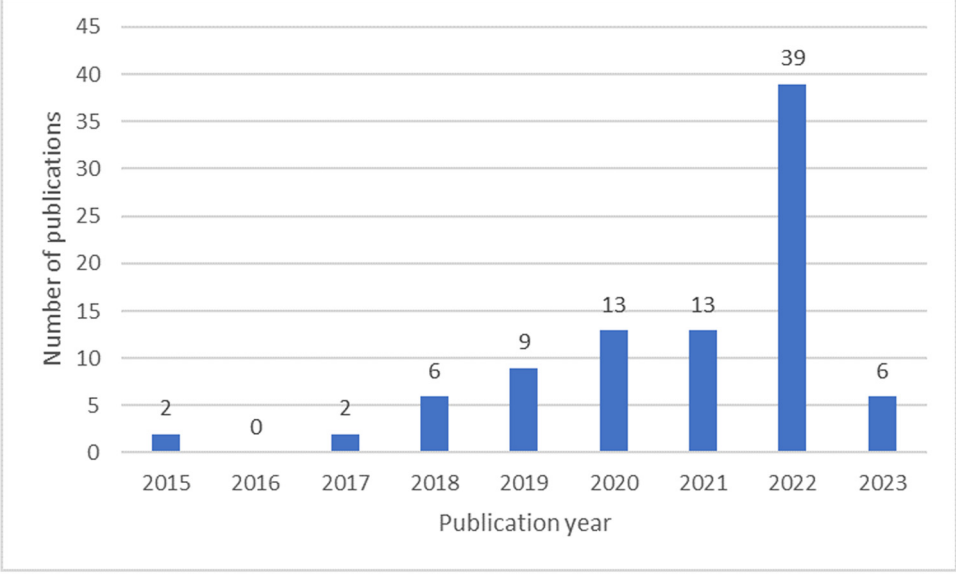

Figure 1: Number of scientific publications per year related to "magnetic nanoparticles" and "3D-printed scaffolds," according to the ScienceDirect (accessed 25 January 2023).

Superparamagnetic Fe_3O_4 NPs exposed to an alternating magnetic field (AMF) can generate heat through mainly Néel and Brownian relaxations, together with hysteresis losses, which are used in MH [10]. However, despite the many advantages of IONPs, there are also some disadvantages, such as a relatively high Curie temperature. The latter might be a problem in biomedical applications (e.g., MH) since it can lead to overheating of the surrounding tissue if there is no external temperature control to turn off the magnetic field [11]. For this reason, nickel–copper (NiCu) NPs with a Curie temperature within the therapeutic range (42–46°C) seem even more promising when using MH [12–15]. NiCu NPs are chemically stable, biocompatible, and exhibit desired magnetic properties, which makes them highly interesting for use in biomedicine [11]. Many groups have already investigated the use of NiCu MNPs as mediators for MH, but Stergar *et al.* were the first to report the potential of NiCu NPs as bimodal therapeutic systems, capable of simultaneous MH and targeted drug delivery [16].

Porosity, pore size and interconnectivity, biocompatibility, biodegradability, and mechanical properties are important parameters to be considered in developing suitable scaffolds [17]. Various techniques have been used to fabricate scaffolds, including freeze-drying, solvent casting, particulate leaching, phase separation, electrospinning, melt moulding, and gas foaming [18]. However, in these techniques, it is often difficult to precisely control the pore size, pore geometry, porosity, and connectivity of the pores [19]. Three-dimensional (3D) printing technology is among the methods developed to overcome these limitations through its layer-by-layer deposition, which enables the fabrication of complex and precise structures [20]. 3D Printing brez pomišljaja technology offers several advantages over traditional scaffold fabrication methods. Among them is the ability to fabricate versatile scaffolds with complex geometries and desired overall shapes. Such possibilities are ideal for designing materials for homogeneous cell distribution, mimicry of the extracellular matrix, and fine-tuning the microenvironment to promote cell adhesion, proliferation, and differentiation [21]. 3D Printing technology has revolutionized many areas of biomedical research and clinical practice. From creating patient-specific implants to printing tissue constructs for drug screening, 3D printing has opened up new possibilities for personalized and precision medicine. Extrusion-based 3D printing is one of the simplest and most cost-effective techniques used in 3D printing of polymers with potential application in TE [22]. Other 3D printing technologies currently being used in the preparation of different tissue scaffolds mainly include selective laser sintering, stereolithography, electron beam melting, 3DP technology, and biological 3D printing [23].

Since 3D printing is one of the most widely used techniques nowadays in TE, a lot of related research focuses on tissue-specific material choice. This is crucial from two perspectives: finding the optimum materials to grow specific cell types and suitable printability. Some natural polymers studied for this purpose are collagen, fibrin, chitosan, hyaluronic acid, alginate, gelatin, and gelatin methacrylate [24–26]. Despite their excellent bioactivity and biodegradability, low potential for immune defence and ability to form scaffolds that maintain the extracellular matrix composition of host tissues are the reasons that they are not ideal for TE. Their main disadvantages include their low mechanical strength and rapid degradation rate, which hinder their use in load-bearing applications. Although natural materials are beneficial for cellular processes, synthetic polymers are a better choice for tissue support due to their better mechanical properties, easily modifiable biological properties, and controlled degradation rate. Some of the most commonly used synthetic polymers are poly-L-lactic acid (PLLA), polyglycolic acid (PGA), poly(lactic-*co*-glycolic acid) (PLGA), and polycaprolactone (PCL) [21,27]. Bioceramic materials such as hydroxyapatite (HA) and beta tri-calcium phosphate (β-TCP) are also widely used in 3D printing. Due to their chemical and structural similarity to the mineral phase of natural bone, they exhibit excellent osteoconductivity and biocompatibility. However, they often have disadvantages due to low fracture toughness, extremely high stiffness, and low elasticity [27]. The disadvantages of the above biomaterials, which limit their use in the biomedical field, have led to the development of biocomposite materials that include particles, fibres, or nanomaterials to reinforce their mechanical and functional properties [24].

The use of MNPs for the fabrication of magnetically responsive scaffolds is one of the most recent strategies in the field of TE. Several studies have reported that incorporating MNPs into a scaffold material can improve the mechanical properties of the scaffolds, such as increased strength and toughness [28–32]. In addition, recent studies have shown that the presence of MNPs in scaffolds significantly affects biological properties and cellular responses. Under the influence of an external magnetic field, MNPs were shown to significantly promote cell adhesion, proliferation, and differentiation [33–35] (Figure 2).

This review focuses on the importance of incorporating MNPs into 3D-printed scaffolds and on the recent advances in the use of MNPs in 3D-printed scaffolds in TE.

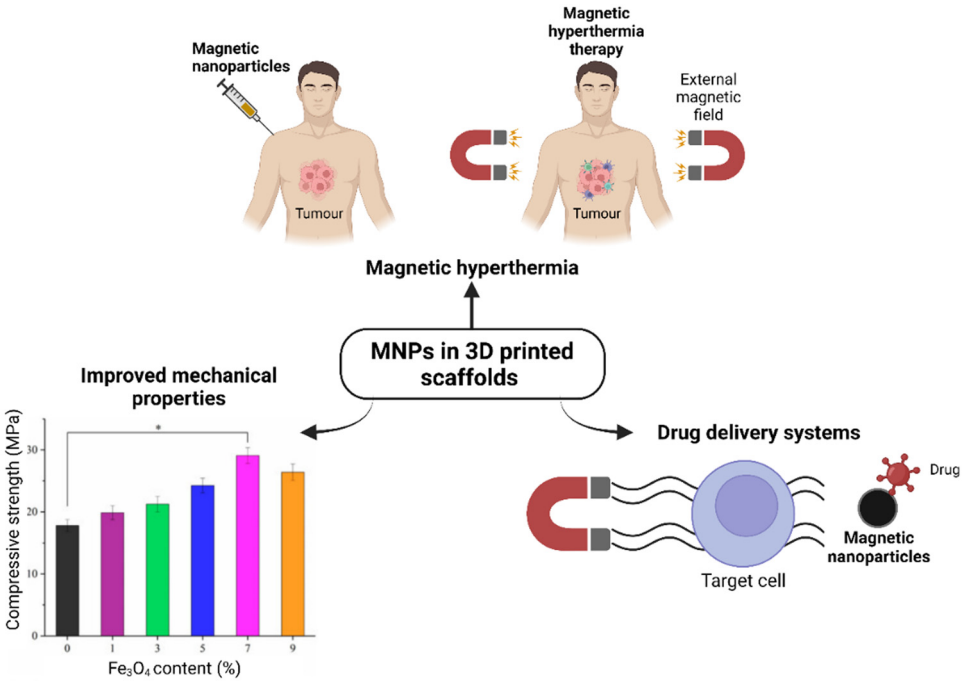

Figure 2: Various applications of MNPs in 3D-printed scaffolds in biomedicine. The picture was produced using BioRender. The LEFT BOTTOM graph was taken with permission from [36].

4 Contribution of MNPs to specific properties of 3D-printed scaffolds

4.1 Fe_3O_4 NPs

Considering the advantages of Fe_3O_4 NPs, several researchers have incorporated Fe_3O_4 NPs into 3D-printed scaffolds for TE applications. For example, Bin *et al.* fabricated a magnetic scaffold for bone tissue applications by incorporating Fe_3O_4 NPs into PLLA by selective laser sintering (Figure 3(a)). The incorporation of Fe_3O_4 NPs, which acted as nanoscale reinforcement in the polymer matrix, not only improved the mechanical properties of the scaffold, such as compressive strength, modulus, and Vickers hardness, but also significantly improved the biological activity (improved cell adhesion) of the scaffold. The compressive strength and Vickers hardness increased with the Fe_3O_4 content and reached a maximum value at 7 wt% (Figure 3(b)). The results showed that the PLLA/Fe_3O_4 scaffold improved MG63 attachment, proliferation, and interaction (Figure 3(c and d)), which promoted the desired cell phenotype [36].

To tailor the degradation rate of PLLA/PGA scaffolds, Shuai *et al.* incorporated magnetic Fe_3O_4 NPs into the scaffolds by selective laser sintering. The saturation magnetization of the scaffolds increased from 1.66 to 8.51 emu/g when the content of Fe_3O_4 NPs increased from 2.5 to 10 wt% and was proportional to the Fe_3O_4 content. Moreover, the water contact angle decreased with the increase of Fe_3O_4 NPs, indicating that the incorporation of Fe_3O_4 NPs significantly improved the hydrophilicity of the scaffold. Although the results indicate that adding Fe_3O_4 NPs improves the compressive strength and modulus of the scaffold, excessive addition of NPs leads to agglomeration, which reduces the mechanical properties of the matrix. The scaffold with 7.5 wt% was selected for further biological experiments. It was shown that the scaffold promoted cell adhesion, proliferation, and differentiation *in vitro* and significantly accelerated the formation of new bone tissue *in vivo* [37].

Chen *et al.* investigated the effect of water-based magnetic fluids with different Fe_3O_4 concentrations on 3D-printed Fe_3O_4/$CaSiO_3$ composite scaffolds for bone TE and obtained similar results. Scaffolds were prepared with Fe_3O_4 NPs at concentrations of 2.6, 3.5, 5.4 and 10.5 w/v%. The results showed that the composite scaffolds had the highest surface content of Fe_3O_4 NPs, the highest saturation magnetization of 69.6 emu/g, and the best stability in dynamically stimulated body fluid when the Fe_3O_4 concentration was 5.4% [38].

In a recent study, Kao *et al.* fabricated porous calcium silicate/PCL scaffolds with various concentrations of Fe_3O_4 NPs (0, 2.5, and 5 wt%) using 3D printing and evaluated their capability to regenerate bone tissue. A favourable combination of compressive strength and rate of decomposition was

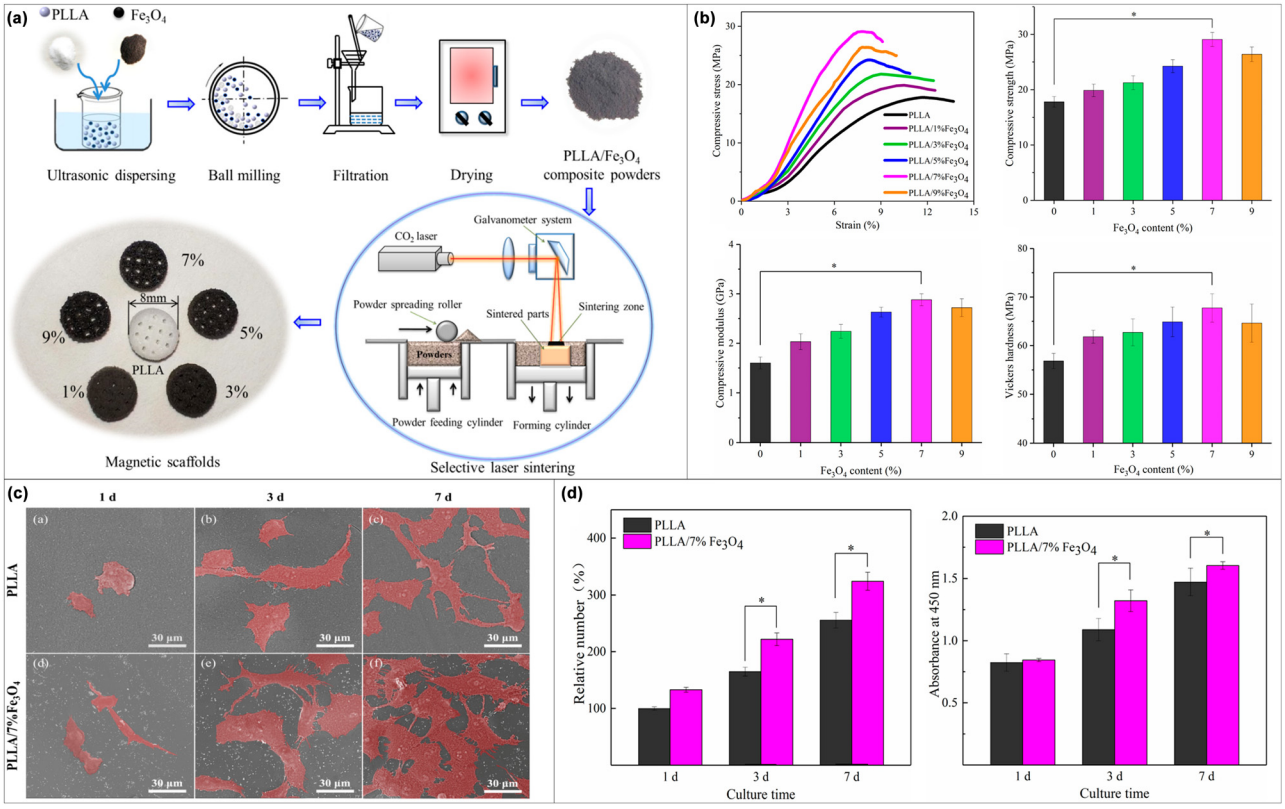

Figure 3: (a) A schematic of the preparation of PLLA/Fe_3O_4 magnetic composite scaffolds. (b) Mechanical properties of the PLLA/Fe_3O_4 scaffolds. (c) SEM pseudocolor image of MG63 cell adhesion on the scaffolds. (d) The relative number of living cells and CCK-8 test in the fluorescence graph. Reproduced with permission from [36].

observed with 5 wt% Fe_3O_4. Results showed that the incorporation of Fe_3O_4 into scaffolds further enhanced the mechanical strength and increased the secretion of osteogenic-related markers, such as alkaline phosphatase, bone sialoprotein, collagen I, and osteocalcin [39].

De Santis *et al.* fabricated magnetic nanocomposite scaffolds based on PCL and poly(ethylene glycol) by 3D fibre deposition technique to regenerate complex tissues such as osteochondral bone. The incorporation of Fe_3O_4 NPs strongly affected the mechanical properties of both PCL- and poly(ethylene glycol)-based scaffolds by increasing the compressive modulus while decreasing ductility [40].

Han *et al.* demonstrated that introducing magnetic IONPs into 3D-printed PLGA scaffolds improved osteogenic differentiation *in vitro* and promoted bone regeneration *in vivo*. These improvements were attributed to enhanced cell adhesion to the magnetic scaffolds due to changes in hydrophilicity, increased surface roughness, and chemical composition of the scaffold. In addition, magnetic effects may also play a role in cell adhesion [41]. 3D-Printed PLGA scaffolds coated with super-paramagnetic iron oxide nanoparticles (SPIONs) were also used in a recent study by Jia *et al.* Their palate-bone regeneration was investigated in a rat model. It was found that SPIONs-coated scaffolds improved bone regeneration, which was partly related to a change in the oral microbiota due to the antibacterial effect of SPIONs [42].

In the presence of SPIONs, Ko *et al.* successfully prepared a self-healing ferrogel based on glycol chitosan (GC) and oxidized hyaluronate (OHA) without using additional chemical crosslinkers. The addition of SPIONs decreased the elastic modulus of the GC/OHA hydrogel, and the storage shear modulus of the GC/OHA/SPIONs ferrogel decreased with an increase in SPIONs concentration. In addition, the properties of the ferrogel also depended on the [GC]/[OHA] ratio and the total polymer concentration. Cytotoxicity was evaluated using ATDC5 cells. Since no significant cytotoxicity of the GC/OHA/SPIONs ferrogel was observed, the authors concluded that the ferrogel could be useful for drug delivery systems and TE applications [43].

In a recent study, adipic acid dihydrazide was added to OHA/GC/SPION ferrogels to improve their 3D printability. By combining a self-healing hydrogel and a self-healing ferrogel without subsequent crosslinking, Choi *et al.* fabricated a

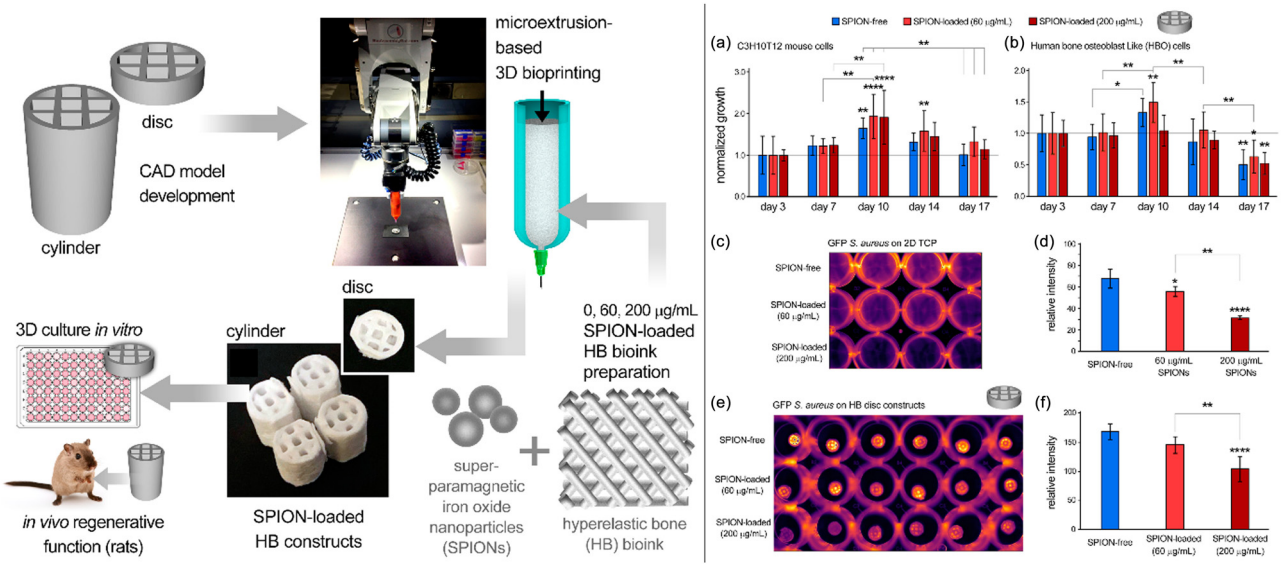

Figure 4: LEFT: Schematic summary of the experimental method used in this study. RIGHT: Characterization of cellular and bacterial response to bioprinted HB constructs *in vitro*. (a and b) Cellular growth (normalized to day 3) for C3H10T12 mouse cells (a) and human bone osteoblast (HBO) cells (b), measured by the noninvasive AlamarBlue assay for 17 days of *in vitro* culture. (c–f) Bacteriostatic effects of SPION in 2D culture (c and d) and SPION-loaded HB constructs (e and f) were evaluated by culturing GFP + S. aureus onto scaffolds for 24 h (c and e) and measuring fluorescence signals (d and f). * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$ (reproduced with permission from Shokouhimehr *et al.* [48]).

3D-printed dynamic tissue scaffold that can be used to stimulate and regulate cell phenotype under magnetic stimulation [44].

4.2 Hydroxyapatite in combination with magnetic nanoparticles

In addition to the mentioned natural and synthetic polymers, bioceramic materials such as hydroxyapatite (HA) are also widely used for bone TE due to their chemical and structural similarity to the mineral phase of natural bone.

Saraiva *et al.* fabricated a novel 3D-printed polylactic acid platform loaded with HA and IONPs to promote bone tissue repair and regrowth. Their results showed that the presence of two types of NPs (IONPs and HA) altered the nanomorphological properties of the 3D platforms and increased the osteogenic functionality of the cells [45].

Petretta *et al.* used 3D printing technology to develop PCL-based scaffolds to which HA and different concentrations of SPIONs were added. These additions aimed to improve the efficiency and control of cell attachment. Two different concentrations of SPIONs, 0.5 and 1%, were chosen, while HA accounted for 10% of the total weight. The addition of SPIONs resulted in higher cell seeding efficiency, activated through an external magnetic field, which was dependent on the degree of scaffold magnetization. The best results in terms of cell entrapment time and adhesion rates were obtained with the 1% SPIONs formulation with a high degree of magnetization. This study showed that PCL-HA-1% SPIONs scaffolds are promising candidates for bone tissue repair and regeneration because they have no toxic effects on fibroblasts and mesenchymal stromal cells and exhibit good cell proliferation and intrinsic osteogenic potential [46].

De Santis *et al.* also developed 3D-printed magnetic nanocomposite scaffolds for bone TE by incorporating iron-doped hydroxyapatite (FeHA) NPs into a PCL matrix. Previous studies have shown that incorporating FeHA NPs improves magnetic properties (i.e., saturation magnetization, temperature values due to hyperthermia), hydrophilicity (indicated by lower water contact angle values), and stiffness while decreasing their mechanical strength. Since the introduction of FeHA NPs led to discontinuities at the interface between the NPs and the matrix, which could be due to the difference in ductility between the polymer matrix and the inorganic nanofillers, the mechanical properties of PCL/FeHA scaffolds are limited. However, compared with pure scaffolds, PCL/FeHA scaffolds showed greater bone marrow mesenchymal stem cells (BMSCs) growth, resulting in improved bone regeneration [47].

To improve the bacteriostatic properties of the implants, Shokouhimehr *et al.* incorporated SPIONs into a hyperelastic bone bioink, which consisted of 90 wt% HA and 10 wt% PLGA (Figure 4). Although the incorporation of 200 mg/ml SPIONs

increased antibacterial activity compared to the 60 mg/ml SPIONs, the 60 mg/ml group showed the most optimal *in vitro* cell response [48].

In addition to various polymers and HA, 3D-printed porous titanium–aluminium–vanadium (pTi) scaffolds are also promising materials for reconstructing large bone defects due to their good mechanical properties, high corrosion resistance, and excellent biocompatibility. However, their restricted induction of bone ingrowth compared to some other materials limits their application in the clinic. To overcome the limitation of the poor osteogenic activity of 3D-printed porous pTi scaffolds, Huang *et al.* fabricated a magnetic coating by applying Fe_3O_4 NPs and polydopamine to the surface of the scaffolds. This new coating significantly improved cell adhesion, proliferation, and osteogenic differentiation of human BMSCs *in vitro* and new bone formation *in vivo*. Moreover, these improvements could be further enhanced by a static magnetic field [49].

5 Magnetic nanoparticles and their use to manipulate 3D-printed materials

Since induced hyperthermia can cause tumour cell death, MH also presents a potential cancer treatment. Zhang *et al.* successfully prepared a multifunctional 3D-printed β-TCP bioceramic scaffold by modifying the surface with Fe_3O_4 NPs/graphene oxide (GO) layers. The resulting β-TCP-Fe-GO scaffold presented a highly ordered macroporous structure with super-paramagnetic behaviour and hyperthermia effects. The porosity of the scaffolds did not change significantly after modification with Fe_3O_4/GO, while the magnetic intensity of the scaffolds increased with increasing Fe_3O_4 content, as previously found in other studies. Therefore, by controlling the magnetic intensity and Fe_3O_4 content, the temperature of the scaffolds could be easily modulated/tailored in the range between 50 and 80°C. The results indicate that such scaffolds have the potential to be used in the therapy and regeneration of bone defects caused by bone tumours due to their excellent magnetic and osteogenic capabilities [50].

In a recent study, Li *et al.* prepared a novel hydrogel composite scaffold of polyvinyl alcohol/sodium alginate/HA by 3D printing. They optimized its properties by varying the concentrations of magnetic graphene oxide (MGO), with Fe_3O_4 NPs uniformly distributed on the surface of GO (Figure 5). Adding MGO improved the composite material's thermal stability and imparted magnetic properties. The prepared composite scaffolds not only improved the biological functions and supported the differentiation of rat BMSCs *in vitro* but also showed favourable anti-tumour effects *in vivo* [51].

Yang *et al.* developed implantable magnetocaloric mats capable of hyperthermia for cancer treatment. These properties were achieved by incorporating Fe_3O_4 NPs into PCL using E-jet 3D printing technology. When the PCL/Fe_3O_4 mat was exposed to an AMF, it resulted in efficient heating without loss of heating capacity or leakage of Fe_3O_4 NPs. The mats containing 6 mmol/L Fe_3O_4 NPs were the most effective, as they peripherally raised the temperature under an AMF to 45°C within 45 min and could inhibit tumour

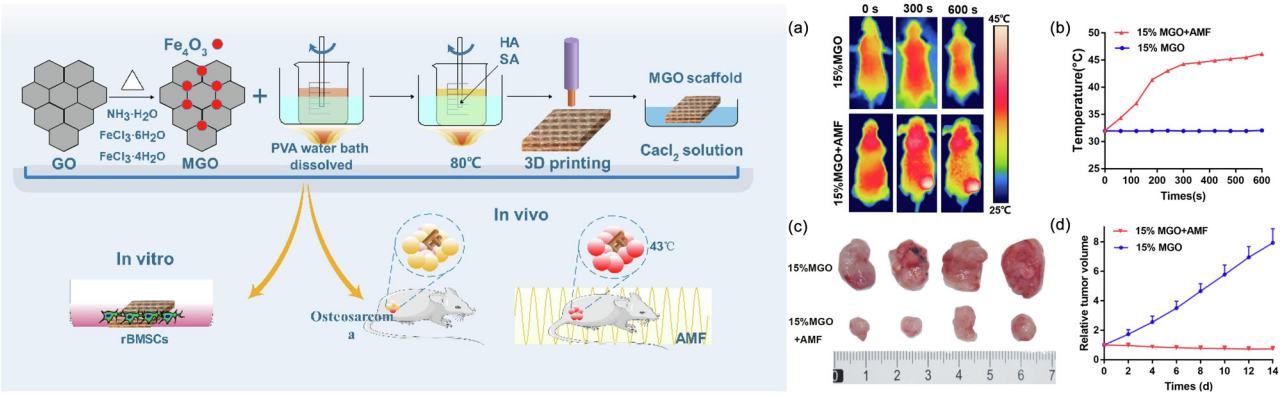

Figure 5: LEFT: Schematic diagrams of MGO hydrogel composite fabrication (above) and application to bone tumour defect regeneration *in vitro* and *in vivo* (below). RIGHT: Inhibition of osteosarcoma tumour growth *in vivo*. (a) *In vivo* infrared thermography of 143b-tumour-bearing nude mice after intratumorally implantation with MGO hydrogel composite under AMF at various time points. (b) Temperature versus time at the tumour sites implanted with MGO hydrogel composite with and without AMF. (c) Digital photographs of the dissected tumours. (d) Relative tumour volume changes over time after the different treatments (reproduced with permission from Li *et al.* [51]).

growth *in vivo*. Such magnetic mats are ideal for hyperthermia treatment of easily accessible tumours [52].

Another interesting study was published by Dong *et al.*, in which the authors report an excellent synergistic therapeutic effect in osteosarcoma treatment. The latter was achieved through a combination of MH with an elaborate catalytic Fenton reaction by Fe_3O_4 and calcium peroxide (CaO_2) NPs. Fe_3O_4 NPs were loaded into a 3D-printed akermanite scaffold to initiate MH through an AMF and catalyse the generation of hydroxyl radicals from hydrogen peroxide (H_2O_2). At the same time, the co-loaded CaO_2 NPs acted as an H_2O_2 source [53].

In addition to magnetothermal cancer therapies, magnetic field application could also be used to stimulate osteogenesis for bone repair. Shuai *et al.* fabricated porous super-paramagnetic PGA/Fe_3O_4 scaffolds that exhibit favourable mechanical, magnetic, and degradation properties. The magnetic moment of Fe_3O_4 NPs rearranged along the direction of the self-developed external static magnetic field applied as an external magnetic source, resulting in a locally enhanced magnetic field. As a result, cell adhesion, proliferation, and differentiation were promoted, and bone regeneration was significantly accelerated [54].

Zhang *et al.* used 3D printing to fabricate mesoporous bioactive glass (MBG)/PCL composite scaffolds containing magnetic Fe_3O_4 NPs. The saturation magnetization of the Fe_3O_4/MBG/PCL scaffolds increased with increasing Fe_3O_4 content, and a positive correlation between the heating rate and Fe_3O_4 content in the scaffolds was also observed. Although the incorporation of magnetic Fe_3O_4 NPs into the scaffolds did not affect apatite mineralization ability, it resulted in excellent magnetic heating and significantly stimulated cell proliferation and differentiation. The composite scaffolds also exhibited excellent bioactivity in apatite formation and increased compressive strength. Therefore, there is great potential for using Fe_3O_4/MBG/PCL scaffolds in the treatment and regeneration of bone defects through a combination of enhanced osteogenic activity, local delivery of anticancer drugs, and MH [55].

The agglomeration potential of IONPs has necessitated the development of strategies to modify the surface of IONPs. Lin *et al.* chemically modified the IONPs with sodium citrate to obtain a negative charge on the surface before embedding them in a chitosan hydrogel so that the surrounding cells could not directly contact the NPs. The study showed that the inductive coupling magnetic force successfully promoted bone cell growth, as evidenced by higher osteoblast cell proliferation, type I collagen production, alkaline phosphatase expression, and mineralization [56].

6 Magnetic nanoparticles in tailoring delivery of drugs and cells from 3D-printed materials

Scaffolds with incorporated MNPs are also quite interesting for the development of advanced, stimuli-responsive drug delivery systems as they can be guided and triggered by external magnetic fields. In these systems, therapeutic compounds are attached to biocompatible MNPs, which are directed to specific targets *in vivo* using an external magnetic field, resulting in enhanced delivery to the target site and reduced side effects of drugs by reducing their systemic distribution. For example, when an external magnetic field is applied to MNPs bound to cellular surface receptors, the MNPs generate mechanical forces that can be transmitted to the membrane to activate mechanosensitive ion channels [57]. Zhao *et al.* incorporated IONPs into alginate hydrogels to control the release of various drugs and cells by causing large deformation and volume change of over 70% under the control of external magnetic field (Figure 6) [58].

Such on-demand release of cells from porous scaffolds can be also very useful for tissue regeneration and cell therapies.

Wang *et al.* developed a magnetically driven delivery system for precise control of drug, protein, and cell release based on 3D-printed alginate/IONPs hollow fibre scaffolds. In this system, drugs, proteins, and even cells can be extruded from the core of the hollow fibres based on the deformation of the scaffolds under the magnetic field, which could prove useful for disease treatment and TE applications. The scaffolds' deformation behaviour (and ability to release on demand) can be influenced by several factors, such as the concentration of alginate inks, crosslink density, and the content of incorporated NPs. A higher amount of NPs resulted in more deformation under magnetic stimulation. Furthermore, adding Fe_3O_4 NPs to the inks did not significantly affect the printing behaviour of the hollow fibre scaffolds [59].

7 Magnetic nanoparticles for remote magnetic actuation of cells in tissue engineering applications

In addition to already described applications, MNPs have recently been explored to enable remote magnetic actuation for targeting and activating specific mechanosensitive

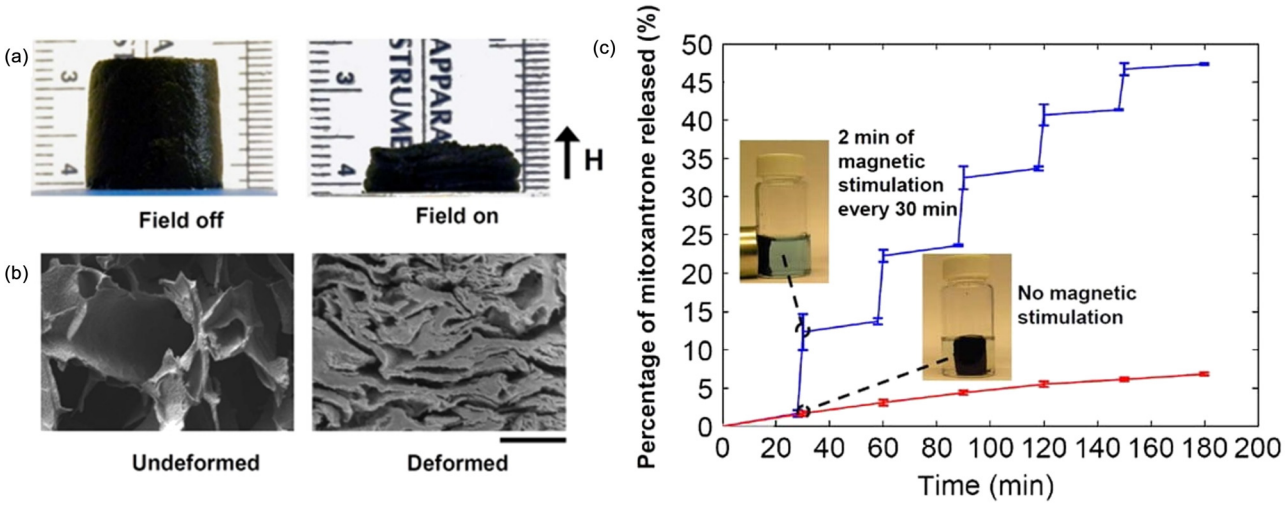

Figure 6: (a) A cylinder of a macroporous ferrogel reduced its height ~70% when subjected to a vertical magnetic-field gradient of ~38 A/m^2. (b) SEM images of a freeze-dried macroporous ferrogel in the undeformed and deformed states. Scale bar: 500 μm. (c) Cumulative release profiles of mitoxantrone from macroporous ferrogels subject to 2 min of magnetic stimulation every 30 min, or no magnetic stimulation. Reproduced with permission from [58].

membrane receptors and ion channels to regulate cell signalling pathways and consequently control cell behaviour [60]. In TE, this approach has been applied to different types of stem cells, as stem cell-based therapies offer great potential for regenerating and repairing damaged tissues *in vivo* [60]. Using 3D printing technology, Gonçalves *et al.* fabricated the magnetically responsive scaffold from a biodegradable polymer blend of starch, and PCL incorporated with IONPs with potential for tendon tissue engineering. *In vitro* studies showed that incorporation of MNPs did not negatively affect the viability or differentiation of human adipose stem cells (hASCs) and may even enhance cells' metabolic activity. Furthermore, applying an external magnetic field enhanced the biological performance of hASCs cultured on developed magnetic scaffolds regarding cell proliferation and differentiation. The developed scaffolds were also cytocompatible in an ectopic rat model [61]. Results of another study suggested that Activin receptor type IIA (ActRIIA) in hASCs is a mechanosensitive receptor that can be remotely activated using anti-ActRIIA functionalized MNPs, whose action is stimulated by an external magnetic field, leading to tenogenic differentiation, which enables successful cell therapy for tendon regeneration [62]. An exciting feature of this approach is the ability of functionalized MNPs to activate cells remotely using bio-magnetic approaches. In a more recent study, this approach was successfully translated into a 3D environment combining magnetically responsive scaffolds, and MNPs-ActRIIA tagged hASCs exposed to the actuation of externally applied AMF, the synergy of which enhanced the tenogenic commitment of hASCs [63]. Their findings, therefore, represent the first step towards the mechanical stimulation of the regeneration of tendon tissue.

8 Other applications of MNPs in the 3D-printed scaffolds

Combinations of scaffolds and MNPs were also shown promising for many other applications [57,64]. In addition to bone TE, Li *et al.* described a method to fabricate biocompatible artificial bile ducts with 3D printing using a tubular composite scaffold based on PCL as a matrix for the organoid cells of the bile duct. A layer of gelatin methacryloyl (GelMA) hydrogel was applied to the outer layer to increase biocompatibility. Ultrasmall super-paramagnetic iron oxide NPs were uniformly dispersed in GelMA to allow monitoring by magnetic resonance imaging [65].

In another recent study, Xiang *et al.* fabricated a novel bilayered artificial bile duct scaffold with a PLGA inner layer and a GelMA outer layer. PLGA with suitable mechanical properties, slow degradation kinetics, and good biocompatibility was used instead of PCL. Moreover, IKVAV laminin peptide was used to improve cell adhesion and ultrasmall super-paramagnetic iron oxide NPs were used again for magnetic resonance imaging [66].

9 Other magnetic nanoparticles combined with 3D-printed materials

In addition to IONPs, which are the most commonly used due to their relatively simple synthesis, high magnetization, biocompatibility, and chemical stability [8], other MNPs, such as NiCu and $CuFeSe_2$ were also already successfully incorporated into 3D-printed scaffolds to improve their properties [32,67].

To tailor the desired properties of the scaffolds, such as printability, surface roughness, swelling, degradation, and mechanical properties, Milojević *et al.* incorporated variable concentrations of NiCu NPs into hybrid hydrogel formulations of alginate, carboxymethyl cellulose, and nanofibrillated cellulose. The results showed that NiCu NPs were an effective means of controlling hydrogel viscosity, scaffold swelling, degradation, and topographic properties. In addition, all the scaffolds not only promoted cell adhesion, aggregation, and migration but also supported the long-term growth of pancreatic cells, and thus could be used in the field of pancreas-related disease research [32].

Furthermore, Dang *et al.* were the first to combine the photothermal performance of semiconductor nanocrystals of $CuFeSe_2$ with the bone-forming activity of bioactive glass (BG) scaffolds. The photothermal performance of the BG-$CuFeSe_2$ scaffolds could be well regulated by controlling the $CuFeSe_2$ content and the laser power density. Due to hyperthermia induced by the $CuFeSe_2$ nanocrystals, the BG-$CuFeSe_2$ scaffolds could not only effectively ablate the bone tumour cells *in vitro* but also suppress the growth of bone tumour tissue *in vivo*. Moreover, the BG-$CuFeSe_2$ scaffolds could support the attachment and proliferation of rabbit BMSCs. Finally, the scaffolds were shown to stimulate the formation of new bone in bone defects. The authors concluded that scaffolds with such dual functions (bone tumour therapy and bone defect regeneration) might represent a promising treatment strategy for tumour-induced bone defects [67].

As recently pointed out in a review article by Palenzula and Pumera [68], 3D printing can also be used as a perfect platform for developing sensors and biosensors. An example of the latter is microfluidic platforms for the detection of bacterial pathogens [69]. The vast opportunities enabled by nanoparticle use in the 3D printing of electronic and bioelectronic devices are also highlighted in Hales *et al.* [70].

10 Conclusion and future perspectives

This review summarizes relevant studies and recent progress on incorporating MNPs into 3D-printed scaffolds for biomedical applications. Several studies have reported that the incorporation of MNPs and their concentration affect the mechanical properties of the scaffolds. MNPs have shown great potential for use in bone TE, as they play several important roles in stimulating and modifying cellular responses that are beneficial for bone formation. The results of several studies indicate that MNPs incorporated into the scaffolds promote cell adhesion, proliferation, and differentiation *in vitro* and significantly accelerate the formation of new bone tissue *in vivo.* Moreover, MNPs can potentially be used in MH and drug delivery. However, as most of the research on 3D-printed scaffolds is limited to bone tissue, more research on other tissues will be needed to prove their worth in TE further. Among additional applications are also studies related to the incorporation of NiCu MNPs into polysaccharide-based dressings with antimelanoma activity, conducted by our research group. In addition to MNPs, which have already been successfully incorporated into 3D-printed scaffolds, other nanocomposites such as FeNi, FeCu, or different ferrites, with appropriate mechanical and hyperthermal properties are being investigated.

MNPs incorporated into 3D-printed scaffolds hold great promise for various biomedical applications, and several future perspectives can be explored. The magnetic properties of MNPs can enable magnetic manipulation of the 3D-printed scaffold and the cells within it, which is a highly interesting property in TE. The magnetic field can be used to guide cell migration and promote tissue regeneration. Furthermore, MNPs can be incorporated into 3D-printed scaffolds as carriers for targeted drug delivery. The drug can be attached to the surface of the MNPs and released in response to an external magnetic field. Incorporating MNPs into 3D-printed scaffolds can also enhance the contrast in magnetic resonance imaging (MRI), providing a more detailed and accurate image of the scaffold and the surrounding tissue. It can also enable biosensing applications, such as detecting specific biomolecules or pathogens within the scaffold. MNPs can enhance the sensitivity and selectivity of such biosensors.

Overall, incorporating MNPs into 3D-printed scaffolds for biomedical applications has great potential for enhancing TE, drug delivery, imaging, magnetic manipulation,

and biosensing. Continued research in this field will likely lead to further advancements and innovations in the future.

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